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TITLE: A Fetus-Targeted Antibody Therapy to Prevent Zika Virus Infection During Pregnancy

PRINCIPAL INVESTIGATOR: Diogo Magnani

CONTRACTING ORGANIZATION: University of Massachusetts, Worcester, 01655-0002

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14. ABSTRACT

Interventions are urgently needed to prevent the severe fetal complications of Zika virus infection. In the US, one in ten pregnant women infected with Zika virus delivered babies with severe birth defects, including small head size (microcephaly) and other neurological problems. Once the fetus is infected, there is nothing we can do to stop viral replication with current technologies. An approach considered safe for the fetus is to administer antibodies that can neutralize Zika virus. The problem, however, is that these effective antivirals, cannot reach the fetal tissues early in the pregnancy in sufficient amounts to be effective. Here, we are proposing to engineer antibody molecules so that they will cross the placenta effectively and reach the fetus. We will test our approach in rhesus monkeys, the best animal model of Zika virus infections during human pregnancy, by administering our 'fetus-targeted' antibody to pregnant macaques and challenging with Zika virus. If the antibodies can indeed prevent fetal infection on macaques, this proposal might lead to one of the first effective therapies for Zika virus infection.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:

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1. **INTRODUCTION:**

Our ultimate objective is to prevent fetal Zika virus (ZIKV) infection. We are testing a novel approach for preventing fetal ZIKV replication, by engineering monoclonal antibody (mAb) delivery to the fetus. The proposed experiments are straightforward: we are engineered a ZIKV-neutralizing antibody with neonatal Fc receptor (FcRn)-enhancing mutations. The ability of this antibody to cross the placenta will be tested *in vivo*. Our major goal is, therefore, to **define mAb mutations that result in enhanced transplacental transfer of therapeutic monoclonal antibodies.** Importantly, this award had delays due to difficulties of scheduling experiments with pregnant nonhuman primates in 2020 and is currently on a no cost extension.

2. **KEYWORDS:**

Monoclonal antibody therapy; Zika virus; rhesus macaque; congenital disease; neutralizing antibody

3. **ACCOMPLISHMENTS:**

Table. 1 Major goals of the project as stated in the modified SOW and current status (as November/25/2020)

STATEMENT OF WORK – November/25/2020
PROPOSED START DATE Aug 01, 2019

Site 1: Massbiologics of University of Massachusetts Medical School

460 Walk Hill St
Mattapan, MA 02124, USA

PI: Diogo Magnani

Site 2: California National Primate Research Center
University of California, Davis
One Shields Avenue
Davis, CA 95616-8542, USA

Partnering PI: Koen Van Rompay

Specific Aim 1	Timeline	Status	Site 1	Site 2
Produce monoclonal antibodies	Months			
1.1. Express and purify antibodies	1-4	Completed	Dr. Magnani	
1.2. Measure Fc-FcRn binding affinity	1-4	Modified	Dr. Magnani	
1.3. Produce *in vivo* grade material	3-7	Completed	Dr. Magnani	
Milestone(s) Achieved	5-7		Dr. Magnani	
Perform animal experiment				
2.1 Set up subaward contracts	3	Completed	Dr. Magnani	Dr. Van Rompay
2.2. Local IRB/IACUC Approval	3	Completed	Dr. Magnani	Dr. Van Rompay
Milestone Achieved: HRPO/ACURO Approval	6	Completed	Dr. Magnani	Dr. Van Rompay
Identify a pool of pregnant macaques in the second trimester of pregnancy	3-6	Postponed		Dr. Van Rompay
Select pregnant animals in the gestation day 125	6-7	Postponed		Dr. Van Rompay
Administer antibody to pregnant macaques (intravenous infusions)	7-9	Postponed		Dr. Van Rompay
C-section (gestation day 154)	7-9	Postponed		Dr. Van Rompay
Blood collections	7-9	Postponed		
Measure antibody levels in mother and neonates	7-9	Postponed	Dr. Magnani	Dr. Van Rompay
Milestone(s) Achieved:	8-9	Postponed	Dr. Magnani	Dr. Van Rompay

Our initial experiments were set to design and characterize monoclonal antibody constructs to enhance the FcRn function (Table 1). Our major activities, specific objectives, and significant results were as follows:

- We used our DNA vectors to express the ZIKV-neutralizing antibody SMZAb2 in wild-type and mutated versions to modify the interactions with FcRn.

We also constructed and expressed our control antibody molecules:

- *anti-HIV [F240] as a human antibody*
- *anti_HIV[CH58R1L] as a rhesus antibody*
- *anti_SARS-CoV-2[CR3022] as a rhesus antibody*
- *anti-Tetanus as a human antibody*
- *anti-DSP as a rhesus antibody*
- *anti-HIV [MN215] as a rhesus antibody containing a N297G Fc mutation*
- *anti-HIV[3D6] as a rhesus antibody containing a LS Fc mutation*

We produced a total of 1.2 grams of *in vivo*-grade recombinant monoclonal antibodies, purified by protein A and size-exclusion chromatography. These antibodies are now ready for infusion into animals.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

To accomplish our goals, we will need to test our constructs in the rhesus macaque model of monoclonal antibody therapy during pregnancy. We already produced the recombinant antibodies, however, our projects are currently halted due to COVID19 pandemic. As soon as we are cleared to resume activities, we will perform a pre-infusion biochemical characterization of the recombinant products (confirming molecule purity, identity, and sterility). We anticipate macaques in the second or third trimester of pregnancy becoming available in the next

breeding period of December-March. We will then perform the preclinical animal experiments to determine if an engineered antibodies with FcRn-enhancing mutations can cross the placental barrier at higher rates than wild type.

4. **IMPACT:**

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

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

Nothing to Report.

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

The experiments proposed here are currently halted due to the COVID19 pandemic. We will request a no-cost extension to perform the activities whenever possible.

Changes that had a significant impact on expenditures

Nothing to Report.

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

Nothing to Report.

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

Nothing to Report.

6. **PRODUCTS:**

Nothing to Report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	*Diogo Magnani*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1773-2406*
Nearest person month worked:	<1
Contribution to Project:	*Dr. Magnani oversees all steps of the project, including experimental planning, execution, analysis and reporting.*
Funding Support:	*No change.*

Name:	*Walter Flores*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*Not available*
Nearest person month worked:	<1
Contribution to Project:	*Mr. Flores is responsible for producing and qualifying the recombinant proteins used in this project. He also performs the in vitro assay analyses.*
Funding Support:	*No change.*

Name:	*KOEN VAN ROMPAY*
Project Role:	Site 2 Principal Investigator
Researcher Identifier (e.g. ORCID ID):	*0000-0002-7375-1337*
Nearest person month worked:	<1
Contribution to Project:	*Dr. Koen has established the animal protocols (IACUC) and completed ACURO reviews.*
Funding Support:	**ACTIVE (2020)** P51 OD011107-54 (PI: Dr. H. Lewin) 5/01/97-4/30/23 2.4 cal months Source: NIH/NCRR *"California National Primate Center"* This is the base operating grant of the California National Primate Research Center and support for scientific expertise and collaborative contributions. Role: Co-investigator 1P01 AI117915-05 (PI: Dr. Sallie Permar, Duke University) 4/1/15-3/31/21 2.04 cal months NIH (subcontract only) *Maternal and Infant Immunization to eliminate breastmilk transmission of HIV.* The goal of this project is to use an animal model mother-to-child HIV transmission to determine if immunization of pregnant female macaques and/or their newborns is able to protect their infants against oral infection. Role: Co-investigator NIH U420D010990 (PI: Dr. Jeffrey Roberts) 5/1/16-1/30/21 0.6 cal months SPF colony of CNPRC The purpose of this grant is to manage and improve specific pathogen free (SPF) nonhumanprimate colones. Role: Co-investigator Research agreement A18-1417 2/16/18-5/31/20 0.12 cal months PaxVax, Inc. *Immunogenicity and protective efficacy of PXVX0317 against Chikungunya virus challenge in rhesus macaques*. The purpose of this study is to test whether active immunization of macaques with a CHIK vaccine protects against virus infection. Role: Principal Investigator 5R01DE025444-05 (PI: Sallie Permar, Duke University) 7/1/18-6/30/20 0.24 cal months NIH (subcontract only) *Innate antiviral factors in breast milk and the oral HIV-1 reservoirs* The role of this study is to test anti-latency therapies in SHIV-infected infant macaques as an animal model of pediatric HIV infection. Role: Co-investigator RF1AG061001 (Iyer/Morrison, MPIs) 9/01/18 - 8/31/23 0.6 cal months NIH, NIA *Immune Mechanisms Underlying Age-Related Neurodegeneration in HIV Infection*

The role of this study is to test immune-related mechanisms of neurogeneration in aging SIV-infected macaques.
Role: Co-investigator

1K01OD024877-01 (PI: Justin Pollara, Duke University) 9/01/2017-8/31/2020
0.72 cal months
NIH
Combined Hepatitis B and HIV-1 envelope vaccination to augment T cell help via linked recognition of unrelated antigens.
The role of the study is to test the immunogenicity of novel HIV vaccine constructs linked to hepatitis B vaccine in infant macaques.
Role: co-mentor

NIH/5R01HL131696 (PI: Bryce Chackerian, Univ. of New Mexico) 01/01/2019 - 12/31/2020 0.3 cal months
NIH
A Nanoparticle-Based Vaccine Targeting PCSK9
The goal of the study is to demonstrate proof-of-concept of a novel vaccine that targets PCSK9 as a mechanism to lower cholesterol levels for patients that are resistant to statins.
Role: Co-investigator

1R01DE028146-01 (PI: K. De Paris, Univ. North Carolina, Chapel Hill) 9/6/18 to 6/30/23 1.44 cal mos
NIH
Sublingual-parenteral vaccination to prevent oral HIV transmission in infants.
Role: Co-investigator
The goal of this study is to test whether a combination of sublingual and parenteral vaccination is more effective than either one of both routes in inducing systemic and mucosal immune responses that protect infants macaques against oral SHIV infection.

Research agreement A20-1316 9/1/19-8/31/20
3 cal months
Emergent Biosolutions Inc/PaxVax, Inc.

Preclinical testing of Chikungunya vaccine
The goal of this study is to test whether passive immunization with anti-chikungunyna virus antibodies prior to virus inoculation is able to protect macaques against infection or the development of pathology.
Role: Principal investigator

5R01HD096436-02 (PI: E. Bliss-Moreau, UC-Davis) 8/9/18-5/31/23 0.3 calendar months
NIH
Cognitive, Socioaffective, and Neural Development Following Fetal Zika Virus Infection
The goal of this study is to test whether infection of pregnant macaques with Zika virus leads to longterm neurodevelopmental effects in their infants that are monitored after birth for several years.
Role: Co-investigator

W81XWH1810094 (PI: Magnani, Diogo, Univ. of Massachussets) 08/01/19-10/31/21
0.24 cal mos
Department of Defense (subcontract only)
Fetus-Targeted Antibody Therapy to Prevent Zika Virus Infection During Pregnancy

The goal of this study is to look at the kinetics of transplacental transfer of anti-zika antibodies in pregnant macaques.
Role: Principal Investigator (subaward).

Research agreement A19-2496 1/01/19-12/31/20 0.0 calendar months (no animal studies at this time)
Novartis Institute for Tropical Diseases
Discovery of novel antimalaria drugs
The goal of this study is to develop a model of Plasmodium cynomolgi infection of rhesus macaques to subsequently be able to test novel antimalarial drugs.
Role: Principal investigator (of UCD research agreement)

R01AI141181-01 (PI: Peter Rice, Univ. of Massachusetts) 12/01/18-11/30/21 0.0 cal mos (animal studies have not started yet and are on hold)
NIH (subcontract only)
A novel vaccine against multi-drug resistant gonnorhea
The goal of this study is to determine the immunogenicity of a novel vaccine against gonorrhea in macaques.
Role: Co-investigator

R01AI145016-01 (PI: Genevieve Fouda Amou'ou, Duke University) 04/01/2019-03/31/2021 0.0 cal mos (animal studies have not started yet and are on hold)

NIH subcontract only)
Supramolecular pediatric HIV vaccine design
The goal of this study is to test a novel HIV vaccine regimen in the infant macaque model of pediatric HIV infection.
Role: Co-investigator

Research Agreement (PI: Brian Bird, UC-Davis) 07/01/2019 - 6/30/2021 0.0 cal months (animal studies have not started yet and are on hold)

Coalition For Epidemic Preparedness Innovations (G-19803-01). (direct costs)

Rift Valley Fever Vaccine Assessment in Rhesus Macaques

The goal of this study is to study the immunogenicity of a live-attenuated Rift Valley Fever vaccine in macaques.
Role: Co-investigator

1R01AI34868 (PI: Dan Granoff, CHORI) 5/5/19-5/14/2024 0.0 cal months (animal studies have not started yet and are on hold)
NIH (subcontract only)
Improved protein-based meningococcal serogroup B vaccines
The goal of this study is to test the immunogenicity of novel vaccines directed against meningococcal infection.
Role: Co-investigator

PENDING:

1P01 AI117915-06 (PI: Sallie Permar, Duke; Kristina De Paris, Univ of North Carolina, Chapel Hill): 4/1/20-3/31/25 0.6 calendar months
Early life vaccination to prevent HIV acquisition during adolescence
(subcontract only)
The goal of this study is to investigate whether starting HIV immunizations in infant macaques is more effective than to starting immunizations
Role: Co-investigator

R01 (PI: Guo, Haitao; Univ. of North Carolina, Chapel Hill): 7/1/20-6/30/25
1.2 calendar months
NIH (subcontract only)
Inflammasomes in Central Nervous System Comorbidity between Opioid Drug Abuse and HIV
The goal of the study is to study the interaction between morphine and SIV infection on the development of brain pathology in SIV-infected macaques.
Role: Co-investigator

Research Agreement- Gilead Sciences 05/01/20-04/30/21
3 calendar months
Gilead nonhuman primate vaccine studies
The goal of the study is to test novel HIV vaccine strategies against SIV infection in macaques.
Role: Principal Investigator

R01 (PI: M. Martins) 7/1/20-6/30/25 0.6 calendar months
NIH (subcontract only)
eCD4-Ig for preventing and treating obstetric HIV infection
The pre-clinical experiments proposed here will help us gauge the prophylactic and therapeutic potential of eCD4-Ig for combatting obstetric HIV infection. Time-mated pregnant RMs will receive intravenous injections of parental eCD4-Ig or Fc-mutated versions of this molecule. Puerperal RMs will be treated intramuscularly with AAV vectors encoding eCD4-Ig. Serum, plasma, and mucosal secretions from female monkeys will be collected at regular points for immunological and virologic measurements.

INACTIVE

5R37AI037526-23 rev (Subcontract Rockefeller University) 10/30/18-1/31/20
0.72 cal months
NIH
Preclinical testing of neutralizing antibodies against Zika virus
The goal of the study is to test whether neutralizing antibodies are able to protect macaques against Zika virus infection.
Role: Principal investigator of subcontract.

HHSN268201100001I (PI: Michael Busch, BSRI) 9/1/16-1/31/20
1.32 cal months
Recent Epidemiology and Donor Evaluation Study-III (REDS-III)
The goal of this study is to test the risk of transmission of Zika virus via blood transfusion and test countermeasures.
Role: Co-investigator
HHSF223201610018C (PI: Nolan, Garry; Stanford Univ).
9/30/19 10/01/17-
FDA
only) (subcontract
Sequelae and immunopathology of Ebola virus infections
The goal of this study is to use archived tissues of Zika-virus infected macaques to study immunopathology of Zika virus infection.

1R01AI114701 (PI: Dan Granoff, CHORI) 12/1/16-3/31/19

	NIH (subcontract only) *The effect of binding of fH to meningococcal fHbp vaccine on antibody protection* The goal of this study was to test the immunogenicity of novel vaccines directed against meningococcal infection. 1R21AI129479-01. (PI: Van Rompay) 11/14/16-10/31/2018 NIH/NIAID *Macaque model of Zika virus infection* The goal of this study is to develop a non-human primate model of fetal Zika virus pathogenesis and to define markers of severe disease in pregnant macaques. 1R21AI129479-01-Supplement (PI: Van Rompay) 11/14/16-10/31/19 NIH/NIAID *Development of a nonhuman primate model of fetal zika virus infection and disease* The goal of the study is to test if a ZIKV DNA vaccine can protect pregnant macaques and their fetuses against ZIKV infection. Role: Principal Investigator

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

The PI (Diogo Magnani) has been awarded the following new NIH grants:

P40OD028116 (Magnani/Engelman) 01/01/2020–12/31/2024 2.76 cal. mo. *NIH/OD*
Nonhuman Primate Antibody Resource for Immune Cell Depletion
This program develops immune cell-depleting antibodies for nonhuman primate research.

R24OD028257-01, NIH OD (PI Engelman; Role: Co-PI 0.6 cal. mo.) 09/01/2019–08/31/2024 *Neotropical Primate Reagent Resource*
In this resource application, we propose to develop a number of marmoset and squirrel monkey antibodies to address the gap between project goals and available reagents. Species-matched antibodies can deplete targeted lymphocyte subsets and allow investigators to probe the impact of specific immune populations on progression, protection, and recovery. Diagnostic antibodies that distinguish antibody classes and subclasses, cell surface biomarkers, and soluble mediators will reduce limitations imposed by poor reagent accessibility. To derive these reagents, the squirrel monkey and marmoset immunoglobulin and Fcg receptor repertoire will be exhaustively defined and characterized.

U24AI126683-05S1 NIH NIAID SUP (Magnani/Engelman; Role: PI, no effort) 04/13/2020– 04/12/2021 We will leverage our tools, expertise, and infrastructure to produce recombinant reagents for research on COVID-19. Our proposal will also establish large-scale industrial methodologies for producing and purifying viral antigens, shall they become necessary in the future.

U24AI126683, NIH NIAID SUP (Magnani/Engelman; Role: PI, no effort) 05/31/2019–05/31/2021 Preclinical evaluation of rhesus ATG as a depletional induction agent in rhesus macaques

P40OD028116, NIH OD SUP (Magnani/Engelman; Role: PI, no effort) 07/01/2020–12/31/2024 We will produce and distribute defined coronavirus-specific monkey immunoglobulin preparations. Our recombinant

standard reagents will support the development of rigorous coronavirus-specific antibody assays for nonhuman primate research and colony management.

R01AI154559-01 (PI: Jonah Sacha; Role: SubawardPI) 07/23/2020–06/30/2025 *A Solid-Phase, Long-Acting CCR5 Monoclonal Antibody for HIV Transmission*
The proposed studies are aimed at understanding the potential of a half-life extended antibody against the co-receptor of AIDS viruses, CCR5, in prevention of viral acquisition against challenges via different routes.

What other organizations were involved as partners?

Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. **APPENDICES:**

Nothing to Report.