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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.					
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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 engineering a ZIKV-neutralizing antibody with neonatal Fc receptor (FcRn)-enhancing mutations and testing *in vitro*. The ability of this antibody to cross the placenta and prevent ZIKV infection 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 unanticipated delays 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 approved modified SOW (June/26/2019) and current status (as April/18/2020)

STATEMENT OF WORK – June/26/2019 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 <i>in vivo</i> 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	Completed		Dr. Van Rompay
Select pregnant animals in the gestation day 125	6-7	Completed		
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). During the current reporting period, our only major task as per our approved Scope of Work (SOW) was to: Design, synthesize, express antibody constructs; DNA sequencing of plasmids and viruses. 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-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:	<i>Diogo Magnani</i>
Project Role:	<i>Principal Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>0000-0002-1773-2406</i>
Nearest person month worked:	<i><1</i>
Contribution to Project:	<i>Dr. Magnani oversees all steps of the project, including experimental planning, execution, analysis and reporting.</i>
Funding Support:	<i>No change.</i>

Name:	<i>Walter Flores</i>
Project Role:	<i>Research Associate</i>
Researcher Identifier (e.g. ORCID ID):	<i>Not available</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Mr. Flores is responsible for producing and qualifying the recombinant proteins used in this project. He also performs the in vitro assay analyses.</i>
Funding Support:	<i>No change.</i>

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:	<i>Dr. Koen has established the animal protocols (IACUC) and completed ACURO reviews.</i>
Funding Support:	<p>ACTIVE</p> <p>P51 OD011107-54 (PI: Dr. H. Lewin) 5/01/97-4/30/23 2.4 cal months</p> <p>Source: NIH/NCRR \$11,407,363 "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</p> <p>1P01 AI117915-05 (PI: Dr. Sallie Permar, Duke University) 4/1/15-3/31/21 2.04 cal months NIH \$630,323 (subcontract only) <i>Maternal and Infant Immunization to eliminate breastmilk transmission of HIV.</i> 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</p> <p>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 colonies. Role: Co-investigator</p> <p>Research agreement A18-1417 2/16/18-5/31/20 0.12 cal months PaxVax, Inc. \$1,189,733 <i>Immunogenicity and protective efficacy of PXVX0317 against Chikungunya virus challenge in rhesus macaques.</i> The purpose of this study is to test whether active immunization of macaques with a CHIK vaccine protects against virus infection. Role: Principal Investigator</p> <p>5R01DE025444-05 (PI: Sallie Permar, Duke University) 7/1/18-6/30/20 0.24 cal months NIH \$173,240 (subcontract only) <i>Innate antiviral factors in breast milk and the oral HIV-1 reservoirs</i> 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</p> <p>RF1AG061001 (Iyer/Morrison, MPs) 9/01/18 - 8/31/23 0.6 cal months NIH, NIA \$2,342,690 <i>Immune Mechanisms Underlying Age-Related Neurodegeneration in HIV Infection</i></p>

	<p>The role of this study is to test immune-related mechanisms of neurogeneration in aging SIV-infected macaques. Role: Co-investigator</p> <p>1K01OD024877-01 (PI: Justin Pollara, Duke University) 9/01/2017-8/31/2020 0.72 cal months NIH \$223,314 <i>Combined Hepatitis B and HIV-1 envelope vaccination to augment T cell help via linked recognition of unrelated antigens.</i> 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</p> <p>NIH/5R01HL131696 (PI: Bryce Chackerian, Univ. of New Mexico) 01/01/2019 - 12/31/2020 0.3 cal months NIH 391,578 (direct costs) <i>A Nanoparticle-Based Vaccine Targeting PCSK9</i> 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</p> <p>1R01DE028146-01 (PI: K. De Paris, Univ. North Carolina, Chapel Hill) 9/6/18 to 6/30/23 1.44 cal mos NIH \$480,799 <i>Sublingual-parenteral vaccination to prevent oral HIV transmission in infants.</i> 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.</p> <p>Research agreement A20-1316 9/1/19-8/31/20 3 cal months Emergent Biosolutions Inc/PaxVax, Inc. \$812,815 <i>Preclinical testing of Chikungunya vaccine</i> The goal of this study is to test whether passive immunization with anti-chikungunya virus antibodies prior to virus inoculation is able to protect macaques against infection or the development of pathology. Role: Principal investigator</p> <p>5R01HD096436-02 (PI: E. Bliss-Moreau, UC-Davis) 8/9/18-5/31/23 0.3 calendar months NIH \$633,255 <i>Cognitive, Socioaffective, and Neural Development Following Fetal Zika Virus Infection</i> 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</p> <p>W81XWH1810094 (PI: Magnani, Diogo, Univ. of Massachussets) 08/01/19-10/31/20 0.24 cal mos Department of Defense \$56,903 (subcontract only) <i>Fetus-Targeted Antibody Therapy to Prevent Zika Virus Infection During Pregnancy</i></p>
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	<p>R01 (PI: Guo, Haitao; Univ. of North Carolina, Chapel Hill): 7/1/20-6/30/25 1.2 calendar months NIH \$60,916 (subcontract only) <i>Inflammasomes in Central Nervous System Comorbidity between Opioid Drug Abuse and HIV</i> 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</p> <p>Research Agreement- Gilead Sciences 05/01/20-04/30/21 3 calendar months <i>Gilead nonhuman primate vaccine studies</i> The goal of the study is to test novel HIV vaccine strategies against SIV infection in macaques. Role: Principal Investigator</p> <p>R01 (PI: M. Martins) 7/1/20-6/30/25 0.6 calendar months NIH \$1,767,382 (subcontract only) <i>eCD4-Ig for preventing and treating obstetric HIV infection</i> 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.</p> <p><u>INACTIVE</u></p> <p>5R37AI037526-23 rev (Subcontract Rockefeller University) 10/30/18-1/31/20 0.72 cal months NIH \$320,000 <i>Preclinical testing of neutralizing antibodies against Zika virus</i> 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.</p> <p>HHSN268201100001I (PI: Michael Busch, BSRI) 9/1/16-1/31/20 1.32 cal months <i>Recent Epidemiology and Donor Evaluation Study-III (REDS-III)</i> The goal of this study is to test the risk of transmission of Zika virus via blood transfusion and test countermeasures. Role: Co-investigator</p> <p>HHSF223201610018C (PI: Nolan, Garry; Stanford Univ). 10/01/17-9/30/19 FDA \$20,653 (subcontract only) <i>Sequelae and immunopathology of Ebola virus infections</i> The goal of this study is to use archived tissues of Zika-virus infected macaques to study immunopathology of Zika virus infection.</p> <p>1R01AI114701 (PI: Dan Granoff, CHORI) 12/1/16-3/31/19</p>
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	<p>NIH \$286,051 (subcontract only) <i>The effect of binding of fH to meningococcal fHbp vaccine on antibody protection</i> The goal of this study was to test the immunogenicity of novel vaccines directed against meningococcal infection.</p> <p>1R21AI129479-01. (PI: Van Rompay) 11/14/16-10/31/2018</p> <p>NIH/NIAID \$250,000 <i>Macaque model of Zika virus infection</i> 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.</p> <p>1R21AI129479-01-Supplement (PI: Van Rompay) 11/14/16-10/31/19 NIH/NIAID \$1,110,149 <i>Development of a nonhuman primate model of fetal zika virus infection and disease</i> 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</p>
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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 a new NIH grant:

P40OD028116 (Magnani/Engelman) 01/01/2020–12/31/2024 2.76 cal. mo. *NIH/OD* \$449,823
Nonhuman Primate Antibody Resource for Immune Cell Depletion
This program develops immune cell-depleting antibodies for nonhuman primate research.

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.