# Fiscal Year 2016-2017 Zika Research Initiative

| Principal Investigator | Principal Investigator's Organization | Project Title | General Audience Abstract |
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| Sylvia Daunert, PharmD, PhD | University of Miami | Antibody-Based Zika Diagnostics | Zika virus (ZIKV) infection has become a serious public health concern with the potential to impact millions of individuals by the end of 2016. Of particular concern is the link between ZIKV infection of pregnant women and microcephaly, neurological impairment and distress in their offspring. We need rapid diagnostics for both the acute and convalescent phase to prevent or control ZIKV spread. It is particularly important to distinguish ZIKV infection from that of the structurally related dengue virus (DENV) in areas where DENV is endemic and ZIKV is increasing in prevalence. Regions with the highest incidence of ZIKV infection also tend to be resource-limited, so there is an urgent and unmet need for rapid, simple, and cost-effective diagnostics that can specifically identify ZIKV and ZIKV-specific Ab responses in body fluids. To address this unmet clinical need, we have developed assays that can both detect ZIKV and serological reactivity against this virus. In collaboration with the Kallas laboratory we have isolated a novel ZIKV-specific monoclonal antibody (mAb) P1F12. We will use this unique mAb and other mAbs in assays that will have the following characteristics: • Rapid. Direct visualization in ~30 min. • Easy to use. Point-of-care (PoC) paper (nitrocellulose) strip test needs minimal operator expertise. • Simplified detection. A positive result is scored by the presence of a line on the nitrocellulose strip. • Economical. Total cost of reagents and materials ~2$ per test. • Stable and Long-Shelf Life. • Portable. Our PoC test can be used in urban, rural or remote locations in developed and developing countries. In our first Specific Aim I, we will develop a clinical laboratory-based assay for direct detection of virus in bodily fluids of individuals with active infection and the presence of ZIKV-specific Ab responses (indicating prior exposure to the virus) in the convalescent phase. We will accomplish this by using our highly specific mAbs. In our second Specific Aim II, we will develop a rapid lateral flow-type PoC assay for the presence of ZIKV and ZIKV-specific Abs in the blood. We will, therefore, develop rapid and cost-effective ZIKV diagnostics that, within a year, could be implemented in resource-limited regions. This assay will permit widespread and affordable testing for ZIKV infection. The assay would be suitable for large scale population screening, would allow couples to make informed decisions about future pregnancies. If we are successful, we will: (1) Establish a rapid and economical laboratory-based test for ZIKV during the acute phase and ZIKV-specific antibodies during the convalescent phase; (2) Develop a rapid PoC diagnostic that can be used in the Doctor's office and community health centers. The development of these simple assays will be important for ZIKV diagnostics. Our team of investigators has expertise in the clinic, virology, isolation of antibodies, and development of molecular diagnostic platforms and PoC tests. Thus, we are ideally suited to accomplish the goals of this proposal. |

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| Eric H. Holmes, PhD | Florida State University | Human Pharmacokinetics of Niclosamide | Recent published reports have demonstrated that the drug niclosamide inhibits Zika virus replication in the sub-micro molar range. Niclosamide is currently approved by FDA for the treatment of tape worm infections in the gut and has an established wide safety margin. The marketed drug, Niclocide (also marketed as Yomesan, Bayer Pharmaceuticals), is an oral chewable tablet. The adult dose is 2 grams of niclosamide per day (either as a one-time dose or daily for a week), or a dose of about 25- to 33 mg/Kg body weight per dose. Studies in rats demonstrated that a 5 mg/Kg niclosamide dose yielded a blood level of 1.08 micromolar. Thus, despite the poor oral bioavailability of niclosamide (about 10%), it is possible that the approved 2 gram/day dose would be adequate to achieve a blood level in humans in the 3- to 10 micromolar range. Should this level be achieved, it would be several multiples of the concentration recently shown to inhibit Zika replication in vitro. Given the significant public health threat posed by Zika virus infections, especially in pregnant women, the availability of an easily adapted marketed drug for Zika therapeutics represents a major opportunity. In particular, use of an off-the-shelf therapeutic could make a rapid and critical impact and buy important time while a more efficient niclosamide formulation could be developed. The first step is to determine the human pharmacokinetics profile of the marketed formulation Niclocide through a small human clinical trial involving 20 to 24 normal volunteers. A fed/fasted cross-over study will be conducted in Australia using Clinical Network Services (CNS), a CRO that specializes in early and mid-stage clinical research, is capable of managing all phases of the trial, and with whom FSU has successfully worked with in the past. The advantage of doing this trial in Australia is in the simpler regulatory path to initiation of the trial and its overall lower cost. The results obtained will be suitable to support further IND-directed clinical trials and will provide specific information to establish the blood level in humans that is derived from the Niclocide product. From that a judgement can be made regarding if a therapeutic level is reached which will be used as a justification for a subsequent therapeutic efficacy trial in Zika infected individuals. |

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| Natasa Strbo, MD, PhD | University of Miami | Development and testing of novel Secreted GP96-Ig Zika virus (ZIKV) vaccine | ZIKV infection in most instances, results in a self-limiting febrile illness associated with rash and conjunctivitis. Severe neurological phenotypes can also occur, including Guillain-Barre syndrome and meningoencephalitis. Infection in pregnant women is of major concern, as it is linked to catastrophic fetal abnormalities including microcephaly, spontaneous abortion, and intrauterine growth restriction due to placental insufficiency. Currently, there is no effective treatment or prevention of ZIKV infection other than avoidance of its mosquito vectors. Accordingly, there remains an urgent need for ZIKV vaccines that could prevent and/or mitigate ZIKV infections and which will not only protect adults from ZIKV infection but also pregnant women and their embryos and fetuses. The placenta acts as a barrier against infections, due to multiple unique structural, cellular, and immune properties. The detrimental effects of congenital viruses, including ZIKV, on pregnancy and fetal outcomes occur in part because of impaired placental function and profound pathological changes in ZIKV-infected placentas. Our hypothesis is that induction of appropriate vaccine-induced immune responses in placenta are key to successful prevention of viral infections in developing fetus. Induction of ZIKV virus-specific responses by secreted gp96-Ig in the placenta will lead to clearance of the ZIKV and prevention of the ZIKV virus transmission to the fetus. To explore the contribution of vaccine induced immunity in ZIKV prophylaxis during pregnancy, we have adapted a heat-shock protein (HSP) based vaccine approach for protection at the maternal/fetal interface. HSP gp96 is a biological adjuvant that activates antigen-presenting cells via TLR2 and TLR4 and simultaneously delivers antigen specific peptides to MHC I via CD91-receptor mediated endocytosis. To take advantage of these properties, gp96 was genetically engineered to become secretory fusion protein gp96-Ig. Secreted Gp96-Ig vaccine approach has been adapted in different vaccine platforms including therapeutic allogeneic adenocarcinoma cell line secreting gp96-Ig together with relevant non-small cell lung cancer (NSCLC) tumor antigens as well as preventive cell-based vaccine that secretes gp96-Ig and simian immunodeficiency virus (SIV) antigens in non-human primates. Importantly, our experience developing and testing secreted gp96-Ig based vaccines has therefore reached several proof-of-concept milestones (safety and immunogenicity in humans, immunogenicity against tumor and infectious disease antigens in primates, prophylactic immunity against SIV infection in rhesus macaque, Strbo et al 2013. J.Immunol.) which warrant extending this vaccine approach to ZIKV infection. Here we developed a novel secreted gp96 vaccine for induction of ZIKV-antigens specific CD8+ T cell responses in placenta and decidua. Proof of principle experiment preformed in mouse model with gp96-Ig vaccine confirmed a high frequency of antigen specific CD8+ T cells in placenta and decidua of pregnant B6 mice. Following specific aims will be carried out in order to generate a ZIKV-specific vaccine and test its preclinical immunogenicity: 1. Construction of vaccine cells expressing secreted gp96-Ig and ZIKV antigens 2. Immunogenicity of the secreted gp96-Ig ZIKV vaccine in pregnant mice 3. Protective efficacy of gp96-Ig ZIKV vaccine against ZIKV Challenge in the pregnant mouse model. |

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| Qun Huo, PhD | University of Central Florida | Point of care assay development for diagnosis of Zika viremia | Recent outbreaks of Zika virus (ZIKV) underscore the importance of surveillance and early detection strategies that can quickly identify infected patients at the point of contact, especially women of child bearing age, and to stymie widespread viral transmission. In the proposed study, we intend to develop and evaluate a rapid point of care, field-deployable assay for detecting ZIKV in human blood, urine, and saliva during the acute period of infection (< 5 days). The proposed assay is built upon an innovative, portable diagnostic device platform, developed by our Florida-based Industry Collaborator Aviana Molecular Technologies (AMT). The diagnostic approach is based on the adaptation of sensors found in cellular communication with proprietary hardware, software and biological binding of human biomarkers. Over a period of ten years, AMT has built and refined the portable diagnostic system, rooted in UCF-initiated and NASA-sponsored research, with the intention of adapting it as a cost-effective infectious disease diagnostic to be used in scenarios such as the current goal of acute ZIKV detection and for other emerging diseases. Reagents for testing will be obtained from the laboratories of Dr. Michael Diamond at Washington University and from commercial sources. The proposed approach represents a dramatic improvement over current acute viremia testing methods which have poor sensitivity (e.g., lateral flow assays) or require expensive equipment, specialized reagents, a controlled laboratory setting , complex and lengthy protocols, and highly educated trained staff capable of interpreting results (e.g, qRT-PCR assays). The proposed portable diagnostic system will facilitate point of care testing for ZIKV infection in hospitals, clinics, and primary care settings, providing fast and precise diagnostic results that can be used to inform clinical care decisions, viral control, measure implementation, and prevention strategies in near real time. Minimal sample processing is required in this highly sensitive, and simple to use system. Connectivity of the system and small size are additional advantages to the diagnostic platform as it will pair with any smart device. Success with the proposed project also will create a clear path for developing assays to sensitively and specifically measure chronic ZIKV infection, another critical goal in the effort to prevent and control viral transmission. Because ZIKV substantially impacts pregnant women and unborn babies, this study fits well with the mission and priorities of FL DOH. |

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| Karin Chumbimuni-Torres, PhD | University of Central Florida | A Universal Nucleic Acid Recognition Platform for Detection of Zika Virus | In this proposal, our goal is to develop a new technology for the specific and sensitive point of serving testing (POST) platform for the rapid detection of ZIKV. Electrochemical detection will be used along with an amplification technique (Nucleic Acid Sequence Based Amplification, NASBA). To accomplish this goal we will use a binary probe that has proven to have high specificity for SNS differentiation in RNA. RNAs are considered key biomarkers for a variety of viral infections. However, these RNAs form secondary structures, complicating their detection. Current approaches, such as NAATs, offer high sensitivity and rapid results, but they are prone to contamination and frequently produce false-positive results and require technical expertise. Furthermore, these approaches are incapable of differentiating single nucleotide substitution, especially, at ambient temperatures, which is necessary to distinguish among other flaviviruses. The project research is divide in two specific aims: 1. To optimize a protocol for the rapid electrochemical detection of ZIKV RNA using NASBA. First synthetic ZIKV RNA with highly conservative sequences will be used. The specific sequences will be chosen using GENBAK and Biodefence and Emerging Infections resources. The sequences chosen will be obtained from American and African lineages. Then NASBA will be used to amplify the specific synthetic sequences. The amplified RNA products will be further analyzed with the optimized electrochemical sensor. 2. To apply the developed methodology for the detection of ZIKV RNA in cell culture and blood samples. ZIKV will be cultivated and the viral RNA will be extracted from purified ZIKV. This purified strain of ZIKV RNA will be amplified using NASBA and used as a standard to develop further the methodology towards the end goal, which is the direct analysis of plasma or blood samples. Highly conservative sequences will be used to screen for ZIKV, and sequences with an SNS that can differentiate between the two lineages. The limit of detection will be determined by assaying a serial dilution with known amounts of ZIKV in the CFU of the virus. We expect to achieve detection of around 50 to 100 CFU using this methodology. The new methodology proposed here will exceed the performance of current state-of-the-art approaches for RNA sensing in the following aspects: (i) it will exhibit zero false-positive responses, thus improving sensitivity and limit of detection (LOD); (ii) it will enable accurate recognition of SNS at ambient temperatures in RNA; (iii) it will allow for detection of multiple analytes using a single universal probe in a re-usable. The proposed work plans to: i) identify conservative sequences of a variety of Zika strains ii) enhance the number of specific strains using NASBA and iii) perform the detection using a developed universal electrochemical sensor. The advantages of the propose platform are the capability of performing multiplex analysis with zero signal background and the potential for on-site testing while offering fast, highly specific, sensitive, user-friendly, low power, portable, and affordable. For this reason, a novel sensor platform with the ability to detect RNA would have an unprecedented impact for the diagnosis of ZIKV. Since the platform will recognize any Zika strains by identifying the specific conservative sequences and differentiation among other flavivirus data will allow us to explore associations between their brain imaging metrics and neurodevelopmental outcomes. |

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| Timothy L. Tellinghuisen, PhD | The Scripps Research Institute | Development of High Throughput Screening Tools to Search for Compounds Inhibiting the Essential Zika Virus NS3 Protease | Zika virus (ZIKV) is an important emerging human pathogen. ZIKV is mosquito-borne virus of the genus Flavivirus in the Flaviridae family of enveloped, positive strand RNA viruses. ZIKV has dramatically expanded from Africa and Asia to the Pacific in the last decades, with outbreaks in 2007 on Yap Island, Micronesia and French Polynesia in 2013-14. ZIKV first emerged in the Americas in Brazil in May of 2015 and quickly spread across the Americas to at least 25 different countries reporting direct viral transmission, including, more recently, local transmission in Florida. ZIKV was originally believed to produce little to no human pathology, but recent outbreaks have suggested a link between ZIKV infection and the development of Guillain-Barré Syndrome, a debilitating disease of the peripheral nervous system. Additionally, ZIKV infection results in increased cases of the congenital neurological malformation microcephaly when pregnant women become infected. Surprisingly, the virus is sexually transmissible, and maintains reservoirs in the testes of infected men, resulting in the potential of sexual transmission many months post exposure. There is currently no effective vaccine or specific antivirals to prevent or treat ZIKV infection. Like all small RNA viruses, ZIKV produces a single, large protein molecule during infection that is cleaved by a combination of viral and host scissor-like enzymes called proteases to produce the mature viral products that replicate the virus. The major protease enzyme for ZIKV is called NS3, and this protein is absolutely essential for ZIKV to replicate in both humans and mosquitos, making it an ideal therapeutic target for new drug development. Our objective for this project is to generate a series of laboratory assays to measure the activity of NS3 that can determine when small molecule drugs bind to and block the ability of NS3 to allow virus to replicate. Using these assays as a starting point, we will adapt these tools to a format that is compatible with the high throughput screening facility available at Scripps Florida. This would allow for the screening of the Scripps Florida small molecule drug library of approximately 3,000,000 unique drug-like molecules for inhibitors that block the activity of ZIKV NS3. Compounds that inhibit NS3 in our assays will be evaluated for their ability to inhibit ZIKV infection in cell culture and animal models of ZIKV infection as a prelude towards developing NS3 inhibitors into therapeutic drugs for human use to treat ZIKV. Completion of the proposed research would deliver an effective drug screening platform for ZIKV NS3 activity and allow for preliminary screening efforts to search for NS3 based ZIKV treatments. |

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| Timothy L. Megraw, PhD | Florida State University | Mechanism of centrosome activation by Zika Virus and the evaluation of pharmacological | The Zika Virus (ZIKV) outbreak of 2015-2016 has alerted health officials in the Americas to a new and unprecedented infectious disease public health crisis. ZIKV, an arbovirus transmitted mostly by mosquitoes of the Aedes genus, is the first known mosquito-transmitted virus that causes birth defects. ZIKV can cause severe impairment of fetal brain development when women are infected during pregnancy, resulting in babies born with severe microcephaly. Previously regarded as a virus with little public health impact, ZIKV has rapidly emerged as a serious threat to public health in the US, and in Florida in particular. In June 2016, mosquito-borne infections were first detected in North Miami. As of early November 2016, more than 215 mosquito-transmitted infections have been reported, and at least 143 pregnant women have been infected in Florida. Why ZIKV has become more virulent toward developing fetuses is not clear, but this phenomenon exposes the possibility that other widespread related flaviviruses and other arboviruses like Dengue, Chikungunya, etc. could also acquire newfound virulence and pose new and elevated threats to public health. Inherited developmental microcephaly is caused by a spectrum of relatively rare syndromes that affect the centrosome, a cellular structure that organizes the microtubule cytoskeleton to support intracellular trafficking of molecules and macromolecular assemblies. Any one of at least 16 centrosome protein-encoding genes is mutated in one or more microcephaly syndromes. We hypothesize that ZIKV alters centrosome functions, which then disrupts neural stem cell proliferation or survival, contributing to microcephaly in developing fetuses. Our current data show that the centrosome microtubule-organizing center (MTOC) activity is activated following ZIKV infection and appears to involve Polo-like kinase 1 (PLK1). We will test ZIKV-infected human cells, including neural progenitor cells, for changes in the levels and localization patterns of centrosome proteins mutated in microcephaly syndromes. We will further determine whether PLK1 is activated, and whether its activation is required for ZIKV to activate centrosomal MTOC activity. A variety of pharmacological and genetic engineering experiments will test the requirement of the centrosome and its MTOC activity to support ZIKV propagation. Achievement of this aim will show how the centrosome MTOC activity is augmented in ZIKV infected cells, and whether the centrosome and its MTOC activity are essential for ZIKV replication. Our second goal is to identify the ZIKV protein(s) that are responsible for interfering with host cell centrosome function. We will express each ZIKV-encoded protein separately or in combinations in human cell culture, and determine which protein(s) elicit centrosome MTOC activation. Once we identify the ZIKV protein responsible, we will then identify the relevant host protein using protein co-purification and mass spectrometry. Once identified, we will determine how the interaction between ZIKV protein and host proteins results in centrosome MTOC activation, and its requirement for ZIKV proliferation. The outcome of this project will be a clear and novel understanding of the mechanisms by which ZIKV impacts centrosome activity to promote ZIKV replication, and the evaluation of pharmacological targeting of PLK1 to acutely block ZIKV pathogenesis. |

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| Emmalee S. Bandstra, MD | University of Miami | ZIK-Action: A Prospective, Longitudinal Assessment of Infants of Mothers with Zika Infection in Pregnancy | This Dynamic Change Team Science proposal will be submitted in response to the State of Florida's Department of Health Funding Opportunity Announcement. Evidence indicates that Zika virus is a teratogenic agent linked with potential life-long consequences involving infant brain growth, structure, and function, resulting in deficits in cognition, motor function, and special senses of vision and hearing. Although guidelines have been established for the care of confirmed infants with congenital Zika infection, very little is known about maternal-fetal transmission and the full spectrum of defects in Zika positive and exposed infants. In addition, asymptomatic infants have the risk of developing latent complications within the first year of life as observed with other congenital infections, such as cytomegalovirus. Prior to the Florida endemic Zika outbreak, our institution was confronted with travel-associated Zika infection in pregnant mothers. Realizing the magnitude of the situation, the Departments of Obstetrics and Pediatrics developed a Zika Response Team (ZRT), led by Dr. Christine Curry and Dr. Ivan Gonzalez. Our ZRT includes an onsite network of adult and pediatric subspecialties, including Maternal Fetal Medicine and Pediatric subspecialties such as Infectious Disease, Neonatal-Perinatal Medicine, Neurodevelopmental Follow-up, Ophthalmology, Audiology, Neurology, Cardiology, Nephrology, and Neuroradiology. The objective of this proposal is to determine the effect of maternal Zika infection in pregnancy on the acute and long-term outcomes of infants of Zika-positive mothers using serial comprehensive infant growth, physical, neurodevelopmental, ophthalmological, and audiological assessments from birth through age 30 months. Our team members in Virology at Florida Gulf Coast University will be pivotal in helping us address a related objective to determine the the incidence and duration of viral shedding in urine, breastmilk and saliva, and hopefully whether Zika infection can be transmitted via breastfeeding as documented in other viral infections. We will recruit study infants at the University of Miami Miller School of Medicine-affiliated Jackson Memorial-Holtz Children's Hospital which delivers 5,000 infants per year. Although we cannot predetermine the total number of Zika-positive women as this is an area of ongoing transmission, we expect a pool of about 250 Zika-positive pregnant women per year, with 5% seroconversion rate. During the grant period, we anticipate enrolling 200 infants born to Zika-infected mothers and 100 controls matched (2:1) for sex, race, birth weight and gestational age. Assessments will include comprehensive physical and neurologic examinations, NICU Network Behavioral Scales, the Bayley Scales of Infant Development-3rd Edition, ocular and fundus examinations, and age-dependent auditory testing. This Dynamic Change Team Science Proposal aligns with the objectives in the FOA as it involves a collaborative investigation between University of Miami multidisciplinary investigators and our partners at Florida Gulf Coast University with whom we will perform the Zika virus laboratory work. Statewide collaboration also includes the Florida Department of Health and Children's Medical Services. This proposal will provide valuable data for correlation with other proposals from our institution as well as other institutions across the state. |

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| Nazira El-Hage, PhD | Florida International University | Development of nanoscale approaches for Zika virus and therapeutics | There is a growing evidence that Zika-virus (ZIKV) infection is associated with microcephaly in infants following maternal infection. The present application by a consortium formed by the Florida International University and the University of Miami investigators is focused on the entry of ZIKV into the brain, neuropathology induced by the virus, and novel therapeutic approaches to protect against these events. The trafficking of ZIKV into the brain raises a question on the role of the bloodbrain barrier (BBB) in this process. We hypothesize that ZIKV infects the brain via underdeveloped or disrupted BBB. This hypothesis will be addressed by infected pregnant mice at different stages of BBB development in embryos. The outcomes of ZIKV infection will be addressed by focusing on neurogenesis of the hippocampal neural progenitor cells and autophagy responses in the brain. Autophagy is a highly conserved biological process responsible for the lysosomal degradation of long-lived proteins, damaged organelles and parts of the cytosol, and has also been implicated in innate and adaptive immune response. Studies have shown that ZIKV exploits autophagy to enhance its replication, and pharmacological inhibitor of autophagosome formation, strongly reduced viral copy numbers in infected fibroblasts. We hypothesize that autophagy plays a key role in the pathogenesis of ZIKV-associated primary microcephaly. Preliminary data supporting this hypothesis show (i) increased expression of the autophagic proteins Beclin1 and LC3, (ii) increased dendritic varicosity in neurons and (iii) increased inflammation with the release of cytokines (IL-6 and TNF-α) and chemokines (MCP-1 and RANTES) in astrocytes infected with ZIKV. Inducing the autophagy pathway with rapamycin increases ZIKV replication, whereas silencing the autophagy with siRNA against the beclin1 gene decreases viral replication in astrocytes. In this proposal, we plan to use a ZIKV infected-autophagy deficient (B6.129X1-Becn1tm1Blev/J) mouse model treated with anti-ifnar antibody to further evaluate the role of autophagy in the underlying mechanism(s) in ZIKV replication and associated neuropathogenesis. In terms of translational approach, we will use state-of-the- art and recently patented nanotechnology tools to deliver nanoparticles loaded with ZIKV specific anti-ifnar, Beclin 1 and AXL receptor antibodies across BBB to treat or prevent ZIKV induced defects. Thus this multidisciplinary approach between scientists from the University of Miami and Florida International University exploring a) the role of ZIKV infection on the integrity profile of BBB, b) mechanisms of ZIKA induced pathogenesis and c) nanodelivery of ZIKA specific therapeutic cargo across BBB is uniquely suited to the stated goals of this Dynamic Change Team Science announcement of FLDOH. |

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| Sapna Deo, PhD | University of Miami | Rapid RNA Test for Zika Virus | Direct detection of Zika virus RNA in patient samples is challenging and time consuming. The current strategy for ZIKV RNA detection is reverse transcription polymerase chain reaction (RT-PCR), and requires specialized laboratories and equipment. Additionally, no specific and sensitive immunoassays for ZIKV detection currently exist. This results in a significant delay in obtaining information on infection status, and may induce additional anxiety in pregnant women in Florida and around the world who remain at risk of infection. This necessitates the development of testing platforms for ZIKV that are simple, cheap, and rapid; can be easily mass-produced; and easily utilized in locations beyond traditional clinical settings. To solve this significant challenge, we are developing a portable, rapid, equipment-free detection technique employing rolling circle amplification of ZIKV RNA followed by paper-strip-based visual detection of the amplified product. Our preliminary data demonstrates that our technology works near room temperature and is capable of identifying the presence of ZIKV RNA on a paper strip using gold-nanoparticle conjugated probes that are observable with the naked eye. Moreover, because there is no need for specialized laboratory equipment, the entire test can be performed on-site. Successful detection of ZIKV infections in a rapid manner will meet the substantial need for assisting pregnant women in Florida and general population who currently have to wait several months to get the result. To accomplish our goal, we have formulated the following two Specific Aims: Specific Aim 1. Design, development, and analytical optimization of an easy to use, rapid, and cost-effective portable test for Zika virus detection. Specific Aim 2. Evaluation of the test to detect Zika virus spiked in buffer, serum, saliva, and urine. Stability analysis of the developed test. Validation of the test via analysis of clinical samples. The proposed work should result in a rapid, reliable, portable, and cost-effective Zika virus detection test that will facilitate Zika virus detection in developed and developing countries for use in hospitals, community health clinics, and remote locations where the Zika virus is spread. Our team of investigators has 20+ years of expertise in the design and development of portable molecular diagnostic platforms and point-of-care tests. |

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| Waseem Asghar, PhD | Florida Atlantic University | Development of a diagnostic assay for rapid detection and quantification of Zika virus | Zika virus (ZIKV) is an emerging mosquito-borne virus that belongs to the genus flavivirus. In 2015, there was a dramatic increase in reports of ZIKV infection in the Americas. Brazil is the most affected country, with preliminary estimates of 440,000 to 1.3 million cases of ZIKV infection reported through December 2015. Centers for Disease Control and Prevention (CDC) has also reported the transmission of ZIKV to the United States. As of November 16, 2016, the CDC has reported 32,068 ZIKV disease cases in the United States and US territories, with Florida reaching 1188 ZIKV cases where Miami Dade county is severely affected. Recent reports suggest the association between ZIKV infection and microcephaly and other neurological disorders among newborns. Additionally, ZIKV has been found in blood, fueling growing concerns about the risk of transfusion-transmission with particular concern over severe outcomes in at-risk transfusion recipient populations such as pregnant women. Current ZIKV diagnosis assays are based on measuring early antibody immunoglobulin (Ig) M using enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR). There is a substantial serological cross-reactivity between ZIKV and other flaviviruses. Current IgM antibody-based ELISA assays cannot reliably distinguish between ZIKV and Dengue Virus (DENV). Therefore, an IgM positive result in a Dengue or Zika IgM ELISA test should be considered solely indicative of a recent flavivirus infection. Plaque-reduction neutralization tests (PRNT) can be performed to measure virus-specific neutralizing antibodies and can distinguish between infection by ZIKV and other flaviviruses. Although IgM ELISA followed by PRNT assay can identify the cause of viral infection, PRNT assays are time-consuming and take several days. RT-PCR is a more commonly used method and can distinguish between ZIKV and DENV with high specificity. However, RT-PCR based methods are complex, time consuming, and require multiple labor-intensive sample preparation and processing steps, hence not suitable for rapid testing at airports, community health centers, urgent care centers, and other point-of-care (POC) settings. To increase access to ZIKV testing and to reduce the disease spread, there is an urgent need to develop a reliable device for rapid ZIKV detection. To our knowledge, currently no rapid ZIKV viral load technology exists at the POC. Our goal is to develop a novel, low-cost (using transparency paper and plastic materials, <$2) and automated (on-chip virus lysis and impedance sensing) tool for rapid (~15 minutes) detection of ZIKV from human serum and saliva samples at POC settings. Rapid ZIKV detection at POC settings will potentially reduce the risk of transfusion-transmission in pregnant women and will also help in reducing disease transmission by potentially identifying the travel-associated cases and recommending them to refrain from activities which might pass it on to other people—for example, not engaging in sexual activity. |

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| Cuong Q. Nguyen, PhD | University of Florida | Identification of potent neutralizing Zika virus antibodies using single-cell analysis technology | Transitioning from Africa to the Americas via the South Pacific, Zika virus (ZIKV) infections have become an emerging health pandemic of significant medical importance. Recently, concern about ZIKV infections has increased as the virus has become linked to devastating neurodevelopmental defects in the newborns of infected pregnant women throughout the Americas. Over the past year, doctors in Brazil have documented over 4,000 cases of microcephaly in which infants were born with abnormally small heads. The detection of ZIKV in fetal brain tissues and anti-ZIKV antibodies in the mothers and/or infants establishes a possible causal link between ZIKV and microcephaly. Furthermore, there could be an additional link between ZIKV and the dramatic increase in the reported cases of Guillain-Barré syndrome. This rare disorder of the peripheral nervous system is characterized by muscle weakness and paralysis. In severe cases, some Zika patients have required life support. The spread of ZIKV has reached an alarming rate particularly in the state of Florida. Both the influx of travelers from ZIKV-infected areas and the warm tropical climate in this state promote the survival of the ZIKV-carrying mosquitoes and accelerate the spread of the virus. Florida is just behind New York with the highest number of travel-associated cases; however, it is the only state with recorded locally-acquired ZIKV infections (n=139 cases to date). Unlike other well-known flaviviruses like Dengue, West Nile, and Yellow Fever viruses, there are no treatments or vaccinations against ZIKV, and diagnostic reagents are very limited. Although many investigations using immune-based therapies for arboviral infection have been pursued and have shown promise, there are no commercially available immune-based products for ZIKV. One critical challenge in the development of effective vaccines is our incomplete understanding of the protective humoral or antibody immunity against ZIKV. This challenge is attributed to limitations of the current technologies to provide a comprehensive profile of protective neutralizing antibodies against ZIKV infection. In this application, as a team of experts with multiple disciplines in the field of immunology, virology, and public health in the state of Florida, we propose to use an innovative single-cell technology, referred to as single-cell antibody nanowells (SCAN), to quickly and efficiently screen individual B-cells for antigen specific products capable of neutralizing ZIKV. The antigen specific products will be isolated and expanded via cloning and recombinant DNA technologies. This extremely efficient process uses much smaller samples than conventional methodologies, and, most importantly, it allows for the identification of even rare cross-neutralizing epitopes. We propose using SCAN technology to identify and isolate ZIKV-specific antibodies that will be evaluated for viral neutralizing properties. Antibodies with neutralizing properties will then be characterized to determine their mechanisms of neutralization. Results will generate a complete repertoire of ZIKV-specific antibodies. While these antibodies will be screened for neutralizing capacity and mapped for antigenic epitope reactivity, on a more fundamental level, we expect our results to establish a simple approach for creating immune- therapeutics and eventually vaccines. |

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| Vladimir Beljanski, PhD | Nova Southeastern University | A comparative analysis of Zika virus-induced antiviral response mechanisms in under-studied cell populations | Zika virus (ZIKV) is an arbovirus belonging to the Flaviviridae family, which also includes dengue and West Nile viruses. Major public health concerns have been initiated following the discovery that ZIKV infections are linked to serious neurological birth defects. Once ZIKV reaches the developing brain, it selectively infects and kills neural stem cells that would eventually become the neurons that are absent in these children. ZIKV enters these specific cells using a receptor named AXL. While most research has been targeted at understanding how ZIKV contributes to microcephaly, mounting evidence indicates it also contributes to other conditions, including ones affecting adults. Interestingly, cell type-based profiling of AXL can readily explain these other ZIKV related conditions such as ocular defects, Guillain-Barre Syndrome, and sensory polyneuropathy, as well as predict effects on other biological systems with potentially long-term clinical manifestations. Blocking of AXL has been shown to almost completely prevent ZIKV infection. However, AXL signaling also plays critical roles in normal development and immunity, so targeting it directly would probably have multiple adverse consequences. Based on what is known about the effects of AXL signaling on immune responses, it is possible that in addition to using AXL as an entry receptor, ZIKV may also use it to enhance its own infectivity and suppress antiviral mechanisms. We hypothesize that ZIKV infectivity can be reduced without interfering with cellular maturation processes by targeting the interface of AXL signaling and intracellular antiviral responses. We will test the modulation of these pathways in a variety of AXL expressing cell types, as they are differentiated in vitro, because the mechanisms contributing to antiviral responses can vary between cell types as well as across developmental stages. Specifically, we propose the following aims: 1. Investigate the effects of antiviral signaling pathway modulation downstream of AXL on ZIKV infectivity using selective small molecule inhibitors. ZIKV infection and replication will be measured in the presence of agonists or antagonists of several key antiviral pathway components. 2. Determine the effect these modulators in the presence and absence of ZIKV on the maturation and survival of AXL expressing cells types. Critical populations of potentially affected vascular, neuronal, glial, retinal, and immune cell types will be used, and the expression of differentiation and viral response-related protein markers specific for each respective lineage will be analyzed. 3. Characterize additional viral response mechanisms in AXL expressing cell types that can be targeted in ZIKV infections. Detailed comparative analysis of gene expression and high-throughput proteomic changes specific to immune signaling will be assessed upon virus exposure. The interdisciplinary research program established at the NSU Cell Therapies Institute will make use of our unique library of stem cells and chemically defined differentiation platforms coupled with techniques for modulating intracellular viral defense mechanisms that have been developed in collaboration with Karolinska Institutet. Altogether this project will establish a strong preclinical rationale for the development of new treatments for improving ZIKV clearance as well as improve our understanding of how ZIKV may contribute to morbidities in systems beyond the fetal brain. |

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| Gaurav Saigal, MD | University of Miami | Longitudinal Brain MRI Characterization of Zika-positive and exposed children using advanced MRI techniques and Correlations with Neurodevelopmental Outcomes | The impact of Zika virus (ZIKV) infection on the developing brain of children who were prenatally infected i.e., Zika-positive group (Zika PCR or IgM positive) is just beginning to unravel. Though ZIKV is already linked to catastrophic fetal brain abnormalities, its further impact on the developing infected-brain is yet to emerge. Most importantly, the brain development of those children who were prenatally exposed to ZIKV i.e., Zika-exposed group (Zika PCR and IgM negative and maternal PCR/IgM positive) is not yet known. Therefore, the aim of this proposal is to characterize longitudinal changes in the brain of children infected with or exposed to Zika virus using advanced brain imaging techniques, and correlate these changes to their neurodevelopmental outcomes. This study will be conducted in the University of Miami, Miami, Florida. The Zika-PCR positive (n= 15; age range: 0-3 years) and exposed (n=45; age range: 0-3 years) groups will be recruited from the Zika Clinic of University of Miami, Miami. A matching control group (n=15; age range: 0-3 years) will be recruited for comparisons. All subjects will be scanned three times, i.e., at 1-month, 12-month, and 24-30 months of their age. The MRI scans will be performed using a 3 Tesla MRI scanner. The MRI protocol will include conventional diagnostic imaging methods and advanced imaging methods such as MR spectroscopy (MRS), diffusion kurtosis imaging (DKI) and high-resolution tissue structural imaging. Neurodevelopmental outcomes will be assessed at each MRI scan visit and the imaging measures will be associated with the outcomes to evaluate associations between these measures. MRS is a noninvasive diagnostic method for measuring biochemical or metabolite concentration changes in the brain. The metabolites of interest include N-acetyl aspartate, creatine, choline, myo-inositol, glutamate, and lactate. DKI permits us to evaluate alterations in tissue micro-structures, and the metrics obtained include fractional anisotropy, mean diffusivity, axial diffusivity, radial diffusivity, mean kurtosis, axial kurtosis, and radial kurtosis. The high-resolution structural images will be used to obtain morphological metrics such as thickness of cortical gray matter structures, volume of gray matter and white matter sub-structures, and total gray matter and white matter and ventricular volumes. Volume of calcium deposits will be quantified from diagnostic imaging data. Of great interest is to evaluate the brains of the Zika-exposed children i.e., Zika PCR negative children, to ascertain whether they are normally growing based on their conventional imaging and neurodevelopmental outcomes. Any subtle brain abnormality that is missed by conventional diagnostic imaging will be assessed by more sensitive MRS and DKI techniques. These abnormalities may include subtle metabolite and tissue micro-structural alterations. The findings of this application will establish baseline and longitudinal brain imaging abnormalities in Zika-positive and exposed groups of children. Furthermore, the resulting data will allow us to explore associations between their brain imaging metrics and neurodevelopmental outcomes. |

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| Barry W. Alto, PhD | University of Florida | Point of Sampling Rapid Detection of Zika and Other Mosquito-Borne Pathogens. Science, Technology, and Product Delivery | Firebird scientists are leaders in developing chemistry to support human diagnostics. These include products for use in high resource environments, including branched DNA diagnostic assays that measure viral load in patients infected with HIV, hepatitis B, and hepatitis C viruses, which had lifetime sales approaching $1 billion. Other products include a HRE kit that allows the detection of up to 24 mosquito-borne viruses in a single assay, and kits to detect a variety of exotic pathogens, including SARS, MERS, and other respiratory pathogens. The Florida Medical Entomology Laboratory is a resource for mosquito entomology. The FMEL is one of the world's largest research institutions devoted to improving our understanding and control of medically important insects. To date, more than 1,100 scientific papers have been published in national and international journals. The scientists at the FMEL have extensive experience in studies on mosquito-borne arboviruses affecting human health in Florida, including West Nile, St. Louis Encephalitis, Dengue, Chikungunya, and Zika viruses. The FMEL has a repository of mosquito strains and a variety of common and exotic arboviruses available for use in this project. Firebird chemistry is being developed to detect pathogens in low resource environments. Activity includes collaboration with Columbia University to detect pathogens carried by ticks, work with the Department of Defense to detect norovirus environmental samples, and NIH-sponsored work with the University of Florida veterinary school to detect malaria in asymptomatic carriers, all at points of sampling. We seek to extend this to Zika with Firebird's chemistry and FMEL's entomology skills. With the modest $200,000 budget in the "rapid pilot" program, Firebird's chemistry will be adapted to detect Zika virus at points of sampling in either mosquitoes (for public health surveillance) or human samples (urine, for example). This will be possible by the collaboration between Firebird scientists and FMEL scientists This collaboration has already generated a breadboard product. The breadboard product informs a user within 30 minutes of the presence or absence of Zika virus, for just pennies a test. Here, it targets the genome of the virus. It requires no upfront sample preparation, and is so simple that it can be used by operators having no particular expertise. It functions on trapped mosquitoes, and can be used by public health service personnel in their surveys. It functions without sample preparation on urine, and can be used by first responders, personal physicians, and high school nurses, among others. We will provide (by the end of the one-year project period) public health services battery-powered portable devices to determine, within the hour, the infectious state of mosquitoes, animals, or human patients. Firebirds chemistry allows this product to be expanded in multiple ways. First, the product can easily be expanded to include other mosquito-borne pathogens that are found together in tropical ecosystems, including dengue, chikungunya, O'nyong nyong, and Mayaro, associated with human illness. Additionally, it is flexible enough to be adapted to new variants of Zika. Further, it can manage "forward contamination" that makes DNA- and RNA-targeted assays difficult to move to points-of-sampling. |

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| David G. Meckes, PhD | Florida State University | Fetal Brain Exosomes in the Maternal Circulation for the Detection of Zika Virus Infected Fetuses | Exosomes are small vesicles secreted from cells and circulate in the blood and other bodily fluids including urine, saliva, and cerebral spinal fluid. Exosomes carry proteins and other cellular factors that allow cells to communicate with each other. This communication is likely essential for important processes including cell growth, development, and immune responses. Evidence also suggests that exosomes play a role in the progression of many diseases including cancer, Alzheimer's, and viral infection. The molecular information contained within exosomes that originates from diseased cells has proven to be useful in early diagnosis of certain cancers. Exosomes are secreted from nearly every cell type investigated; therefore, exosomes in the blood represent a complex mixture from diverse sources. Recent data suggests that exosomes from the fetus are present in the mother's circulation and may represent a new means to non-invasively monitor the health and development of the fetus. Zika virus is an emerging infectious disease that is rapidly spreading across the Caribbean and South America with confirmed cases of locally-acquired Zika in the state of Florida. Infection of pregnant women during the first trimester has been linked to microcephaly, a neurological condition where babies are born with significantly smaller heads due to abnormal brain development. Babies born with microcephaly can develop convulsions and suffer physical and learning disabilities as they grow older. Currently, there is no non-invasive test available to determine whether a fetus has been infected with Zika virus or will develop associated disease. This study will take advantage of novel approaches to detect fetal Zika infection and to monitor the health and development of the growing fetus. Our objectives for the study are twofold: 1) to compare and characterize fetal-derived exosomes present in blood of healthy and Zika infected pregnant women; and 2) to compare molecular information in exosomes to fetal imaging data acquired during pregnancy. We are well positioned to make significant advances on this area of reseach as our group has already developed new methods for the isolation and characterization of exosomes from blood, including brain exosomes. Overall, the proposed research will provide a novel way to detect microcephaly risk due to Zika infection by isolating fetal-specific exosomes for early characterization from pregnant women. |

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| Griffith D. Parks, PhD | University of Central Florida | Zika Virus Activation and Inhibition of Human Complement Immunity | This project will form a partnership between the University of Central Florida College of Medicine in Orlando and the Moffitt Research Institute in Tampa to address the Research Priority "Health Effects of Zika Virus." The deleterious health effects of Zika can be clearly seen because it evades the human immune system, and subsequently affects a large number of other human organ systems including reproductive, cardiovascular, developmental, and central nervous systems. All viruses (including Zika virus) must face normal pre-existing immune responses in the human host known as "innate immunity." One of the strongest of these innate immune systems in humans is called Complement, which is a series of human proteins that recognize viruses and infected cells to inactivate them. The complement system is a critical front-line defense against viruses, but also acts to modulate the formation of later protective immunity such as antibodies and immune cells. We have a long history of studying Complement activation and inhibition by a number of human pathogenic viruses. Thus, we are well positioned to apply our expertise to attack the unique problems in Zika virus biology and immunity. In our ongoing laboratory studies with Zika Virus, we have found that the virus is resistant to Complement-mediated neutralization, a result that is in strong contrast with other related viruses we have worked with before. Most importantly, this resistance to neutralization depends on which particular human donor serum we test. Thus, we hypothesize that Zika virus has novel mechanisms to resist neutralization by human serum, but the effectiveness of this inhibition varies from person to person. In addition, these results suggest that there may be human factors that differ between individuals that can control whether we inactivate Zika virus or whether it spread in the body. The long term goal of our work is to elucidate the interactions of Zika virus with human innate immune systems and how the virus inhibits these pathways to survive and spread through the host. The short term aims of this project are to build on our new findings to: 1) define the activation and inhibition of human complement pathways by Zika virus, 2) to partner with researchers at Moffitt Research Institute to define the human serum proteins that are associated with Zika virus using cutting edge proteomics technology, and 3) test the hypothesis that Zika virus inhibits Complement-mediated lysis of infected cells. There is no current vaccine for Zika virus and pre-existing innate immunity is the frontline defense against virus infection. Thus, our work has strong implications for design of novel therapeutics or repurposing of existing drugs that harness the power of Complement to control or prevent Zika infections. |

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| John G. Morris, MD, MPH & TM | University of Florida | Rapid diagnostic test for Zika virus in dried blood spots with low demands on instrumentation | Zika virus (ZIKV) is a mosquito-borne virus that began spreading widely in the Americas in 2015 and the cause of Zika Fever ('Zika'). Infection by ZIKV can lead to the brain diseases acute disseminated encephalomyelitis, Guillain Barre Syndrome, and the birth of babies with microcephaly to mothers infected with ZIKV. Zika is commonly diagnosed by testing acute-phase serum or plasma for ZIKV genomic RNA (vRNA) by reverse-transcription polymerase chain reaction (RT-PCR). This requires collection of blood and separation of the blood cells from serum or plasma. Currently, the CDC state's that the methods authorized for diagnosing Zika under the FDA's Emergency Use Authorization are the Trioplex RT-PCR assay and IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA). The Trioplex assay tests for Chikungunya, Dengue, and ZIKV vRNAs. Both assays require collection of blood specimens, and for maintenance of specimen integrity, temperature-controlled transport of the blood specimens to testing laboratories. However, the Trioplex assay is less sensitive than single-plex assays, and IgM tests can be cross-reactive among flaviviruses. Moreover, it has recently been shown that ZIKV can adhere to red blood cells, which are discarded during the preparation of serum or plasma. Thus, standard procedures as practiced introduce significant bias and likely result in false negative tests in many instances. To address the challenges of diagnosing Zika in a rapid and cost-effective manner, we propose to diagnose ZIKV-infection using specimens in the form of dried blood spots coupled with reverse transcription strand invasion based amplification (RT-SIBA). Unlike the common practice of spotting blood onto Flinders Technology Associates (FTA) filter paper ( "FTA cards"), researchers at the CDC report that for blood-borne RNA viruses, superior vRNA detection occurs using blood spotted onto high-quality filter paper instead of FTA cards. Moreover, by using whole blood instead of serum or plasma spotted onto paper, a higher concentration of viruses are deposited and preserved on the filter paper. Finally, RT-SIBA affords rapid and low-cost detection, with results obtainable in around 20 minutes after the vRNA is extruded from dried blood spots (standard real-time RT-PCR requires perhaps 2 hrs). During RT-SIBA, ZIKV vRNA is first reverse-transcribed to cDNA, followed by amplification and detection under low and constant temperature conditions. SIBA relies on recombinase-coated single-stranded invasion oligonucleotides that separate complementary target duplexes that then act as templates for amplification by a DNA polymerase. End-point reads can be performed in real-time PCR machines or on a commercially available battery-operated portable fluorescence detection system. Potentially, then, the proposed system is relatively low-cost, rapid, and may lead to CLIA-waived tests in the future. The AIMS are: 1. Validate filter paper as an appropriate medium for the preparation, stability, and shipping of dried blood spots containing ZIKV. Human blood will be spiked with different amounts of ZIKV and spotted onto FTA cards and filter paper, the filters dried, and vRNA extracted and measured by RT-PCR. 2. Design and validate an RT-SIBA method for ZIKV. 3. Integrate the two methods. The combined assay is expected to increase accuracy and reduce false positives/negatives. |

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| Thomas B. Casale, MD | University of South Florida | University of South Florida Integrated Clinical Trial Network Structuring and Enhancement for Execution of Zika Virus Vaccine and Diagnostic Clinical Trials, and testing of other Emerging Infectious Disease (EID) Solutions for Florida | An epidemic of Zika Virus (ZIKV) and ZIKV congenital syndrome (ZCS) is a major public health problem in the USA, and Florida is the epicenter of this epidemic with 100% of locally transmitted ZIKV in mainland USA and 881 of the 3,952 international travel related cases reported nationwide as of October 26, 2016. This declared "public health emergency of international concern" by the World Health Organization is causing devastating personal and financial costs due to neurological sequelae such as Guillain-Barre Syndrome (rapid-onset muscle weakness caused by the immune system damaging nerves) and fetal microcephaly (condition in which the brain does not develop properly resulting in a smaller than normal head, mental retardation, poor speech, abnormal movements and seizures and sometimes death). As mosquitoes that transmit ZIKV are widespread throughout much of the USA and especially Florida, the ZIKV presents a severe and possibly persistent health risk to the residents of Florida (especially pregnant women). Thus, building clinical trial network capacity for ZIKV vaccines and diagnostics is critical to stem the tide of this rising health crisis both in Florida and around the world. Such a network is also urgently needed to address similar issues with other emerging infectious diseases (EID) in the region surrounding Florida. The overall goal of this project will be to develop such a clinical trials network. Specific Aims: 1) To amalgamate and strengthen key relevant University of South Florida (USF) clinical trials teams into a Zika Clinical Research Network (ZiCRN) in Florida, with the purpose of testing drugs, vaccines and diagnostics in response to the current ZIKV emergency and other EID. This program will leverage expertise from academic, government and industry experts to achieve a multifunctional, integrated approach to rapidly and effectively execute human clinical trials in response to these needs; and 2) To enhance training about clinical research, global health, and biotechnology expertise within the USF system to better address the ZIKV emergency and future emerging infectious diseases crises. An integrated ZiCRN is important for the development of infrastructure necessary to support ZIKV studies that are more complex and require more targeted patient populations. Integrating several sites into a ZiCRN will give investigators access to greater resources including expanded patient databases, diagnostic shared equipment and other resources individual investigators would not otherwise have available. Ultimately, this will aid in attracting research studies and new investigators by having an established infrastructure, and other necessary tools to conduct both inpatient and outpatient clinical research studies. The basic science laboratories that are proposed as part of the ZiCRN can aid in the development of assays to examine predictors of responses to treatments studied and prognosis of patients. The benefits of a ZiCRN to the state of Florida and USF include the potential innovative contributions to medical science, opportunities to provide cutting-edge therapy to the community, enhancement of the image of USF and the state to tackle public health concerns such as ZIKV and improvement of opportunities for USF and the state of Florida to engage in research aimed at counteracting infectious diseases, especially ZIKV. |

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| Ramzi Tamer Younis, MD | University of Miami | Early diagnosis and rehabilitation for craniofacial disorders in congenital Zika syndrome | Zika virus is an ongoing global public health crisis affecting many countries in the Americas. Epidemiologists speculate that globalization, urbanization, and the tropical weather are factors that heightened Zika virus transmission in the current epidemic. In our great state of Florida, we will likely see a prolonged effect of the current Zika epidemic and future outbreaks. Although Zika virus causes relatively mild and nonspecific disease in adults, it has been reported to result in devastating birth defects. At our institution, we have already seen the delivery of approximately 30 infants with exposure to Zika virus infection at birth. A great concern is whether subtle neurologic deficit, such as swallowing, hearing, and vision problems, will affect the long-term development of Zika-exposed infants. We will need to care for the children affected by Zika infection for many years to come. This great responsibility demands that we pursue immediate investigation. Although microcephaly is the better-known complication, reports of swallowing dysfunction, hearing deficits, and eye diseases have been alarming among infants with congenital Zika infection. In addition, Zika-exposed infants born with normal head circumference have been shown to exhibit slowed head circumference growth after birth, implicating a lasting effect of the virus on neurodevelopment. Subtle but progressive feeding difficulty, hearing loss, or vision impairment can be underdiagnosed or missed in infants, with grave consequences for the developing child. The Center for Disease Control recognized the threat of craniofacial disorders in Zika infants, and has recommended additional swallow, hearing, and eye evaluation for infants born with Zika infection. However, health care providers and family members alike are faced with rapidly changing guidelines, unanticipated medical needs of this new disease, and a lack of coordinated plans from the medical professionals. We propose a comprehensive evaluation program with the goal of achieving early diagnosis and intervention for craniofacial disorders in all infants with congenital Zika infection. Drawing from our experience treating children with other congenital defects, we have developed a clear plan to study the characteristics and progression of craniofacial disorders in congenital Zika infection. The inclusion of both symptomatic and asymptomatic Zika infants will allow identification of risk factors for craniofacial disorders, and improve efficiency in the current clinical guidelines. A rigorous evaluation and follow-up schedule by a dedicated team will ensure that signs of craniofacial disorders in all infants affected by Zika infection will be screened appropriately and addressed immediately. The close collaboration of pediatric otolaryngologists, neurotologists, speech-language pathologists, audiologists, and ophthalmologists will provide the best care possible for the early development of communicative skills. This program also provides critical educational resources and logistic support for the family and caregivers by facilitating navigation of a complex schedule of medical appointments and triaging new symptoms and concerns. Infants and families will benefit from a coordinated evaluation and treatment plan with a wide array of established health care services to help ameliorate long-term developmental impact. |

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| Glen N. Barber, PhD | University of Miami | Evaluation of Novel Vaccines that Prevent Zika Infection | In 2015, outbreaks of Zika Virus (ZIKV) were reported for the first time in Brazil and were associated with abundant causes of microcephaly as perceived in aborted fetuses and in infants born to ZIKV infected mothers. For example, Brazil normally reports approximately 150 cases of microcephaly per year. However, in 2015 alone, approximately 3000 cases were documented, which manifests a raise from 5.7 to 99.7 cases per 100,000 births. ZIKV has now been detected in Southern Florida with numerous documented cases of infection occurring in the Miami region. The possibility that ZIKV could become an epidemic worldwide lead the World Health Organization to declare ZIKV a global public health emergency. ZIKV was first isolated in the Zika forest of Uganda in 1947. The virus is related to Dengue Virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JAV) and West Nile Virus (WNV). The Aedes genus of mosquito is the major vector for ZENV and has been isolated as far away as Malaysia, as well as Africa and South America. Aside from being transmittable by mosquito, however, ZIKV has now been documented as being sexually transmittable. It is presently unclear whether the USA or regions outside of Brazil will experience a microcephaly endemic. There are presently no therapies or vaccines to treat or prevent ZIKV infection, respectively, and thus the development of such measures are naturally of paramount importance. Here, we report that we have developed novel, effective vaccines that may protect against ZIKV infection. We intend to further test the effectiveness of our vaccines with the objective of generating sufficient data to warrant the consideration of clinical trials. |

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| Hugh Fan, PhD | University of Florida | Multiplexed Detection Platform for Point-of-Service Testing of Zika Virus | Zika virus (ZIKV) is a mosquito-borne RNA virus and it is a member of the genus Flavivirus. ZIKV caused an explosive outbreak in Brazil in 2015, and spread into Florida in 2016. ZIKV is a major public health concern primarily because it has been linked to abnormally small heads and brains in newborns, a rare condition known as microcephaly. Since ZIKV-infected individuals have common symptoms such as fever, joint pains, and a rash that also occur in other arbovirus infections, ZIKV infection can be misdiagnosed as diseases associated with dengue and chikungunya. It is important to have a point-of-care testing platform to accurately identify ZIKV infection for clinical management of patients. In addition, ZIKV could cause asymptomatic infections. A point-of-service testing platform in the field can be useful for screening asymptomatic patients and monitoring possible ZIKV transmission, as well as for safeguarding the blood supply through ZIKV testing at the blood-donation stations. According to the Centers for Disease Control and Prevention (CDC), the current methods authorized for assessing ZIKV infection include the Trioplex reverse transcription polymerase chain reaction (RT-PCR) assay and IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA). The RT-PCR assay is used for serum or urine samples, within 14 days after possible exposure such as traveling to an infected region. MAC-ELISA is "intended for the qualitative detection of ZIKV IgM antibodies in human sera or cerebrospinal fluid (CSF)". Both Trioplex RT-PCR and MAC-ELISA have not been approved by the Food and Drug Administration (FDA) except for usage under FDA's Emergency Use Authorization (EUA). In addition, MAC-ELISA positive result is "not definitive for diagnosis of ZIKV infection" because there is cross-reactivity in the immune response to flaviviruses, and therefore confirmatory tests are needed. Moreover, these assays are carried out in laboratories, not at the point of care or in the infected field. To address the challenge, we propose to develop laminated paper-based analytical devices (LPAD) for detecting ZIKV infection. LPAD will be developed by borrowing the concept from pH papers and pregnancy test strips. Either colorimetric reading or optical detection can be used. The LPAD devices are of low-cost, easy to operate by nontechnical personnel, and manufacturable. Moreover, our goal is to develop an integrated platform that tests ZIKV infection based on both nucleic acid amplification and immunoassay. To achieve the goal, we propose the following specific aims: 1. Design, fabricate, and test one LPAD for ZIKV infection based on nucleic acid amplification. The LPAD device consists of components for lysis, RT-LAMP (loopmediated isothermal amplification), and detection. 2. Design, fabricate, and test another LPAD for ZIKV infection based on IgM antibody. The LPAD device consists of components for antibody conjugation, control/test bands, and colorimetric detection. 3. Integrate two LAPD devices into one platform for multiplexed detection based on immunoassay and nucleic acid amplification. The combined assay is expected to increase accuracy and reduce false positives/negatives. The key advantages of the proposed platform include (1) being portable and low-cost, (2) integration of genetic test with immunoassay, and (3) ability to screen asymptomatic infections using non-invasive samples such as urine and saliva over a broader testing window. |

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| Charles J. Lockwood, MD, MHCM | University of South Florida | Cellular and molecular mediators of Zika Virus replication in decidua and mechanisms of Zika Virus transmission from maternal decidua to the placental/fetal unit | Flaviviruses are enveloped, positive-stranded ribonucleic acid (RNA) viruses that are an emerging global health threat. They include dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis, tick-borne encephalitis, West Nile and Zika Viruses. In pregnant women, the impact of mosquito-transmitted Zika Virus (ZIKV) infection on the mother is usually minimal except in cases post-viral Guillain–Barré syndrome. However, in the fetus the virus can cause severe developmental problems, ranging from fetal growth restriction, chronic placentitis and/or severe congenital defects. The later includes abnormal brain development i.e. microcephaly, retinopathy and limb contractures. The virus is also associated with miscarriage and stillbirth. Preliminary observation suggest that the virus can evade local immune barriers in the placenta and brain to infect these tissues which in turn act as reservoirs causing long term shedding of the virus. However, it is unclear how the virus gains access to the placenta and subsequently to the fetus. Such information is vital to prevention of these catastrophic outcomes. The placenta is attached to the uterine decidua. The latter is a specialized tissue composed of equal number decidual cells (50%) and decidua-specific immune cell types including uterine natural killer cells (60-80%), macrophages (20-25%) and T-lymphocytes/ dendritic cells (1-2%). The decidua is the only site of direct cell-cell interactions between the maternal and fetal tissues (aka the maternal-fetal interface). Moreover, the decidua anchors the placenta, facilitates maternal vascular adaptation to pregnancy, contributes to the immunosuppressive state of pregnancy, which prevents rejection of fetal allograft and serves as a barrier to placental and fetal infection. We posit that this decidua-mediated immunosuppression enables ZIKV survival, replication and dissemination into the placenta. Thus, containment and eradication of ZIKV in the decidua should prove crucial to the prevention of subsequent placental transmission. This proposal will first explore the cellular and molecular site(s) of ZIKV that allow its replication in the decidua, then will identify the molecules responsible for ZIKV attachment and infection of decidual cells, resident leukocytes and/or trophoblasts and finally will test novel agents including neutralizing antibodies and small molecule inhibitors that block this attachment and/or infection. In addition, we will test these agents in a novel decidual –placental villi explant co-cultures to identify optimal approaches to the prevention of maternal-fetal ZIKV transmission. This strategy will enable us to reduce the perinatal burden of ZIKV infection. To accomplish these aims we have assembled an interdisciplinary team of decidual-placental biologists and infectious disease experts. |

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| Claudia A. Martinez, MD | University of Miami | Cardiovascular Complications Related to Zika Virus Infection | The World Health Organization has declared the current Zika outbreak and its associated complications a Global Public Health Emergency. Zika virus is a member of the viral family Flaviviridae that is related to other flaviviruses that have been recognized to cause neurological disorders as well as short and long-term cardiovascular complications. Zika virus is a relatively new public health threat; the relationship of Zika virus infection and cardiovascular complications has not been established. Previous reports have documented acute heart muscle inflammation with evidence of abnormal echocardiogram after other flaviviruses infections with confirmation of the presence of viral antigen in cardiac and vascular cells as well as a subsequent increased in the incidence of acute coronary syndrome. This proposal will evaluate the impact of Zika Virus infection on short and long term cardiovascular complications previously described to occur with other flaviviruses. Aims: 1)To determine whether patients between 18-50 years of age with acute or recent ( <90 days) confirmed zika virus infection manifest cardiovascular symptoms as determined by a cardiovascular symptoms questionnaire at baseline and yearly up to 3 years 2) To determine whether these patients manifest objective evidence of cardiovascular involvement evaluated by: EKG, pro-BNP levels echocardiography findings of heart muscle inflammation (myocarditis or pericarditis) and vascular changes identified by brachial artery flow mediated dilation, at baseline and yearly up to 3 years after Zika infection. Results of this proposal will improve scientific understanding of subsequent impact of Zika-related cardiovascular morbidities. |

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| Matthew DeGennaro, PhD | Florida International University | Identifying Molecular Targets for Spatial Mosquito Repellent Design | Current approaches to control the spread of Zika are ineffective. There have been 184 locally transmitted cases of the disease in Florida so far. Aedes aegypti, the principle vector of Zika, is very difficult to eradicate because it is so well adapted to cosmopolitan environments and has shown resistance to insecticides. Personal protection using insect repellents is one of the few tools we possess to protect against mosquito bites and the subsequent transmission of these diseases. The current EPA approved repellents, picaridin, IR3535, oil of lemon eucalyptus (OLE), and DEET are most effective when worn on skin, but are not fully protective. None of these chemicals are particularly good spatial repellents, so cannot repel mosquitoes at distances sufficient enough to prevent mosquitoes from entering our homes or outdoors spaces. The project goal is to identify the genes that allow mosquitoes to smell repellents to facilitate the design of new mosquito repellents. We have developed a technique that allows us figure out which genes "smell" an odor by assessing how the olfactory receptor gene expression is altered after a mosquito is exposed to that odor. By exposing mosquitoes to the insect repellents picaridin, IR3535, oil of lemon eucalyptus (OLE), PMD, and DEET, we will find the genes that enable each of these chemicals' repellency. This proposal uses a new technique developed in mammals and Drosophila, Deorphanization of Receptors based on Expression Alteration of mRNA levels (DREAM), that our unpublished data shows can work in mosquitoes. This will allow us to comprehensively determine which Aedes aegypti mosquito olfactory receptors are activated by repellents in vivo by characterizing the reduction of mRNA levels of these receptors. These genes, which are likely to be olfactory receptors, can then be used to screen for chemicals that are highly effective spatial repellents. The odor-responsive gene sets we will produce will provide insight into the molecular mechanism of mosquito repellency and a list of molecular targets for repellent design. |

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| Ivan A. Gonzalez, MD | University of Miami | ZIK-Action: Evaluation of Infants for Zika Related End Organ Damage, A Team Science Approach | Prior to the onset of our Zika outbreak, our institution faced travel associated Zika infection in pregnant mothers. The Departments of Obstetrics and Pediatrics developed a Zika Response Team (ZRT), with Dr. Christine Curry and Dr. Gonzalez as lead physicians, respectively. The collaboration was developed with one goal in mind, which was to care for both mother and child. This provided the opportunity to create an infrastructure for what we thought was inevitable. As part of our ZRT, we have a network of adult and pediatric subspecialties available onsite. These include specialties in Maternal Fetal Medicine, Pediatric Neurology, Pediatric Ophthalmology, Neurodevelopmental Psychology, Pediatric Audiology, Pediatric Nephrology and Pediatric Cardiology. Once the local transmission of the virus became a devastating reality, our clinical protocols and infrastructure were set in motion. Overall, the birth rate of our health system is well over 5,000 deliveries per year and that number has remained unchanged thus far. The Obstetrics department's faculty provides care for the majority of this population. This will serve as the potential pool from which to recruit subjects into the study. The total number of positive women cannot be determined since there is an area of ongoing transmission. The seroconversion rate thus far is 5%. Extrapolating this rate, of the 5,000 deliveries, it is expected that a pool of about 250 positive women in any given year of on-going local transmission. The extent of end organ damage related to Zika infection in utero has not been completely elucidated. Mouse model confirms the presence of Zika virus which correlates to detection of virus in urine samples of symptomatic patients, renal involvement has not been evaluated in infected or exposed infants. Hypopigmented lesion related to Zika have also been described but the long term effect on the retina development have not been defined. Flavivirus infection can also cause myocarditis, yet no data exist in the evaluation of Zika infected or exposed infants for cardiac dysfunction. Immunological response during maternal Zika infection may reveal risk factors for the development of organ damage. Neonates exposed to an in utero infection with Zika can have subtle end organ damage. Therefore, our team is proposing to investigate newborns infected and exposed to Zika virus in utero. The proposed study will evaluate these newborn over three years to determine links to end organ damage secondary to Zika. End organ damage will be assessed using a combination of established biomarkers associated with organ function and organ images. These will be compared to age specific normative data. Findings will be correlated to Zika immune response and cytokines profiles. The disciplines involved in this study will include virology, infectious diseases, immunology, ophthalmology, cardiology, nephrology and neurology. The institution involved will include Anne Bates Leach Eye Hospital, University of Miami Miller School of Medicine, Florida Gulf Coast University and the Miami-Dade county Department of Health. |

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| Mario Stevenson, PhD | University of Miami | Identification of the duration of ZIKV persistence to guide reproductive health decisions | The Zika virus is passed to humans by infected mosquitos commonly found in Florida, and can be sexually transmitted. One in 4 infected people are symptomatic with fever, rash, joint/muscle pain, headache, and conjunctivitis (red eyes). Pregnant women infected with Zika bear babies with microcephaly (abnormally small head), brain damage, and various congenital birth defects. There is currently no cure nor vaccine available to the public to protect against Zika infections. VRC-ZKADNA085-00- VP is an investigational DNA vaccine developed by the NIH NIAID Vaccine Research Center. This vaccine instructs the body to make a small amount of protein representing two key antigens of the many proteins made by the intact Zika virus. The body may use these two proteins to build a beneficial immune response. The vaccine is composed of a closed-circular DNA plasmid encoding wild type precursor transmembrane M (prM) & envelope (E) proteins from the H/PF/2013 VKIV strain. Preclinical studies showed that two 4 mg doses of this vaccine given intramuscularly completely protected 17 of 18 nonhuman primates against Zika virus challenge, and that protection correlated with serum antibody neutralization. Our proposed Phase II multicenter safety and immunogenicity randomized double-blind clinical study of VRC-ZKADNA085-00-VP Zika DNA vaccine will follow the NIH Phase I trial currently in progress (NCT02840487). RESEARCH OBJECTIVES: There is currently no vaccine to protect Floridians from the growing threat of Zika virus in Florida. To address this gap, we have assembled a research team that will assess whether VRC-ZKADNA085-00-VP Zika DNA vaccine, supplied by Florida pharmaceutical manufacturer FLUCEL LLC as licensed by NIH Vaccine Research Center, is: 1) is safe; 2) causes any adverse side effects; and 3) mounts appropriate and efficacious immunological activity in Floridians living in Miami and in Gainesville, as a follow up to the presently ending national Phase I trial of this same vaccine. HYPOTHESIS: DNA vaccine VRC-ZKADNA085-00-VP will be safe and well tolerated in healthy adult Floridians, and will elicit a ZIKV-specific immune response seroconversion rate with 4-fold rise in reciprocal EC50 above baseline and/or reciprocal EC50 > 1000. SPECIFIC AIMS RELATING TO FLORIDA ZIKA GRANT INITIATIVE: AIM 1 is to meet the Dynamic Change Team Science objectives of the Florida Zika Grant Initiative in assembling a University of Florida College of Medicine based team to conduct a Phase II Clinical Trial of a Zika vaccine in Florida. AIM 2 is to assess the immunity efficacy and safety of the VRC-ZKADNA085-00-VP Zika DNA vaccine in Floridians. AIM 3 is to provide a scientific foundation for steps to protect Floridians in either an emergency Zika vaccine deployment or in Phase III clinical trials in Florida. PRIMARY RESEARCH OUTCOME MEASURES and ENDPOINTS: 1) Immunogenicity will be analyzed in the total intent to treat population and a modified intent to treat population, as quantified by seroconversion rates. Target endpoints: a) 4-fold rise in reciprocal EC50 above baseline; and/or b) reciprocal EC50>1000. Post-sampling statistical analyses will consider the modified intent to treat subjects who were screened as positive for asymptomatic occult dengue or Zika titers. 2) Safety and Tolerability on intent to treat population assessed by reactogenicity and laboratory measures of safety SECONDARY OUTCOMES MEASURES: Possible Adverse Events or new onset of illness. |

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| Shanta Dhar, PhD | University of Miami | Nano-formulations of Anti-helminthic drugs for Zika Therapy and Prevention | The rapid spread of Zika virus infection across the USA is anticipated to have a direct impact on the U.S. health care system as it is known to cause microcephaly as well as a spectrum of neurologic problems including seizures in newborn babies and Guillain-Barre syndrome in adults. The ultimate scale and impact of Zika virus infection remains to be seen. It is likely that these severe abnormalities recognized at birth only represent the tip of the iceberg. There is a great-unmet need to develop strategies to detect Zika early, but more critically to prevent the further spread of Zika by developing treatment strategies to protect newborn babies exposed to the infection. As of November 6th 2016, the Florida Department of Health has reported a total of 924 cases of Zika diagnosed in Florida out of the grand total of 4128 in the US as reported by the CDC. All the locally acquired cases of Zika in the United States have been identified in South Florida. This underscores the importance for South Florida to rapidly develop management strategies since there are no FDA approved methods available to treat Zika infection. The Miller School of Medicine of the University of Miami is uniquely positioned to undertake this challenge with our expertise in infectious diseases, nanotechnology, biochemistry, and conducting clinical trials. The overarching goal of this project is to investigate nanotechnology-based formulations of the currently FDA approved drug Ivermectin to treat Zika virus infection. The optimized formulations will be evaluated through a fast-track clinical trial. Ivermectin, a broadly used anti-helminthic drug, is a highly potent inhibitor of the Yellow Fever Virus. Recent in vitro studies demonstrated inhibitory effects of Ivermectin on Zika infection at a relatively higher dose. We propose to create controlled released nanoparticles of Ivermectin with ability to supply slow therapeutic dose of this drug over a prolonged period of time. Nanoparticles will also result in prolonged circulation thus effectively increasing the therapeutic window. Further, to reduce the spread of virus through sexual transmission, we will develop a topical formulation of Ivermectin for transdermal delivery of the drug. To achieve the goals of this work, we have formulated the following three Specific Aims. 1. Optimization of nano-formulations of Ivermectin for oral and transdermal use. In vitro and preclinical evaluations of Ivermectin nano-formulations. 2. Develop rapid implementation of the clinical trial network using the currently existing facilities and the entire Human Subject Protection Program. 3. Design and rapid implementation of clinical trials on healthy adults followed by Zika infected subjects. We envision that the proposed work will fill a great-unmet need by developing new effective therapies for the treatment of Zika virus infection. In addition, the formulations developed will provide for a pre-exposure prophylaxis during pregnancy and primary prevention by mitigating perinatal, sexual and mosquito transmitted infections. Apart from the medical and societal benefit of the proposed strategy, it will also address important questions raised by the local government, stakeholders, and the community. |

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| Alvaro N. Monteiro, PhD | Moffitt Cancer Center | Cellular targets of Zika-encoded proteins and microcephaly | A recent epidemic of Zika virus (ZIKV) in the Americas, particularly in Brazil, displayed a temporal correlation with an epidemic of microcephaly. Microcephaly is a congenital malformation caused by reduced brain size. In the vast majority of cases individuals with microcephaly will have long-term health effects including delayed neuronal, psychological, and motor development. Several lines of evidence strongly suggest a causal link between microcephaly and ZIKV infection of the fetus. Moreover, ZIKV infection has been shown to lead to disruption of neural progenitor development in mice and in human cells in vitro. Emerging evidence suggests that ZIKV has the potential to cause Alzheimer's style damage to the adult brain as well. Proteins encoded by viruses can bind and inactivate host cell proteins leading to dramatic biological effects such as attenuation of cell growth, gene expression dysregulation, induction of apoptosis, and abrogation of DNA damage responses. Thus, we hypothesize that ZIKV-encoded proteins specifically target host proteins in neuroprogenitor cells leading to microcephaly; and that missense ZIKV variants isolated in Brazil may do so with a higher affinity. To test this hypothesis we will determine the ZIKV protein-Human protein interaction network using a combination of yeast two hybrid screenings against a human brain library and tandem-affinity purification coupled to mass spectrometry. We will focus on non-structural proteins and on Brazilian isolate-specific missense variants, and cellular targets will be confirmed by immunoprecipitation. We will then determine the biological significant of these interactions by CRISPR-mediated deletion in human astrocytes and in a human cerebral organoid model. Our laboratory is well positioned to rapidly complete this pilot due to our systems biology expertise in protein-protein interaction networks, including of microcephaly proteins (MCPH1). In addition we have also implemented and optimized quantitative in vitro assays and CRISPR-based genome editing as well as the human cerebral organoid model. At the end of this project we expect to generate a comprehensive interaction map of ZIKV proteins and their interaction partners in human cells. We will have assessed the extent to which interactions of ZIKV proteins with a cellular protein leads to cell dysregulation and malformations in a human cerebral organoid model. This interaction network will help elucidate the biological effects of ZIKV infections and create a platform for discovery of drugs that could abrogate or attenuate the brain health effects. |

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| Ashley N. Brown, PhD | University of Florida | Identification of antiviral therapies for the treatment of Zika virus using existing drugs | Zika virus (ZIKV) is a mosquito-borne virus belonging to the family Flaviviridae, which includes other clinically significant viral pathogens such as dengue virus and hepatitis C virus. ZIKV has recently been flagged by the World Health Organization as a Public Health Emergency of International Concern due to its emergence and spread into the Americas (resulting in major outbreaks) and the devastating clinical manifestations associated with infection including severe congenital microcephaly and Guillain-Barre syndrome. In the state of Florida alone there have been over 900 confirmed infections (travel-related and locally-spread) to date, 92 of which involve pregnant women, highlighting the seriousness of the ZIKV threat to the United States. There is currently no vaccine or antiviral therapy licensed for the treatment or prevention of ZIKV, demonstrating the pressing medical need for therapeutic options for this viral disease. The research proposed in this project aims to address this serious unmet need by identifying effective antiviral therapies for ZIKV infections. Our research strategy is to explore the antiviral activity of anti-infective agents that are already approved for clinical use, as drug repurposing is an efficient and cost-effective method to rapidly accelerate drug development. We will evaluate four different drugs, all which have demonstrated antiviral potential against ZIKV, as part of our investigation: ribavirin, interferon-alpha, favipiravir, and niclosamide. The antiviral activity of all four agents will be examined against two clinically relevant ZIKV strains (one of South American Lineage and one of African Lineage) using three different cell lines, as the degree of antiviral effect is often influenced by the host cells employed for drug screening. In order for antiviral therapy to be successful in man, one must first identify the optimal dose (how much?) and dosing interval (how often?) for each compound that will maximize antiviral activity. The overall goal of this proposal is design innovative dosage regimens for each compound as single agent and combination therapy against ZIKV that will yield maximal inhibition of viral replication and minimal emergence of drug-resistant viruses. We will use a state-of-the-art model system in which we can simulate fluctuating drug concentrations that are observed in a human following the administration of a drug due to biological processes such as absorption, metabolism, and excretion. This allows us to evaluate the impact of different doses and dosing intervals (e.g.: administering the total daily dose once-a-day versus half the daily dose twice-a-day) on the antiviral activity of each agent. Novel mechanism-based mathematical models will be developed and fit to these data to identify the regimens that are predicted to yield the best therapeutic outcomes in man. These rationally designed regimens will have the greatest likelihood of clinical success, as they will have been optimized to yield maximal viral suppression with minimal emergence of resistance. This work can be directly applied to the design protocol for human clinical trials. Overall, this translational virology/pharmacology approach using currently approved anti-infective agents has great potential to identify an effective antiviral regimen for ZIKV that can rapidly be brought to market. |

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| Mark E. Sharkey, PhD | University of Miami | Development of a rapid diagnostic assay for Zika Virus infection | Zika virus (ZIKV) was first isolated in 1947 in Uganda but remained an obscure and infrequent mosquito-borne human pathogen causing a mild, self-limited viral syndrome confined to a few regions of Africa and Southeast Asia for close to 60 years. It was not until 2013-14 that an epidemic in French Polynesia led to the first descriptions of an association between Guillain-Barre syndrome and ZIKV infection. This was followed in 2015-16 by a rapidly expanding epidemic in South and Central America, the Caribbean, South Florida, and a burgeoning number of cases of microcephaly and other neural tube malformations in infants born to women who contracted the infection during pregnancy as well as additional reports of post-infection Guillain-Barre syndrome. While viremia is short-lived (typically less than one week), it has now become apparent that ZIKV may persist for longer periods of time in fluids such as urine and semen (6 months or more), and there have been reports of sexual transmission of infection weeks after the initial infection in an index patient. Thus, the ability to diagnose the infection in different body fluids is critical for transmission prevention and for reproductive health decisions. Antibody diagnosis of infection is unreliable due to cross reactivity to similar flaviviruses and the inability to distinguish between viral clearance and viral persistence, and diagnosis through traditional RT-qPCR techniques is expensive, complex, and requires central laboratories with turnaround times of several days. Thus, a simple, inexpensive, point-of-care assay that does not require sophisticated equipment or extensive operator experience is needed. We have developed an assay based on standard PCR that uses a novel enzyme with both RNA and DNA-dependent polymerase activities and the capability to amplify RNA directly from clinical samples without a purification step or separate cDNA synthesis. The incorporation of a fluorescent probe into the PCR reaction allows immediate scoring of positive reactions with an inexpensive blue light source. This format brings the cost and complexity of ZIKV diagnosis down to a fraction of what they currently are with standard RT-qPCR assays and makes feasible a cost-effective point-of-care assay. The proposed research will have 2 specific aims: Aim 1: Evaluate the assay for sensitivity and specificity in body fluids (i.e. spiked lab samples and clinical samples) including blood, urine, saliva and semen. Aim 2: Validate a point-of-care kit in clinical samples. |

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| Ulas Bagci, PhD | University of Central Florida | Utilization of in utero diffusion tensor magnetic resonance imaging to evaluate neurological disorders caused by Zika virugers | Although Zika virus was first identified in Uganda (in 1947), there is only now an identified association between Zika and microcephaly. ZIKV has been considered as a Public Health Emergency of International Concern and its clinical spectrum remains a matter of concerted investigation. As of October 26, 2016, there were 32,814 confirmed cases of ZIKV infections reported by the Centers for Disease Control and Prevention, with 4,091 of these cases in the state of Florida. So far obstetrical ultrasound has been used to evaluate neurological damage in the fetus caused by ZIKV. Although ultrasound can adequately diagnose microcephaly, it has limited sensitivity and specificity. Unfortunately, there are variety of other neurological disorders that can develop in fetuses exposed to ZIKV infection, most of which are not identifiable by ultrasound. To date, MRI characteristics of fetuses of mothers infected with ZIKV have not been reported. Specifically, underlying disorders affecting development of the white matter tracts, which can be determined through Diffusion Tensor Imaging model of diffusion MRI, in patients with ZIKV has not been investigated either. Since MRI is the gold-standard for brain imaging and related diseases, and since diffusion imaging may reveal connectivity of neurons and their developmental changes over time, we anticipate to find in-deep alterations in neurological systems. Therefore, our goal in this project is to utilize fetal MRI with DTI as a screening test in ZIKV infected patients to assess for potential subclinical neurological disorders in the developing fetus. To achieve our overall goal, we have gathered a multi-disciplinary team from internationally renowned imaging scientists, radiologist, statistician, and biomedical engineer(s). In the current collaborative effort, our overarching goal is to develop quantitative MRI and DTI analysis platform to explore neurological disorders caused by ZIKV infection. |

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| Yulia Gerasimova, PhD | University of Central Florida | Point-of-care diagnostic platform for Zika virus infection based on visual split deoxyribozyme sensors | Rapid, accurate, sensitive, and low cost tests for Zika virus infection are needed to enable efficient case management and implementation of infection control programs. Currently, the procedure for ZIKV diagnosis involves observation of its clinical manifestation followed, if necessary, by immunoassays or nucleic acid hybridization tests (NAATs). The symptoms of ZIKV infections overlap with those of other flaviviruses (Dengue and Chikungunya virus), which complicates the clinical diagnosis. Immunoassays, which detect the presence of virus-specific antibodies, suffer from cross-reactivity in case of a previous infection with other flaviviruses. WHO recommends confirming the presence of ZIKV by NAAT, such as RT-PCR, using whole blood, serum, plasma or saliva samples during the acute infection stage (<7 days). The presence of viral RNA in urine is shown to be longer. In addition, parallel detection of dengue and chikunguya is also recommended. RT-PCR diagnostics offers the advantages of high sensitivity and is thus considered the gold standard of NAAT-based diagnostics. At the same time, this method is sensitive to contaminations and produces false-positive results. Therefore, it requires highly trained personnel and expensive equipment, which does not satisfy the criteria for a point-of-care test. Our goal is to develop a simple diagnostic platform for the detection of ZIKV infection with visual signal output. and can be used at point-of-care settings and even at home by untrained users. The platform is based on our original development, which offers highly selective and sensitive, low-cost and instrument-minimized format for detection of natural RNA molecules. The ZIKV defection procedure will include the following stages: (i) brief pre-processing of the biological specimen (blood, serum etc.); (ii) incubation of the analyzed sampled with reagent 'A' at 65oC for 15-30 min; (iii) adding reagent 'B' followed by incubation for 15-30 min at 65oC; (iv) adding reagent 'C', incubation at room temperature for 5-10 min; (v) registering color change if ZIKV infection is present. The platform will take advantage of an isothermal amplification of viral RNA followed by its detection by our original split deoxyribozyme (sDz) sensors capable of generating visual signal. The advantages of sDz technology include (i) tolerance to sample-derived inhibition and, therefore, does not require extensive sample preparation; (ii) high selectivity with the ability to discriminate between two RNA sequences differing in one nucleotide. This may allow for accurate diagnosis of ZIKV even in the presence of closely related RNA from other flaviviruses. (iii) modularity and straightforward design. This feature allows minimal modifications of sDz sequence and optimization of its performance to easily adjust the existing sensor so that it could detect RNA of another ZIKV lineage or another flavivirus. (iv) color change as a signal, which eliminates the need to use instrumentation for the analysis. The applications of the proposed platform can be extended to other flaviviruses in the frame of follow up studies. Our collaborative team is very suitable for the project implementation. Dr. Gerasimova is an expert in the development of hybridization probes for diagnostics of infectious diseases. Dr. Choe is an expert in pathogenesis of viral diseases including West Nile, dengue and Zika viruses. |

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| Michael Teng, PhD | University of South Florida | Rapid identification of natural products with antiviral activity against Zika virus | Zika virus (ZIKV) is a member of the flavivirus family, which includes a number of human pathogens such as West Nile virus, dengue virus, and hepatitis C virus. ZIKV has emerged recently as a significant public health threat in the Americas and has been designated ZIKV infection a public health emergency by the World Health Organization. Initially isolated in Africa in 1947, ZIKV was not associated with significant human disease until 2007 when an outbreak occurred on Yap Island in Micronesia. ZIKV has since migrated eastward, culminating in a serious epidemic in South America and spreading to Central and North America. Recent epidemiological evidence has shown a number of importations of ZIKV and transmission of the virus in south Florida. ZIKV is spread by Aedes aegypti, which is also the mosquito vector for dengue and yellow fever viruses. In addition to vector-mediated transfer, ZIKV has been documented to be transmitted by sexual contact. It is estimated that 80% of ZIKV infections are asymptomatic; however, ZIKV has been associated with significant neurological defects, such as Guillain-Barre Syndrome and the newly described congenital Zika syndrome (CZS). CZS encompasses a wide range of neurological abnormalities associated with acquisition of ZIKV infection during pregnancy. The long-term implications of ZIKV infection are not known. There is an urgent need to develop antiviral therapies against ZIKV to respond to this threat. Vaccine candidates have been rapidly advanced using currently available platforms. However, in the absence of universal vaccination, development of antiviral drugs is essential to limit infection and thus spread of the virus. Importantly, it is becoming apparent that ZIKV can persist in immunologically privileged sites for extended periods of time. Thus, effective antiviral therapies will be necessary to ensure complete clearance of ZIKV from infected individuals. While some of the current antivirals against flaviviruses may be retargeted for ZIKV, homology among the flaviviruses is not extensive. Therefore, development of ZIKVspecific therapies is essential. We propose to develop novel assays to allow for high throughput screening of compounds to identify potential antiviral drugs against ZIKV. These assays do not require the use of live ZIKV and therefore are easily scalable and adaptable for drug discovery without the need for enhanced biosafety level protections. Further, we propose to use an innovative approach to the discovery of natural products as drugs and/or scaffolds for subsequent medicinal chemistry development. Natural products have long been an integral part of drug discovery, particularly for infectious diseases as evidenced by the awarding of the 2015 Nobel Prize in Medicine for the discovery and development of artemisinin and ivermectin, two natural product-based drugs that have had profound effects on human health. Discovery of lead compounds from our screen will allow development of ZIKV-specific drugs and identify scaffolds for further medicinal chemistry development. |