Drexel University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

The Drexel University received $1,431,165 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Effect of Antiretroviral Therapy on Surrogate Markers of Cardiovascular Disease and Inflammation in HIV-infected Patients - Numerous studies have demonstrated that HIV infected patients are at an increased risk of developing cardiovascular disease (CVD) and myocardial infarction (MI). There is evidence to suggest that HIV infected patients have unique risk factors related to their infection and its treatment that interact with traditional cardiac risk factors to put them at increased risk of CVD. The purpose of this study is to prospectively evaluate measures of endothelial function and subclinical atherosclerosis as well as markers of inflammation in HIV patients in order to elucidate the nature of HIV specific risk factors for cardiovascular disease. This knowledge will allow more effective risk stratification and treatment in this population which is disproportionally affected by cardiovascular disease.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

We will conduct a prospective cohort study examining the affect of initiating anti-retroviral therapy (ART) on structural, immunologic, and functional markers of atherosclerosis. We hypothesize that although some components of ART may carry increased risk of CVD, the net effect of viral suppression will be decreased inflammation with resultant decreased atherogenesis and improved endothelial function. Our study will address the following specific aims:

Specific Aim 1: To determine if HIV infection is associated with increased inflammation decreased endothelial function, and increased risk for atherosclerosis.
Specific Aim 2: To determine if initiation of ART in HIV infected adults is associated with improvement of markers of inflammation, endothelial function and atherosclerosis.
Specific Aim 3: To assess for correlation between changes in immune activation and changes in markers of inflammation and subclinical atherosclerosis.

We will accomplish these aims by conducting a prospective observational cohort study focusing on 3 groups: HIV infected subjects who initiate ART, HIV infected subjects who do not initiate ART, and HIV negative subjects with the following specific objectives: 1) To compare mean change at one year in carotid artery intima-media thickness (IMT) between the three study groups. 2) To compare changes in inflammatory markers, brachial artery Flow Mediated Dilation (FMD), cardiac ejection fraction and pulmonary artery pressure at one year between the three study groups. 3) To assess for correlation between changes in serum markers of inflammation, status of HIV disease (as measured by CD4 and viral load), endothelial activation, physiologic surrogates of CVD such as IMT and FMD and cardiac function.

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Other Participating Researchers

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Expected Research Outcomes and Benefits

As HIV-infected patients live longer, the recognition and treatment of other chronic illnesses have taken on increased significance in this population. Cardiovascular disease (CVD) is of particular importance since several large cohort studies have demonstrated an increased prevalence of CVD, and a higher risk for myocardial infarction (MI) in HIV-infected patients in the ART era.

In this study we are collecting data that will allow us to begin to correlate observations from the clinical with changes at the molecular level to better understand the mechanisms of atherosclerotic disease in HIV infected patients. Insight into the pathophysiology of CVD at the molecular level will be elicited by measurement of circulating markers of immune and endothelial activation. Structural and functional measurements of atherosclerosis will provide a macroscopic correlate. At the end of this study, we expect that through this approach we will gain a better understanding of the pathophysiology of this process with the long term goal of establishing biomarkers of CVD in HIV infected patients that can be used in combination with clinical data to predict and monitor CVD in HIV patients in clinical care.

Summary of Research Completed

Eleven transthoracic echocardiograms were evaluated and interpreted on 5 subjects. For all 5 subjects clinical history, serum lipid measurements, HIV specific labs (viral load, CD4) and anthropometric measurements have been recorded. Serum samples have been frozen so that

testing for inflammatory markers can be run batched to minimize reagent use and laboratory time and to standardize test results. Carotid ultrasound measurement of intima-medial thickness were completed. Descriptive statistics were calculated on the enrolled subjects.

Accrual and follow-up for the study has been completed with 11 subjects enrolled. Accrual goals of enrolling 10 patients was met, however not all subjects completed all 48 weeks of follow-up assessments as defined in the study protocol (see table 2 below).

There were several challenges to maintaining long term follow-up in this underserved patient population including unstable housing and incarceration. Additionally, enrolling HIV positive subjects who were not on antiretroviral therapy became increasingly challenging as national HIV treatment guidelines were revised during the study period with recommendations for initiating antiretroviral therapy at higher CD4 cell counts, resulting in fewer patients that did not meet criteria for initiation of therapy. This limited enrollment for this arm of the study.

Trans-thoracic echocardiograms were completed and interpreted with left ventricular ejection fraction and pulmonary artery pressure measured for each subject as specified by the study protocol. Carotid artery ultrasound was performed for each subject as specified by the study protocol with images stored. Changes in available personnel at our institution with expertise in vascular ultrasound resulted in the need to postpone carotid intima-media thickness measurements.

Serum samples were processed and stored. Cytokine profiling will be performed on samples.

Table 1: Baseline characteristics of enrolled subjects

	Mean (range)
Age (y)	32 (21-51)
Body Mass Index	27.5 (21.8-37.7)
CD4+ T-cells (number/microL)	519 (320-840)
HIV-1 Viral load (copies/mL)	35010 (440-67800)
LDL cholesterol (mg/dL)	88.5 (65-172)
Left ventricular ejection fraction (%)	60 (55-65)

Table 2: Enrolled subjects and completed study assessments

Subject #	1	2	3	4	5	6	7	8	9	10	11
Baseline											
Anthropometric data	X	X	X	X	X	X	X	X	X	X	X
HIV lab studies (CD4/VL)	X	X	X	X	X	X	X	X	X	X	X
Serum for immune activation	X	X	X	X	X	X	X		X	X	X
Carotid IMT ultrasound	X	X	X	X	X	X			X	X	X
Trans-Thoracic Echo	X	X	X	X	X	X			X	X	X
4 week											
HIV lab studies (CD4/VL)		X	X	X		X			X	X	X
Serum for immune activation		X	X	X		X			X	X	X
Trans-Thoracic Echo		X	X	X		X			X	X	X
12 week											
HIV lab studies (CD4/VL)		X	X	X		X			X		
24 week											
Anthropometric data		X				X					
HIV lab studies (CD4/VL)		X				X					
Carotid IMT ultrasound		X				X					
Trans-Thoracic Echo		X				X					
48 week											
Anthropometric data		X				X					
HIV lab studies (CD4/VL)		X				X					
Serum for immune activation		X				X					
Carotid IMT ultrasound		X				X					
Trans-Thoracic Echo		X				X					

Research Project 2: Project Title and Purpose

Modulation of Capsid Stability as an Antiviral Strategy - The project purpose is to identify anti-HIV compounds that work by antagonizing the functions of the viral capsid protein.

Duration of Project

1/1/2010 - 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 3: Project Title and Purpose

sPLA2 Inhibition Therapy for Epilepsy - This project will test the efficacy of new secreted phospholipase A2 (sPLA2) inhibitors as therapy for both the excitability disturbances and inflammatory consequences of epilepsy. These inhibitors, called CHECs, consist of 7mer and 9mer peptides with demonstrated systemic efficacy in traumatic and autoimmune disease models. They are part of a portfolio of intellectual property (composition of matter, applications, diagnostic) held by Drexel University. The goal of this application is to expedite the clinical development of these peptides by expanding potential clinical applications. These compounds are especially suited for commercial development since it has been unequivocally demonstrated that they are active when taken by mouth. The present experiments are proof-of-concept studies for treatment of epilepsy, where the inflammatory consequences of the disease have been largely overlooked. Interestingly, there is also evidence, including our own preliminary studies, that excitability changes during epilepsy are closely related to inflammation, raising the possibility that the CHEC peptides represent a unique monotherapy for this disease.

Duration of Project

1/1/2010 - 6/30/2012

Summary of Research Completed

This project ended during the prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 4: Project Title and Purpose

Role of Septins in Growth Cone Guidance - This project will investigate the roles of three members of the septin family of GTPase (septin 2,6,7) in the guidance of growth cones by extracellular signaling molecules. The correct guidance of axons is crucial to nervous system development and regeneration following injury. The project will determine if septins are involved in growth cone guidance and the extension of axons, and thus whether they may be targets for therapeutics aimed at promoting or regulating axon extension and regeneration.

Duration of Project

1/1/2010 - 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 5: Project Title and Purpose

Development of the Cardiac Ultrasound Pacemaker - There is an unmet need for a technology to reliably and painlessly pace the heart without any surgical implantation or intravascular catheter placement. Our objective is to develop a technology that would provide noninvasive, low infection risk, and reliable pacing for minutes to weeks in conscious patients requiring temporary or emergency pacing. We envision an ultrasound device that could be placed directly on the skin of the chest wall that would function as a pacemaker for the heart.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

We will research and develop a completely non-invasive, fully ambulatory, and safe alternative pacing technology using externally applied ultrasound at FDA approved frequencies and intensities. The applied ultrasound device will be able to initiate periodic myocardial depolarization resulting in cardiac contraction thereby establishing a consistent and reliable pacing rhythm without need for the direct or indirect application of electric energy. In contrast to all other devices on the market, the technology proposed in this development effort is completely extracorporeal; an external, wearable ultrasound pacemaker.

A porcine model will be used for our experiments. The choice of a porcine model is supported by the literature since pigs and humans share similarities in terms of the anatomical heart size, location and position of the heart in the thoracic cavity, and intrinsic heart rate. Moreover, these animals will be available for noninvasive testing at our institution. Porcine heart rhythm will be monitored using surface electrodes and a multi-channel electrocardiogram. An M-mode ultrasound imager will be used to identify a location on the chest wall that provides access to the myocardium with a minimum of obstructions. The external ultrasound probe will be placed on the chest surface with coupling gel. The ultrasound pulses will be synchronized with the native heart rhythm using an electrocardiogram to avoid inducing ventricular arrhythmia. Following FDA guidelines, only ultrasound intensity levels with a spatial peak temporal average of 720mW/cm2 or less will be used for the feasibility studies. For the feasibility studies ultrasound pulses will be delivered for one minute, at a pulse interval approximately 25% below the inherent cycle length, to initiate pacing at 20 beats per minute (bpm) faster than the average underlying heart rhythm of 70bpm. The ultrasound-initiated beats will be identified by the morphology of their QRS complexes and timing relative to the ultrasound pulse. A successful pacing protocol will be defined by a 70-100% capture of the delivered ultrasound pulses for three to five minutes. This will provide data on 270-450 successive stimulations for analysis. The heart rhythm will be monitored with the electrocardiogram for an additional 5 minutes to document whether any residual electrophysiologic effects from the ultrasound are present.

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Expected Research Outcomes and Benefits

Cardiac pacemakers have been in existence for almost 50 years. There are two categories of cardiac pacemakers, temporary and permanent. The most recent statistics indicate there are about 1.3 million temporary cardiac pacemakers used annually in the USA at a total cost of about $3.4 billion dollars. Globally, the figures are much higher. The two current techniques for temporary cardiac pacing involve the surgical use of intravascular catheters and leads or the use of transcutaneous electrical pacing, which is painful and impractical for prolonged utility. These approaches have not significantly changed in the last 40 years and are associated with a myriad of morbidity and mortality outcomes as well as high costs. Complications can include infection, drug toxicity, renal failure due to prolonged use of antibiotics, and death.

Our proposed ultrasound device will operate at intensities that follow current FDA guidelines. It will provide rapidly-deployable, noninvasive, painless pacing for minutes to weeks in conscious patients requiring temporary or emergency pacing. Temporary cardiac pacemakers often represent the first therapeutic approach for the ultimate correction of a serious cardiac problem or malady. Such situations often result from bradyarrhythmias myocardial infarction, drug toxicity, bloodstream infection, during patient transport in emergency situations, or in the aftermath of heart surgery. The medical complications and costs associated with current approaches represent a very poorly met medical need, ripe for improvement both in terms of clinical outcome and cost reduction. As a result of the development of the proposed technology, the need for painful and invasive temporary intravascular pacing catheters or transcutaneous electrical pacers would be reduced or eliminated.

Summary of Research Completed

With our prior lack of success in the porcine model, we decided to reassess our initial assumptions regarding ultrasound and its effects on cells by initiating a series of tissue culture experiments using rat ventricular cardiomyocytes. During this time interval, these tissue culture experiments were expanded and continued. We used the same custom 2.5 MHz high-intensity focused ultrasound (HIFU) probe that was used in the porcine and tissue culture experiments

previously described. In an effort to deliberately attenuate the energy that reached the cardiomyocytes in tissue culture we used a holder for the probe that is setback/spacer from the tissue culture plate, as well as, lower energy parameters. With the information obtained from these experiments in tissue models, we plan to modify our ultrasound parameters in our anticipated rabbit and sheep model experiments planned for 2014-2015.

Additional Tissue Culture Experiments Focused on Analyzing Cardiomyocyte Depolarization Rates

Methods

Neonatal ventricular rat cardiomyocytes were harvested from excised hearts of 1 to 3 day old euthanized Sprague-Dawley rat pups using a cell isolation kit (Cellutron Life Technology, Baltimore, MD). Briefly, cells were isolated from the cardiac tissue of both ventricles. The cardiomyocytes were pre-plated for 1 to 2 h with SureCoat (Cellutron) to purify the cardiomyocytes population from interspersed fibroblasts. The cardiomyocytes were cultured at a density of 7,000 cells/cm2 in high serum medium (10% fetal bovine serum) (Cellutron NS medium) and plated directly on microelectrode arrays (MEAs) embedded with titanium nitrite electrodes for EKG readings. The custom 2.5 MHz high-intensity focused ultrasound (HIFU) probe, used in the previously described experiments, was directed at the cardiomyocytes in a tissue culture model. A piezoelectric metal plate 3cm in diameter was on the exposure end. The transducer was placed in a setback/spacer, which was a water-filled 50ml polypropylene centrifuge tube with a polyethylene pipette attached to the conical end directed at a 45-degree angle over the MEA. There was a continuous fluid column from the surface of the ultrasound probe through the setback/spacer and to the plate filled with medium and monolayer of cardiomyocytes. The pipette tip was inserted into the medium approximately 2-3mm above the monolayer of cardiomyocytes. A function generator, with an amplified signal +50 dB, delivered acoustic energy at variable settings of 0.1, 0.3, 0.5 and 1.0 Vpp, pulse durations of 2, 5 and 10ms, and burst periods of 100, 250 and 300ms. At least five trials were conducted at each setting (180 total trials) with 30s of continuous ultrasound exposure followed by an off interval of 30-60 seconds. Temperature of the medium was monitored throughout the trials. The MEA electrodes were connected to a custom software package that produced recording of continuous electrical activity (action potentials) from pre-specified regions on the surface of the MEA plate. The continuous recordings of cardiomyocyte electrical activity was recorded and subsequently analyzed.

Results

The interval durations (ID) between action potentials were measured throughout the recording period and subsequently analyzed. Prior to ultrasound delivery, the IDs were highly irregular, ID range = 0.3-2.7 s. As ultrasound was delivered in an asynchronous manner, using 0.1 and 0.3 Vpp and PD = 2 and 5 ms, there was suppression/inhibition of cellular depolarization for the first 5-10 s. Then 10-15 s after the start of ultrasound delivery, the depolarization rate increased and demonstrated less interval duration variability (ID=0.88-1.03 s, P value<0.05), even after the ultrasound exposure. The data analysis of this series of experiments is ongoing.

Research Project 6: Project Title and Purpose

Human Lung Progenitor Cells for Pulmonary Tissue Engineering and Regenerative Medicine - The purpose of this interdisciplinary research is to isolate and study human lung-specific somatic progenitor cells. These cells can be used to engineer high-fidelity distal pulmonary constructs (alveolar forming units, AFUs) for studying basic mechanisms of lung development and ultimately, for treating currently incurable lung diseases, such as in newborns (e.g., pulmonary hypoplasias) and/or in adults, (e.g., chronic obstructive pulmonary disease (COPD), such as emphysema). Moreover these AFUs can also lead to a commercializable tissue-engineered product for use as models in accelerated drug discovery (e.g., improved anti-asthmatics), in understanding common lung infections (e.g., pneumonia/tuberculosis), or in toxicity studies (e.g., asbestos/vapor inhalation).

Duration of Project

1/1/2010 - 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 7: Project Title and Purpose

Novel Oligomers Targeting AMPA Receptors as Therapeutics for ALS - In this project we will evaluate the efficacy of compounds we have developed called splice modulating oligomers (SMOs), which dramatically decrease flip/flop levels of targeted GluR AMPA subunits, to improve motor function and prolong lifespan in transgenic ALS mice. SMOs are compounds that function by binding to their target pre-mRNA, and redirecting pre-mRNA splicing by the spiceosomal complex in a predictable fashion. Due to recent improvements in chemistry, SMOs show extraordinary specificity, potency, and resistance to degradation. We propose that our SMOs could ultimately be used clinically as neuroprotective agents for treating amyotrophic lateral sclerosis (ALS), by reducing calcium influx in motor neurons (MNs) and breaking the feedback cycle involved in glutamate-mediated excitotoxicity.

Duration of Project

1/1/2010 - 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 8: Project Title and Purpose

Neuronal Substrates of Cognitive Flexibility - Cognitive flexibility is the capacity to alter behavior to meet unexpected challenges, and is an essential aspect of adaptive behavior. Unfortunately, deficits in this capacity are hallmarks of various psychological and neurodevelopmental disorders including schizophrenia, obsessive compulsive disorder, and autism. Furthering the development of effective treatments for individuals with these disorders requires a more comprehensive understanding of the physiological processes mediating behavioral adaptability. The overarching aim of this project is to develop a novel experimental paradigm to explore the detailed neurophysiological substrates of cognitive flexibility using the laboratory rat as an experimental model.

Duration of Project

1/1/2010 - 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 9: Project Title and Purpose

Microfabricated QLISA Biosensor with Embedded Heating and Mixing Elements – The purpose of this project is to develop a novel, low cost microfabricated poly (methyl methacrylate) (PMMA) Quantum-dot-Linked-ImmunoSorbent-Assay (QLISA) biosensor with embedded mixing elements. The significant advantages of this microfluidic platform with integrated electrokinetic mixing are: *a)* Higher optical detection sensitivity *b)* Improved detection limit *c)* Higher signal to noise ratio *d)* Reduction of assay *e)* Multiuse system - repeated use of the sensor is possible, and *f)* Platform technology – can be used to detect any biomarker. The bioassay platform developed will use only 1-2 microliters of sample and achieve detection sensitivity of picomolar quantities of antigen.

Duration of Project

1/1/2010 - 6/30/2011

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 10: Project Title and Purpose

The Role of miRNAs in Modulating Alzheimer's Disease Risk - We will test the hypothesis that genetic variation in the 3' untranslated region (3'UTR) of the human amyloid precursor protein (APP) gene may modulate the risk of developing Alzheimer's disease by disrupting miRNA mediated regulation APP levels. The genetic basis of Alzheimer's disease (AD) is not fully understood. Identifying polymorphisms that increase risk will allow for early prediction of who may develop disease. This knowledge may also lead to a novel therapeutic approach to treating AD with molecules that act like miRNAs to decrease APP levels.

Duration of Project

1/1/2010 - 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 11: Project Title and Purpose

Suppression of Monocyte Migration by Inducible CCR2 Silencing - The purpose of this work is to develop a new method to intervene in the progression of neurologic disease that often occurs in HIV-1 infected individuals. We will investigate a method designed to reduce the ability of HIV-1 infected immune cells to migrate into the brain. This migration is enhanced by signals released from brain-resident infected cells in a positive feedback loop that we will disrupt using a strategy based on RNA interference. Certain receptors that are present on the surface of immune cells, and that allow these cells to respond to the migratory signals, will be down-regulated specifically in HIV-1 infected cells. If successful, this will lead to therapies designed to reduce the overall inflammatory immune response to infection in the brain and help to curb the progression of neurologic disorders.

Duration of Project

1/1/2010 - 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.