Diseases on Which the Indiana Center for Vascular Biology and Medicine Is Focusing

2023 Update

1. **Peripheral Arterial disease**.
    a. PAD is a greater health care burden than coronary artery disease in the U.S. Approximately 8 million Americans are afflicted with PAD.
    b. Critical limb threatening ischemia, (CLTI), is the most severe form of PAD and is characterized by foot pain and gangrene. CLTI is a significant risk for amputation of the leg and has a quality of life equivalent to terminal cancer.
    c. Surgical interventions have provided excellent results in treating CLTI however up to 30% of patients with CLTI will not be candidates for angioplasty or bypass and the only treatment option for relief of pain or removal of an infected ulcer is amputation. Over 153,000 amputations are performed each year ranking as the 6th costliest operation at 10.6-billion-dollar expense to our health care system.
    d. Diabetic patients are especially at risk for limb amputation due to infection and more extensive hardening of the arteries. The incidence of diabetes and its vascular complications continues to rise each year.
    e. **The American Heart Association has recently noted that amputations due to PAD and diabetes has increased over the past ten years. The AHA issued a policy statement that there needs to be an international effort to reduce amputations by 20% by the year 2030.**
    f. With the support of the CMMRF the ICVBM is addressing the critical need for effective therapies to prevent amputations and has been a recognized international leader in this field.
    g. With research funds we have created a diabetic mouse model of CLTI and we are testing new cell types and preparations
    h. Dr. Stephen Miller, PhD., has discovered that mesenchymal[1] stem cells grown in clusters, called spheroids, are able to regenerate skeletal muscle in the diabetic mice with limb ischemia. More excitingly, we have found that those mice treated with MSC spheroids have complete return of muscle function and limb function. Thus, a discovery that we plan to quickly translate into a clinical trial.
    i. We are collaborating with Dr. David Mooney from Harvard University who has developed a gel material that can envelope the stem cell and then be injected into the muscle of a leg with PAD. In addition, with the Mason's research funds, we have purchased the Buchi Encapsulator. This device encapsulates cells [2]which protects them from getting cleared by the recipient's immune system yet still allows the cells to secrete growth factors that stimulate new blood vessels to grow.
    j. A recent paper in Nature Medicine showed that encapsulated human islet cells when injected into a diabetic mouse survived for 175 days and controlled the mouse's blood sugar.

[1] Mesenchymal refers to the cell type. We could call these bone marrow derived stem cells.
[2] Mesenchymal stem cells but also other cell types, such as beta cells that secrete insulin and can control diabetes.

k. We plan to use this encapsulating shield for our MSC spheroids. If the cells can survive longer, they may be more effective in restoring blood flow to the leg and promote wound healing.
l. What is even more exciting is that the Buchi encapsulator is a sterile system and thus whatever we create can be used in humans in clinical trials.
m. Furthermore, the Buchi device allows us to package other cargo in capsules such as growth factors or genes, that can target diseased tissue.
n. The ICVBM is leading the field in stem cell biology to treat PAD by developing an induced pluripotent[3] stem cell that can directly grow into a functioning blood vessel when injected into muscle. This iPSC mesodermal[4] cell is a discovery of Dr. Mervin Yoder at IU and a close collaborator of the ICVBM. We are currently assessing these iPSCs in our mouse models of limb ischemic and plan to move this novel cell population into a clinical trial.
o. Update: The ICVBM has been awarded a $2.4 million grant to assess the safety and feasibility of using MSCs derived from induced pluripotent stem cells in a Phase I clinical trial that will treat 20 veterans with diabetes and CTLI. This will be the first time iPSC-MSCs will be used to treat cardiovascular disease. .
p. We are now using induced pluripotent stem cells that have been genetically modified to lack expression of what are called major histocompatibility antigens. Because these cells lack these antigens, they can be transplanted into an unrelated patient without the patient's immune system recognizing them and thus they will be more effective as they will last longer.

2. <u>Abdominal Aortic Aneurysms</u>.

a. Abdominal Aortic Aneurysms (AAA) are a pathologic dilatation of the aorta, the main blood vessel from the heart, that leads to rupture and death if untreated.
b. AAA is the 13th leading cause of death in the U.S. but its incidence is probably much higher as it has no symptoms until it ruptures. Of those patients who rupture half die before reaching the hospital, half will die after emergent surgery, so that ruptured AAA has a 75% mortality rate
c. Approximately 200,000 new AAAs are diagnosed each year and surgical repair is indicated only when the AAA grows to a diameter of 5.5 cm. Thus, many patients with smaller AAA's must wait for years before repair and describe it as if having a ticking time bomb in their abdomen.

[3] Pluripotent refers to the ability of these cells to grow into all tissue of the human body. We are able to take a well differentiated adult cell from the skin and revert it back to an embryonic like state that is pluripotent, that is the potential to differentiate into plural/multiple tissue types. Once in this pluripotent state we then have to get the cell to grow or differentiate into a heart cell or brain cell. In this process the pluripotent stem cell has to be directed to turn into a brain cell by culturing with growth factors. So in effect we go from an adult cell back to a fetal cell then forward again to an adult cell that we need. Induced pluripotent stem cells have the same properties of forming new adult cells for treating disease as embryonic cells without the ethical concerns of collecting cells from a viable embryo

[4] "Mesodermal" is a term used to describe this specific induced pluripotent derived cell. It is similar to the term "mesemenchymal" in that it refers generally to cells that make up connective tissue.

d. There are no current medical strategies to prevent or suppress AAA expansion and rupture to avoid surgery
e. At the ICVBM we are near completion of a Phase I clinical trial in which we are treating patients with small AAA with intra-venous infusions of mesenchymal stem cells obtained from a young healthy donor. Patients are randomized to placebo, low dose MSCs, and high dose MSCs. We have enrolled and treated 31 of 36 planned patients. Our preliminary results show a significant reduction of inflammatory cells that are related to AAA development and a significant increase in regulatory T cells that suppress inflammation, reaching the primary endpoint! More excitingly, using positron emission tomography[5] we have shown that MSCs decrease inflammation in the aorta in a dose-dependent fashion and prevent AAA from enlarging.
f. Update: This trial has been completed and the results were analyzed. The trial reached its declared primary endpoint showing a significant increase in the "good" regulatory T-cells and a decrease in the "bad" Th17 cells. Also, the study showed the patients in the high cell dose group had a reduction in aneurysm volume! The study was hoping to show that MSCs merely slowed the growth of the aneurysm but this data suggests that high doses of MSCs may cause the aneurysm to shrink. This is a major breakthrough and the results will be presented in a plenary lecture at the annual conference of the Society of Vascular surgery**.**
g. Based on these results we are designing a larger Phase II multi-center clinical trial.
h. Katherine Leckie, MD has demonstrated that those aortic aneurysms are a result of an auto-immune response to proteins in the aorta, specifically to a protein called elastin. Working with collaborators at Northwestern University we are using state of the art nanoparticle technology that will in essence be a "reverse vaccine" that wilprevent patients from developing this immune response and prevent aneurysms from even starting.
i. Dr. Leckie has also invented a skin test that may identify patients at risk for AAA before the aneurysm actually develops. This will allow treatment to suppress aortic inflammation and prevent the AAA from starting.
j. We recently received a NIH grant with Teresa Zimmers, PhD focusing on muscle wasting and heart disease in patients with abdominal aortic aneurysms. We previously discovered that 50% of patients who had evidence of muscle wasting prior to surgical repair for their aneurysm died at 3 years. In the mouse models of aortic aneurysm, we have developed through Cryptic Masons funds we are testing our hypothesis that the chronic inflammation associated with aneurysm formation leads to heart failure.

3. <u>Cancer</u>.

a. The Indiana Center for Vascular Biology has teamed up with the IU Center for Immunotherapy, based on mutual interests in modulating the patient's immune system to fight disease and also because the ICVBM is building a cell manufacturing facility. The Center for Immunotherapy needs expertise in creating antigen specific T cells to fight cancer, what is known as CAR-T cells. Our group has developed the technology to create

[5] Positron emission tomography is a type of scan that can measure inflammation.

antigen specific regulatory cells to prevent inflammation in cardiovascular disease and we will be working with our colleagues in oncology in creating CAR-T cells.

4. **Diabetes**.[6]
    a. Treating Type I diabetes with islet cell transplants from donors has not been successful as the patient's immune system recognizes the islet cells as foreign and clears the cells from the patient's body. A recent publication in Nature Medicine has shown that encapsulation of human islet cells with aliginate, a hydrogel approved for clinical use, protected the human islet cells from the mouse immune system and corrected the diabetes.
    b. Thanks to CMMRF support the ICVBM was able to purchase the Buchi encapsulation device that can make a clinical grade capsule. We plan to begin encapsulating human islet cells and test their ability to control blood sugar levels in our polygenic diabetic mouse model, the TALLY-HO mouse strain. This genetic diabetic mouse more closely duplicates the human diabetic condition then that used in the Nature Medicine paper. Furthermore, if we are able to demonstrate success, we will be able to move this to a clinical trial in patients as the materials we are using are FDA approved.

5. **Heart failure.**
    a. We participated in the CONCERT-HF trial and the SENECA trials through the Cardiovascular Cell Therapy Research Network, a consortium of 7 centers in the US recognized for their expertise in stem cell research. The SENECA study was a Phase I trial that recruited 60 patients with heart failure after receiving chemotherapy for cancer. Unfortunately, the chemotherapy, besides killing cancer cells, also kills heart muscle cells and at times patients survive their cancer but end up with heart failure. SENECA was designed to assess the effects of mesenchymal stem cells injected into the heart muscle of these patients. The study reached completion and the results show that the stem cells improved all 7 measures of heart function and plans are underway for a larger and more definitive Phase II trial.
    b. The CONCERT-HF trial was designed to assess the ability of stem cells in improving heart function in patients with heart failure after a heart attack. In this study patients underwent a biopsy of their heart muscle and also a bone marrow aspiration. Both tissue samples were sent to the University of Miami where stems cells called c-kit positive cells were grown from the heart muscle and mesenchymal stem cells were grown from the bone marrow. Then the cells were sent back to the respective medical center the combination of c-kit and MSCs were injected into the heart muscle. This

[6] **We have not made new progress on diabetes recently for several reasons. First, the stock of induced pluripotent stem cells from which we create the insulin producing beta cells has been on hold as the National Institute of Health and Rutgers University have had significant issues with folks working in the lab and a shortage of reagents to grow the cells. Also, we have not been able to get the alginate, the material that we use to encapsulate the cells, due to supply line issues. We hope that this will be resolved in the spring.**

study has completed enrollment and all follow-up evaluations. We will be meeting in February to review the results of this exciting study.

c. We are collaborating with Meijing Wang,PhD in cardiothoracic surgery and using our 3D bioprinter to create scaffolds onto which heart cells can be implanted. His "beating" scaffold can then be used to surgically replace parts of the heart that have been damaged from a heart attack.

d. We are working with Phillip Yang, MD at Stanford University to develop new approaches to treating heart failure. Dr. Yang has shown that induced pluripotent heart muscle cells produce small microvesicles that contain mitochondria. Mitochondria are small organelles that are in every cell and they make ATP, which is the energy source[7] for the cell to survive. When these microvesicles are injected into a pig heart with an infart they are able to rescue injured heart cells. We plan to create microbeads with the Buchi encapsulating device that will be loaded with mitochondria and Dr Yang will inject these into his pig model.

6. <u>Stroke.</u>

a. The Veterans Administration Research Consortium has asked the ICVBM to develop new approaches to improving outcomes after a stroke. Key to recovery after a stroke is "rescuing" those brain cells that are injured but not dead. We are working with colleagues in Neurosurgery in a rat stroke model in which we can inject regulatory T cells that we are creating in our lab directly into the injured part of the brain.

7. <u>Arthritis.</u>

a. The Veterans Administration also has called for new innovative approaches in treating arthritis of the knee and hip. We have designed a clinical trial in which patients will be treated with mesenchymal stem cells with injections directly into the knee space. We are awaiting FDA approval and final funding from the VA for this multi-center study.

8. <u>Pancreatitis.</u>

a. Each year 37,000 people are diagnosed with pancreatitis, a debilitating disease associated with significant pain and requiring frequent admissions to the hospital. The Indiana University School of Medicine has one of the largest pancreatitis programs in the U.S. We plan a phase I clinical trial, similar to the aortic aneurysm trial, in which we treat patients with pancreatitis with MSCs.

[7] Adenosine triphosphate (ATP), energy-carrying molecule found in the cells of all living things.