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March 18, 2017

Dear Josh,

I am happy to collaborate with you on your project "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction." Treatment of ischemia-reperfusion injury is a major unsolved program for biomedical research, and your proposed approach represents a novel and very promising idea.

As you know, my lab has worked extensively on vascular regeneration and cardiac repair after myocardial infarction (MI), and thus we have substantial experience with rodent models of MI (*Biomaterials*, 2017 and 2015; *Sci. Adv.* 2016; *J. Controlled Release* 2015). During the collaboration, you'll be working with Daniel Long, who you have met already. Daniel has carried out all of our MI model work since 2016, and has begun training others in the technique. He has also become quite skilled with echocardiography. This collaboration will help you complete your proposed experiments from Aim 2. Also, Daniel and other members of our lab have extensive experience in obtaining rat myocytes for primary culture. We will be happy to add you to our protocol and assist with your proposed cell culture experiments in Sub-Aim 1C.

I should mention that my lab is relocating to Cornell University in late July. We are happy to continue the collaboration after the move. Notably, our location in Ithaca, NY will facilitate your training in how to use pressure-volume (PV) catheters for your proposed experiments in Sub-Aim 2C. Transonic is the leading manufacturer of PV catheters for rodent experiments. At their global headquarters in Ithaca, NY, they host weeklong training seminars on recording PV loops with their devices. PV loops are the gold standard for measuring hemodynamic parameters in the rodent heart, and I expect that you and your proposed work will benefit enormously from the this training opportunity.

I look forward to working with you on this exciting collaboration. As you know, my lab is less than a block away from yours, and you are welcome to stop by my office whenever you would like to discuss progress or find solutions to road blocks. Please feel free to talk to me at any time should you have any questions about myocardial injury and repair, rat models, or treatment approaches for MI.

Sincerely,

Yadong Wang, PhD
William Kepler Whiteford Professor of Bioengineering
University of Pittsburgh



University of Pittsburgh

*Kenneth P. Dietrich School of Arts and Sciences
Department of Chemistry*

March 31, 2017

Joshua S. Wesalo
1314S Chevron Science Center
219 Parkman Avenue
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Stephen G. Weber
*Professor of Chemistry
Director of Graduate Studies
Professor of Clinical Translational Science*

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Dear Josh,

I am happy to serve as a collaborator on your project “Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction.” You have made a strong case for H₂O₂-responsive Nox2 inhibitors based on an extensive evaluation of the clinical and basic science literature. I believe that this approach has significant potential for treating the glaringly unaddressed problem of myocardial ischemia-reperfusion injury.

My group will collaborate with you on your proposed cell culture experiments in Sub-Aim 1C. We have developed techniques to model ischemia-reperfusion in tissue culture by inducing changes in oxygen tension that occur nearly instantaneously. The technique has grown out of my longstanding work devoted to understanding redox biology in living tissue subjected to ischemic injury (see, for example, Wallin et al. 1999, Li et al. 1999, Mitala et al. 2008, Jaquins-Gerstl et al. 2011, Yin et al. 2015, and Yin et al. 2017). Unlike previous approaches, we developed an online oxygenator with microfluidic channels containing a gas-permeable membrane. Applying a vacuum allows for rapid removal of perfusion solution from the tissue chamber. Our system has shortened the time required for tissue oxygen tension to equilibrate from over 12 minutes in the standard setup to below 30 seconds for our system. Consequently, this approach models ischemia-reperfusion much more realistically than previous approaches, as it changes oxygen tension nearly instantaneously. So far, my group has demonstrated this in our model of ischemic stroke, and this collaboration is a great opportunity to expand the scope of our technique to myocardial ischemia-reperfusion. I am excited to collaborate with you and apply our innovative and highly effective superfusion setup to the evaluation of your new approach for treating myocardial reperfusion injury.

My office is a mere 30 second elevator ride from yours, and my door is always open. I am pleased to field any questions you may have. Please note that our group has also extensive experience monitoring cellular redox status using H₂O₂- and GSH-responsive readouts, and we will be happy to answer questions that come up as you use these approaches to complete your proposed experiments. As you know, as the Director of Graduate Studies, I am also happy to help and provide general advice during your graduate training. I am particularly attuned to the unique training requirements you face as an MSTP trainee, having mentored one myself (Amir Faraji, who is currently a PGY4 neurosurgery resident at the University of Pittsburgh and whom you have shadowed). Best wishes as you continue your experimental work, and feel free to stop by any time!

Sincerely,

Stephen G. Weber, PhD
Professor of Chemistry
Director of Graduate Studies
Professor of Clinical Translational Science

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March 25, 2017

Dear Josh,

I would be thrilled to serve as a consultant on your project “Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction.” I’m glad that you’re devoting your research efforts to developing a novel approach for treating reperfusion injury. I feel that you are in a unique position to leverage your skills as a chemist and knowledge of vascular biology for preclinical testing of your approach, and I’m excited to work with you and see your results.

I have been a practicing cardiologist at UPMC for 33 years. I run a busy outpatient general cardiology clinic in addition to directing the inpatient Cardiac Pavilion Service at UPMC Presbyterian, which is our medical center’s flagship hospital. As you know, I have always made teaching a high priority throughout my career, and I have earned six awards for teaching medical students, residents, and fellows. I very much enjoyed having you as a student in my small “problem-based learning” group during your second-year cardiology course. A few years ago, I was lucky enough to get to train an MSTP student, Beth Oczypok, when she did a Longitudinal Clinical Clerkship (LCC) with me as she completed her graduate school training. I think Beth got a lot out of it, and I would be thrilled to take you on as an LCC student. As many of my patients have a history of MI and have undergone reperfusion by thrombolytic therapy or percutaneous intervention, I think it will be a good experience that shows you the clinical aspects of your research. During the LCC, you’ll certainly become familiar with the diagnosis and management of the heart failure, valvular pathology, and electrical abnormalities that arise as sequelae of MI and are driven—at least in part—by reperfusion injury. Additionally, I will make arrangements for you to see the cath lab so that you can see reperfusion in action.

Echocardiography is a major component of my practice, and I have published several articles on the subject. On your LCC, I expect that you’ll gain a lot of experience interpreting echos and will come to understand their role in diagnosis and treatment. Your clinical experience with echocardiography will complement the echocardiographic readings you’ll obtain in your animal experiments.

Please don’t hesitate to contact me to discuss the clinical ramifications of your research as you complete your experiments. As you are learning during your dual-degree training, close cooperation of clinicians and basic scientists has yielded some really exciting results and discoveries over the years, and I’m excited to see where it takes us.

Sincerely,

Frederick W. Crock, MD

Fred Crock, MD
Assistant Professor of Medicine
University of Pittsburgh



University of Pittsburgh

*Kenneth P. Dietrich School of Arts and Sciences
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Kay M. Brummond, PhD
Professor and Chair

April 5, 2017

Joshua Wesalo
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Dear Josh,

I am writing to confirm that the Department of Chemistry supports your F30 proposal entitled "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction." It is excellent to see that you're working on your grant writing skills early-on in your career. Your proposal, in particular, exemplifies the collaborative approach our department takes that leverages our unique position near the medical center to carry out research that would be very difficult to do anywhere else.

I remember meeting you during your second look visit three years ago and was hoping you would come to Pitt. I am thrilled to have more MD/PhD students in the department, since we have a history of successfully training MSTP students. Most recently, Amir Faraji earned his PhD in Steve Weber's group (with whom you're collaborating as I understand it), and is now a PGY4 resident in neurosurgery at our medical center – on track for a career as an academic surgeon-scientist. We also trained Justin Baca, who did his thesis work with Sandy Asher and is now a physician-scientist in the emergency medicine department at the University of New Mexico. You have laid out an excellent training plan and will receive top-notch training working in the Deiters group. Alex is exceptionally well-funded and has established a track record of success in applying for grants from the NIH and from other agencies.

I want to reiterate my enthusiasm for this highly collaborative project that combines medicinal chemistry with vascular biology, and I think the proposed research will help you grow as a scientist. The department pledges its full support. I wish you continued success.

Sincerely,

A handwritten signature in black ink that reads "Kay M. Brummond".

Kay M. Brummond



University of Pittsburgh

School of Medicine
Vascular Medicine Institute

Patrick J. Pagano, PhD
Professor & Vice Chair for Graduate Education,
Pharmacology & Chemical Biology
Director, Molecular Pharmacology Program

March 29, 2017

Dear Josh,

I am pleased to serve as a consultant on NADPH oxidase (Nox) inhibitors and biochemical reactive oxygen species (ROS) assays for your project "Spatially controlled Nox2 inhibition for the targeted treatment of reperfusion injury in myocardial infarction". The treatment approach you are proposing is highly innovative, and represents a promising strategy for inhibiting Nox2, a key producer of harmful ROS in the area-at-risk, without perturbing redox balance elsewhere. The localized inhibition of Nox2 in response to pathologic hydrogen peroxide inhibition will be transformative for the field, as it presents a clever solution to the numerous off-target effects that are observed with systemic Nox2 inhibition.

As you know from your rotation in my laboratory, my group has made significant progress in preparing and testing isoform-specific Nox inhibitors and investigating their therapeutic potential. We were one of three labs to first to identify that Nox was found outside phagocytes (in the vascular wall). Subsequently, we were the first group to publish an isoform-specific Nox inhibitor, and we also were the first to clone p67phox, the activating subunit of Nox2. Our work has led to several fundamental discoveries about the roles specific Nox isoforms play in cardiovascular disease. I have 25 years of experience working on ROS and Nox inhibition and my group has published over 90 papers and reviews in the field. Additionally, Dr. Eugenia Cifuentes from my group co-directs the Free Radical and Reactive Oxygen Species Core Facility. She is an expert in the biochemical assays that you are proposing for measuring ROS production, and will be happy to consult with you on performing the assays and analyzing the data, just as she did when you rotated in the lab.

As you described in your training plan, the Vascular Medicine Institute will serve as a key resource for you, both for completing your experiments and for your training at the intersection of chemistry and vascular biology. I encourage you to take advantage of the VMI's core facilities in Biostatistics and Small Animal Hemodynamics, and I look forward to seeing you at grand rounds and VMI seminars. I am happy to act as your liaison with the VMI.

Please feel free to contact me at any time if you have any questions regarding Nox inhibitors or biochemical assays for ROS production. As you know, my lab and office are just a short walk from your lab, and you're welcome to stop by any time.

Sincerely,

A handwritten signature in black ink that reads "Patrick J. Pagano".

Patrick J. Pagano, PhD, FAHA
Professor and Vice-Chair, Graduate Education, Department of Pharmacology and Chemical Biology
Program Director, Molecular Pharmacology Graduate Program