OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

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NAME: Patrick J Pagano

eRA COMMONS USER NAME (credential, e.g., agency login): ppagano1

POSITION TITLE: Professor, Vice-Chair for Graduate Education
Department of Pharmacology & Chemical Biology
Vascular Medicine Institute
University of Pittsburgh

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Binghamton University-Harpur College, Binghamton, NY.	B.A.	05/1985	Chemistry
New York Medical College, Valhalla, NY.	M.Sc.	05/1988	Pharmacology
New York Medical College, Valhalla NY.	Ph.D.	05/1991	Pharmacology
Boston University Medical Center, Vascular Biology Unit, Boston, MA.	Postdoc.	05/1994	Vascular Biology

A. Personal Statement

A vascular biologist by training, my laboratory explores multiple aspects of cardiovascular cell biology as they relate to tissue dysfunction. The laboratory's primary focus has been the vascular effects of reactive oxygen species. Among the first to identify NADPH oxidase (Nox) in the vasculature, we subsequently cloned phagocyte-like $p67^{phox}$ in cells from the vascular adventitia and demonstrated upregulation of this and catalytic subunits of this complex enzyme in response to the pro-hypertensive hormone angiotensin II. Major research interests of the laboratory include paracrine effects of adventitial Nox-derived reactive oxygen species (ROS) on cell hypertrophy, proliferation and vascular tone dysfunction. My group has had many years of experience in *in vivo* models of systemic and pulmonary hypertension and oxidative stress-induced disease as well as detection of tissue ROS. To elucidate the role of Nox signaling in cardiovascular disease, major emphasis has been placed on novel isoform-specific peptidic and small molecule inhibitor development. Finally, a track record of achievement and extensive collaborative experience over the past 2 decades have afforded me the required skills to serve as a consultant for Josh's project. The truly outstanding environment at the University of Pittsburgh provides myriad opportunities and state-of-the-art facilities serving to greatly enhance the success of the research plan.

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B. Positions and Honors

Positions and Employment

1996-1998 Assistant Professor of Medicine (tenure track), Boston University School of Medicine
1998-2008 Senior Staff investigator, Hypertension & Vasc. Res. Division, Henry Ford Hospital
2003-2008 Director, Vascular Biology Research, Hypertension & Vasc. Res. Division, Henry Ford Hospital
2004-2008 Assistant Professor of Medicine (tenure track), Case Western Reserve University
2004-2008 Associate Professor of Physiology, Wayne State University
2008- Professor, Dept. of Pharmacology & Chemical Biology, Univ. of Pittsburgh School of Medicine
2010- Director, Graduate Program, Molecular Pharmacology. Univ. of Pittsburgh, School of Medicine

2012- Vice Chair, Graduate Education, Dept. of Pharmacology & Chemical Biology, Univ. of Pittsburgh School of Medicine

Other Experience and Professional Memberships

American Heart Association
American Physiological Society
American Association for the Advancement of Science
Society for Free Radical Biology and Medicine (formerly The Oxygen Society)
Inter-American Society of Hypertension (Conference Organizing Committee)

Honors

2011- Standing Member, Hypertension & Microcirculation Study Section, NIH NHLBI
2012- Associate Editor, *Clinical Science*
2015- Editorial Board, *Circulation Research*
2005-2010 Established Investigator Award, American Heart Association
2006- Editorial Board, American Journal of Physiology
2006- Editorial Board, Cardiovascular Research
2002 Invited International Participant, Cold Spring Harbor Laboratory, First International Nox Symposium, "Oxidases in Inflammation and Cellular Signaling" symposium
2001- Fellow, American Heart Association
1996-2001 NIH F.I.R.S.T. Award
1990 Pharmaceutical Manufacturers' Foundation, Advanced Pre-doctoral Fellowship
1991-94 Cardiovascular Training Fellowship, Boston University
1987, 1988 National Science Foundation Honorable Mention
1985 Academic honors, Harpur College, Binghamton University

B. Contributions to Science [84 peer-reviewed publications].

The major focus of the research in the Pagano lab is the study of NADPH oxidases, a complex family of proteins that catalyze the production of reactive oxygen species (ROS). Our research interests have centered on the investigation of the pivotal role this enzyme class plays in physiological cell signaling as well as under pathological conditions. Since the deleterious role of high levels of ROS (a.k.a. oxidative stress) has been described for many disorders in the cardiovascular system as well as in cancer and neurodegenerative disease, great efforts have been dedicated to the development of isoform-specific inhibitors of NADPH oxidases that, in turn, can be used as (a) tools to delineate the role of this class of enzymes in normal and pathological cellular signaling pathways; and (b) therapeutic agents and scaffolds for the development of new drugs.

1. Identification of NADPH oxidase in the vasculature.

NADPH oxidase(s), major source(s) of superoxide in a variety of tissues, was first described in neutrophils as involved in the respiratory burst. The following publications from our group are among the first in the field to describe and characterize Nox in a non-phagocytic cell. These findings constitute the basis of what is now known to be an important proximal signaling pathway and a major culprit in the development of myriad diseases.

1a. Al Ghouleh I, Meijles DN, Mutchler S, Zhang Q, Sahoo S, Gorelova A, Henrich Amaral J, Rodríguez AI, Mamonova T, Song GJ, Bisello A, Friedman PA, Cifuentes-Pagano ME, **Pagano PJ**. Binding of EBP50 to Nox organizing subunit $p47^{phox}$ is pivotal to cellular reactive species generation and altered vascular phenotype. *Proc Natl Acad Sci U S A.* 2016; 113 (36):E5308-17. PubMed PMID: 27540115, PMCID: PMC5018796

1b. Pagano PJ, Ito Y, Tornheim K, Gallop PM, Tauber AI, Cohen RA. An NADPH oxidase superoxide-generating system in the rabbit aorta. *Am J Physiol.* 1995; 268: H2274-80.PubMed PMID: 7611477

1c. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proc Natl Acad Sci U S A.* 1997, 94(26):14483-8.PubMed PMID: 9405639, PMCID: PMC25029

1d. Pagano PJ, Chanock SJ, Siwik DA, Colucci WS, Clark JK. Angiotensin II induces p67phox mRNA expression and NADPH oxidase superoxide generation in rabbit aortic adventitial fibroblasts. *Hypertension.* 1998; 32(2):331-7. PubMed PMID: 9719063

2. First-in-class Nox inhibitor and development of other isoform-selective Nox inhibitors.

The understanding of the specific role of each Nox isoform in a signaling pathway or pathophysiological process has been hindered by the paucity of specific Nox inhibitors. A major focus of our research has been the development of isoform-specific inhibitors, both peptidic as well as small molecules. Nox2ds-*tat,* was the first Nox inhibitor rationally designed to specifically inhibit Nox2-oxidase activity (**2a, 2b**) and to date is arguably the most widely used not only in the cardiovascular field but also in the study of numerous diseases where Nox2-derived ROS are involved, including neurodegenerative disease and cancer. *Nox2ds-tat* is a peptidic inhibitor designed to mimic the docking sequence on Nox2 that is important for its interaction with $p47^{phox}$ and that contains a short amino acid region corresponding to HIV-tat protein, this provides our inhibitor with the capacity to cross plasma membrane and block subunit assembly and thus superoxide generation. A related yet distinct strategy was used to develop a Nox1-specific inhibitor (**2c**). In this case, the peptide *NoxA1ds* blocks the interaction of Nox1 with NoxA1 necessary for superoxide anion production. In the case of small molecule inhibitors, we have identified two compounds that specifically inhibit Nox2, using high throughput screening, rational design and stringent biochemical assays (**2d**).

2a. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, **Pagano PJ**. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O_2^- and systolic blood pressure in mice. *Circ Res.* 2001;89(5):408-14. PubMed PMID:11532901

2b. Csányi G, Cifuentes-Pagano E, Al Ghouleh I, Ranayhossaini DJ, Egaña L, Lopes LR, Jackson HM, Kelley EE, **Pagano PJ**. Nox2 B-loop peptide, Nox2ds, specifically inhibits the NADPH oxidase Nox2. *Free Radic Biol Med.* 2011; 51(6):1116-25. PubMed PMID: 21586323, PMCID: PMC3204933

2c. Ranayhossaini DJ, Rodriguez AI, Sahoo S, Chen BB, Mallampalli RK, Kelley EE, Csanyi G, Gladwin MT, Romero G, **Pagano PJ**. Selective recapitulation of conserved and non-conserved regions of putative NOXA1 protein activation domain confers isoform-specific inhibition of Nox1 oxidase and attenuation of endothelial cell migration. *J Biol Chem*. 2013; 288(51):36437-50. PubMed PMID: 24187133, PMCID: PMC3868757

2d. Cifuentes-Pagano E, Saha J, Csányi G, Ghouleh IA, Sahoo S, Rodríguez A, Wipf P, **Pagano PJ**, Skoda EM. Bridged tetrahydroisoquinolines as selective NADPH oxidase 2 (Nox2) inhibitors. *Med Chem Comm.* 2013; 4(7):1085-1092. PubMed PMID: 24466406, PMCID: PMC3897123

3. Paracrine role of the vascular adventitia

In the early days of vascular biology research, the adventitia (the outermost layer of a blood vessel) was essentially ignored for its effect on vascular wall biology. It was largely considered to play a structural role in maintenance of vessel tensile strength and as a matrix from which innervating neurons and the vasa vasorum subserved large vessel function. Our seminal research in this area helped bring to light a paracrine signaling role for the adventitia in vascular homeostasis by demonstrating cross-talk between adventitial NADPH oxidase-derived ROS and remote cellular signaling effecting tone and medial hypertrophy and proliferation.

3a. Di Wang H, Hope S, Du Y, Quinn MT, Cayatte A, **Pagano PJ**, Cohen RA. Paracrine role of adventitial superoxide anion in mediating spontaneous tone of the isolated rat aorta in angiotensin II-induced hypertension. *Hypertension.* 1999; 33(5):1225-32. PubMed PMID: 10334816

3b. Rey FE, Li XC, Carretero OA, Garvin JL, **Pagano PJ**. Perivascular superoxide anion contributes to impairment of endothelium-dependent relaxation: role of $gp91^{phox}$. *Circulation.* 2002; 106(19):2497-502. PubMed PMID: 12417549

3c. Weaver M, Liu J, Pimentel D, Reddy DJ, Harding P, Peterson EL, **Pagano PJ**. Adventitial delivery of dominant-negative $p67^{phox}$ attenuates neointimal hyperplasia of the rat carotid artery. *Am J Physiol, Heart Circ Physiol.* 2006; 290(5): H1933-41. PubMed PMID: 16603705

3d. Cascino T, Csanyi G, Al Ghouleh I, Montezano AC, Touyz RM, Haurani MJ, **Pagano PJ**. Adventitia-derived hydrogen peroxide impairs relaxation of the rat carotid artery via smooth muscle cell p38 mitogen-

activated protein kinase. *Antioxid Redox Signal.* 2011; 15(6):1507-15. PubMed PMID: 21126185, PMCID: PMC3151421

4. Role of NADPH oxidase in cardiovascular diseases

With the discovery of non-phagocytic NADPH oxidase in the vasculature and other tissues and the development of specific inhibitors, our lab has pioneered exploration of the role of NADPH oxidase in cardiopulmonary diseases including systemic and pulmonary hypertension.

4a. Cifuentes ME, Rey FE, Carretero OA, **Pagano PJ**. Upregulation of p67^{phox} and gp91^{phox} in aortas from angiotensin II-infused mice. *Am J Physiol Heart Circ Physiol*. 2000 Nov; 279(5):H2234-40. PubMed PMID: 11045958

4b. Jacobson GM, Dourron HM, Liu J, Carretero OA, Reddy DJ, Andrzejewski T, **Pagano PJ**. Novel NADPH oxidase inhibitor suppresses angioplasty-induced superoxide and neointimal hyperplasia of rat carotid artery. *Circ Res.* 2003; 92(6):637-43. PubMed PMID: 12609967

4c. Liu J, Yang F, Yang XP, Jankowski M, **Pagano PJ**. NADPH oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. *Arterioscler Thromb Vasc Biol.* 2003; 23(5):776-82. PubMed PMID: 12637340

4d. Quesada IM, Lucero A, Amaya C, Meijles DN, Cifuentes ME, **Pagano PJ,** Castro C. Selective inactivation of NADPH oxidase 2 causes regression of vascularization and the size and stability of atherosclerotic plaques. *Atherosclerosis*. 2015, 242(2):469-75. PubMed PMID: 26298737, PMCID: PMC4818577

4e. Sahoo S, Meijles DN, Al Ghouleh I, Tandon M, Cifuentes-Pagano E, Sembrat J, Rojas M, Goncharova E, **Pagano PJ .** MEF2C-MYOCD and Leiomodin1 Suppression by miRNA-214 Promotes Smooth Muscle Cell Phenotype Switching in Pulmonary Arterial Hypertension. *PLoS One.* 2016,11(5):e0153780. PubMed PMID: 27144530, PMCID: PMC4856285

5. NADPH oxidase and TSP1

Despite increasing evidence supporting the role of matricellular proteins, such as thrombospondin 1 (TSP1) in vascular disease, their potential to stimulate ROS in vascular tissue and their pathological significance in oxidative stress−mediated vascular dysfunction remained unknown. Data from our group suggested that this protein could act as a ligand for NADPH oxidase activation through the specific engagement of integrin-associated protein CD47. This interaction represents a highly regulated process of ROS stimulation and blood flow regulation promoted through the direct TSP1/CD47-mediated activation of Nox1.

5a. Csányi G, Yao M, Rodríguez AI, Al Ghouleh I, Sharifi-Sanjani M, Frazziano G, Huang X, Kelley EE, Isenberg JS**, Pagano PJ**. Thrombospondin-1 regulates blood flow via CD47 receptor-mediated activation of NADPH oxidase 1. *Arterioscler Thromb Vasc Biol.* 2012 Dec; 32(12):2966-73. PubMed PMID: 23087362, PMCID: PMC4394361

5b. Rogers NM, Sharifi-Sanjani M, Csányi G**, Pagano PJ**, Isenberg JS. Thrombospondin-1and CD47 regulation of cardiac, pulmonary and vascular responses in health and disease. *Matrix Biol*. 2014; 37:92-101. PubMed PMID: 24418252, PMCID: PMC4096433

5c. Yao M, Rogers NM, Csányi G, Rodriguez AI, Ross MA, St Croix C, Knupp H, Novelli EM, Thomson AW, **Pagano PJ**, Isenberg JS. Thrombospondin-1 activation of signal-regulatory protein-α stimulates reactive oxygen species production and promotes renal ischemia reperfusion injury. *J Am Soc Nephrol.* 2014; 25(6):1171-86. PubMed PMID: 24511121, PMCID: PMC4033366

5d. Meijles DN, Sahoo S, Al Ghouleh I, Amaral JH, Bienes-Martinez R, Knupp HE, Attaran S, Sembrat JC, Nouraie SM, Rojas MM, Novelli EM, Gladwin MT, Isenberg JS, Cifuentes-Pagano E, **Pagano PJ**. The matricellular protein TSP1 promotes human and mouse endothelial cell senescence through CD47 and Nox1. *Sci Signal.* 2017 Oct 17;10(501): pii: eaaj1784. PubMed PMID: 29042481, PMCID: PMC5679204

5e. Novelli EM, Little-Ihrig L, Knupp HE, Rogers NM, Yao M, Baust JJ, Meijles D, St Croix CM, Ross MA, **Pagano PJ**, DeVallance ER, Miles G, Potoka KP, Isenberg JS, Gladwin MT. Vascular TSP1-CD47 signaling promotes sickle cell-associated arterial vasculopathy and pulmonary hypertension in mice. *Am J Physiol Lung Cell Mol Physiol.* 2019 Jun 1;316(6):L1150-L1164. PubMed PMID: 30892078, PMCID: PMC6620668

Deleted: **5a.** Bauer EM, Qin Y, Miller TW, Bandle RW, Csanyi G, **Pagano PJ**, Bauer PM, Schnermann J, Roberts DD, Isenberg JS. Thrombospondin-1 supports blood pressure by limiting eNOS activation and endothelial-dependent vasorelaxation. *Cardiovasc Res*. 2010; 88(3):471-81. PubMed PMID: 20610415, PMCID: PMC2972685¶

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Complete List of Published Work:
http://www.ncbi.nlm.nih.gov/sites/myncbi/patrick.pagano.1/collections/47859398/public/

D. Research Support

Ongoing Research Support (Selected)

The proposed research project has no overlap with any of my past, current or pending research support.

R01 HL079207-A1, NIH Pagano (PI) 08/14/2015 – 2/29/2020
Reactive Oxygen Species in Vascular Disease
This project aims to open a new field of inquiry by (a) identifying novel pathways involving pulmonary vascular oxidant production leading to vascular thickening and occlusion; and (b) testing promising therapies aimed at abolishing these pathways and alleviating PH and RV failure. **Role: PI**

T32 GM08424-21 Pagano (PI) 07/01/2015 – 06/30/2020
Predoctoral Training in Pharmacological Sciences
The purpose of this Predoctoral Training Program is to provide a broad education in areas relevant to the pharmacological sciences and to train students in the techniques, strategies and philosophy of modern biological research.
Role: PI

Completed Research Support (Past 3 years)

R01 HL112914, NIH Pagano/Isenberg (PIs) 8/13/13- 5/31/17
TSP-1 and ROS: CD47 and SIRP-alpha as Mediators of Vascular Dysfunction
This project focuses on the novel activation of NADPH oxidases by thrombospondin1 (TSP1) in vascular smooth muscle and endothelial cells via CD47 and/or SIRP-alpha. The first and second aims characterize the mechanisms by which CD47- and SIRP-alpha mediate cellular ROS production and dysfunction via Nox1 and Nox4, respectively. **Role: Co-PI**

P01 HL103455, NIH Gladwin (PI) 04/01/11 – 03/31/16
Vascular Subphenotypes of Lung Disease
Project #2 – ROS signaling and NOS uncoupling in Pulmonary Vascular Disease
We hypothesize that sub-phenotypes of common diseases, including pulmonary arterial hypertension (PAH), have a profound influence on outcome and responsiveness to therapy. The overarching translational goal of this program is to define common mechanistic and therapeutic pathways for PAH in the context of major lung and systemic diseases, such as COPD and HIV. Our proposed three major projects and two cores, are designed to integrate and synergize fundamental translational research addressing major current and high impact problems in the PAH and advanced lung disease field.
Role: Project Investigator – Project 2

R01 HL079207, NIH/NHLBI Pagano (PI) 04/01/07 – 03/31/13
Reactive Oxygen Species in Vascular Disease
This grant focuses on the development of specific isoforms of major vascular NADPH oxidase isoforms (Nox1 and 4) and applies them in the characterization of cyclic stretch induced reactive oxygen species production with respect to balloon angioplasty-induced neointimal proliferation. In essence, this grant is expected to develop novel Nox inhibitors to act as conditional suppressors of individual Nox proteins in the process leading to neointimal growth.
Role: PI

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TSP-1 and ROS: CD47 and SIRP-alpha as Mediators of Vascular Dysfunction ¶
This project focuses on the novel activation of NADPH oxidases by thrombospondin1 (TSP1) in vascular smooth muscle and endothelial cells via CD47 and/or SIRP-alpha. The first and second aims characterize the mechanisms by which CD47- and SIRP-alpha mediate cellular ROS production and dysfunction via Nox1 and Nox4, respectively. **Role: Co-PI**¶
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This project focuses on the novel activation of NADPH oxidases by thrombospondin1 (TSP1) in vascular smooth muscle and endothelial cells via CD47 and/or SIRP-alpha. The first and second aims characterize the mechanisms by which CD47- and SIRP-alpha mediate cellular ROS production and dysfunction via Nox1 and Nox4, respectively. **Role: Co-PI**¶ ... [1]
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Vascular Subphenotypes of Lung Disease¶
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Role: Project Investigator – Project 2¶
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