Biomedical research is the engine that drives new discoveries that will improve how human diseases are diagnosed and treated. The U.S. has been a world leader in biomedical research, but stagnant funding levels for federal research grants threaten this leadership position. This trend means that important research does not get done and slows the pace of discovery. Despite these challenges, Center for Molecular Medicine (CMM) investigators remain competitive for scarce research dollars. CMM investigator Don Wojchowski was awarded a renewal of an NIH R01 grant to continue his important work on red blood cell formation, and Peter Brooks was awarded a Department of Defense grant to study ovarian cancer. Another CMM investigator, Leif Oxburgh received a grant from the Maine Cancer Foundation to study renal cancer, and Calvin Vary, also a CMM investigator, received a grant from the American Heart Association to study cell signaling mechanisms in blood vessel development. CMM graduate student Sarah Peterson received a predoctoral fellowship award from the American Heart Association, and her mentor, Lucy Liaw, renewed her NIH R01 that supports the laboratory research focus on vascular remodeling. These grants will ensure that these investigators are able to continue their cutting edge research and make new discoveries that will ultimately benefit patients in Maine as well as across the country.

Even in austere times, it is important to bring new people into CMM to foster collaboration and new discovery. CMM was delighted to welcome Dr. Douglas Sawyer, the new Chief of Cardiovascular Services at MMC, and his colleagues Dr. Sergey Ryzhov, Dr. Oleg Tikhomirov, and Ricky Rath. Dr. Sawyer and his colleagues will be doing research on heart failure and new approaches to treat this disease. CMM also welcomes Dr. Michaela Reagan, a new principal investigator who will be doing research on multiple myeloma and bone. These new research programs and investigators will synergize with existing programs in CMM and strengthen our research enterprise. Thus, despite a challenging funding environment, CMM’s successes and growth in 2014 give us hope for an even better 2015.

We welcome Ricky Rath, Dr. Sergey Ryzhov, and Dr. Douglas Sawyer to Maine Medical Center Research Institute

Celebrating Success and Growth
Calvin Vary, Ph.D.

Project Goal: Identify the functions of endoglin and related signaling pathways in the pathogenesis of vascular disease

Grant Support: American Heart Association Grant-in-Aid

Dr. Calvin Vary is a Principal Investigator with an interest in TGFβ signaling pathways and their alterations in human diseases, including vascular disease and cancer. Endoglin is a co-receptor for TGFβ signaling, and mutation of this gene in humans leads to vascular disease. This past year, a project to understand endoglin interaction with Notch signaling was funded by the American Heart Association. Calvin shares insight into his project and life as a researcher.

Q: Why are you passionate about coming to the lab every day?

A: I enjoy the pursuit of knowledge in general, but especially in attempting to understand disease mechanisms at the molecular level. Most of all, science is a social enterprise and I feel most fulfilled when I can leverage my experience and skills to advance the research of my colleagues, the goals of our institution, and the aspiration of helping understand human disease.

Q: What advice do you have for those interested exploring a career in biomedical research?

A: Pursue a passion in an area of science, but do not become limited in your views of the many possible career path options that build upon scientific inquiry to advance a wide range of medical, economic, and social goals.

Q: How will the results of your research study impact patients with vascular disease?

A: Defects in Notch and endoglin contribute to human vascular diseases. Deficiency in Notch signaling leads cardiovascular problems including aortic valve disease, while endoglin deficiency leads to hereditary hemorrhagic telangiectasia (HHT), which affects 1/5000 people. Our study addresses the relationship between Notch and endoglin in terms of how these molecular signaling pathways interact to promote maturation and quiescence of endothelial cells. Our work will lead us to a better understanding of the major question of how to restore normal blood vessel integrity.

Loss of endoglin in the mouse embryo leads to defects in blood vessel development. A normal embryo is shown on the left, with the arrow pointing to a well developed blood vessel. The embryo on the right has a mutation in the endoglin gene, and has enlarged, abnormally formed vessels. These models help understand genes involved in cardiovascular disease.
Sarah Peterson, M.D.

Project Goal: Understand Notch signaling in blood vessel disease

Grant Support: American Heart Association

Sarah Peterson is a graduate student in the University of Maine Graduate School of Biomedical Sciences and Engineering, working towards her Ph.D. degree. Sarah and her mentor, Lucy Liaw, are interested in the role of the smooth muscle cell layer of blood vessels and how these cells contribute to vascular diseases, such as coronary artery disease and restenosis. Lucy Liaw also received an NIH award in 2014 in this area. Sarah talks about her experiences.

Q: How does your medical training help you with your research project?

A: Exposure to the clinical side of cardiovascular care during my medical training guided my decision to focus on a vessel remodeling research project. My project involves understanding why blood vessel blockage occurs and if there are ways to focus on prevention or designing new treatments. A strong background in anatomy and the opportunity to complete a vascular surgery rotation have both been helpful components of my prior medical training that are directly applicable to this project.

Even on a day to day basis, there are many practical ways in which my medical training helps me in my research position. As a medical student, assisting in an operating room during surgical procedures teaches mastery of the principles of aseptic technique and the consequences of breaching the sterile field. These same principles are applicable when growing human cells or when performing surgery in mice. A second practical aspect of medical training includes planning ahead to have all of the items needed for a task prior to initiating it. One of the first times I performed a lumbar puncture on my own, I had the experience of realizing that I needed an additional item after I had already donned sterile gloves and established a sterile field. I think about this experience every time I make a mental checklist of the items I anticipate needing prior to starting a mouse surgery. There are also advantages to the broad exposure to different fields of medicine. Science is becoming more interdisciplinary and collaborative, so it helps to have a lot of experience effectively communicating with people across specialties and across differing levels of education and expertise.

continued on next page
Q: How does Maine Medical Center Research Institute support your career goals?

A: MMCRI is very supportive of its trainees. We have many opportunities for networking, for taking specialized courses, and for professional development. The Research Fellows Association is supported by the institute and provides a chance for trainees to develop leadership skills and be actively involved in the continual improvement of the training environment. The emphasis on creating and maintaining individual development plans ensures that trainees are engaged in a plan that meets their short and long term professional goals.

Q: What advice do you have for a student who may be thinking about a career in biomedical research?

A: It is never too early to initiate gaining research laboratory experience. I first started volunteering at the Foundation for Blood Research when I was in high school. This helped me make the connections necessary to arrange a summer internship and a senior project there. These early experiences strengthened my application for participation in the MMCRI summer research program in 2000. I am grateful to MMCRI for investing time and resources in the development of high school outreach programs and undergraduate summer research programs. These types of programs provide a pathway for Maine students to obtain the early exposure necessary for successfully pursuing careers in science and medicine. For those students already involved in research, my best advice is to keep an open mind about where your career may take you. Even if you are sure you wish to eventually pursue a career in medicine, laboratory research experience will open doors at many different stages of medical training.

Supporting biomedical research training in Maine

Maine Medical Center Research Institute is a cooperating partner of the Graduate School of Biomedical Sciences and Engineering (GSBSE) at University of Maine, Orono. This statewide Ph.D. program supports training in the biomedical sciences and engineering, and consists of partners at research institutes and universities across the state.

Our faculty scientists in the Center for Molecular Medicine are also graduate faculty in the Sackler School of Graduate Biomedical Sciences at Tufts University. This collaboration allows Tufts Ph.D. students to perform their thesis research at Maine Medical Center Research Institute.

Commencement at University of Maine, Orono. Left to right: David Neivandt (UMaine), Director of GSBSE; Pradeep Sathyanarayana, mentor of Melanie Ufkin; Calvin Vary, mentor of Kira Young; Rachel Kennedy, and Justin Guay (mentored by Leif Oxburgh, not pictured). Melanie, Kira, and Justin completed their Ph.D. thesis work at Maine Medical Center Research Institute and graduated in May 2014.
Peter Brooks, Ph.D.

Project Goal: Study the tumor environment of ovarian cancer and use a novel therapeutic to treat ovarian cancer in pre-clinical studies

Grant Support: Department of Defense

Peter Brooks is a Principal Investigator studying cancer biology and how the tumor cell environment regulates tumor aggressiveness. The Brooks laboratory discovered that biochemical changes in the structure of collagen, which surrounds tumor cells, will affect tumor cell growth and metastasis. His team has developed antibodies that are being used as potential cancer-inhibiting therapies. This year, the Department of Defense funded this project to study an antibody therapy and its effects on ovarian cancer.

An antibody recognizing remodeled collagen was used to stain tissues with green fluorescence. The top panel is a benign ovarian granuloma, and the bottom is a malignant ovarian tumor. The ovarian cancer has about 10-fold more of the biochemically altered collagen compared to the non-cancerous tissue.

NEWLY FUNDED PROJECTS IN 2014

Peter Brooks (below) works with UMaine Ph.D. student Jacquelyn Ames to understand the tumor microenvironment and impact on cancer progression. Jacquelyn is part of the Graduate School of Biomedical Sciences and Engineering and is completing her thesis research in the Brooks laboratory.

Tumors are embedded within a mesh-like network containing collagen. These collagen molecules form the “soil” of the tumor, and can provide important cues for cancer cell growth and survival.
Leif Oxburgh, D.V.M., Ph.D.

Project Goal: Uncover the role of the gene FOXD1 in blood vessel recruitment in renal cell carcinoma

Grant Support: Maine Cancer Foundation

Leif Oxburgh is a Principal Investigator with expertise in kidney development and regeneration. His research on a gene required for embryonic kidney development, FOXD1, led to the finding that FOXD1 regulates blood vessel recruitment, with potential application to understanding kidney cancer. Leif discusses the impact of this project.

Q: How will your research findings impact patients with renal cell carcinoma?

A: Our working hypothesis is that the level of FOXD1 in a tumor relates to tumor aggressiveness. We are also investigating the possibility that FOXD1 expression level is a predictor of a tumor’s response to pharmacological therapy. The aim of the project is to understand if FOXD1 could act as a diagnostic biomarker, providing early guidance in the choice of therapy for clear cell renal cell carcinoma.

Q: How did you get started on this project?

A: We have been interested in FOXD1 for many years because it is essential for blood vessel development in the embryonic kidney. Developmental mechanisms are frequently re-activated in cancer and for that reason we decided to study the role of FOXD1 in clear cell renal cell carcinoma, which is a very blood vessel rich tumor.

Q: What are some of the most exciting things about life as a researcher?

A: The ability to spend time understanding basic biological processes. Mapping these processes in cancer has provided new therapy targets and important biomarkers that have dramatically increased life expectancies for patients with many different forms of cancer.

Clear cell renal cell carcinoma (left panel) often arises within an otherwise healthy kidney. The Oxburgh lab has shown that FOXD1 expression in tumor tissue associates with poor clinical outcome in individuals with clear cell renal cell carcinoma. Expression of FOXD1 (dark brown stain) in tumor cell nuclei (red arrows) is shown on the right. Study performed by Jennifer Fetting, a Staff Scientist working in the Oxburgh lab.

Photo of Leif Oxburgh courtesy of the Maine Cancer Foundation. Visit mainecancer.org for more information about their programs.
In healthy individuals, tissue oxygenation depends upon the ability of bone marrow progenitor cells to give rise daily to ~200 billion hemoglobinized red blood cells. When this process is hindered, resulting anemia compromises health, especially among individuals with chronic renal disease, those receiving chemotherapy, and those with mutations in genes encoding (hemo)globin. Physiological stress is also exerted systemically, and within red blood cell progenitors. This is particularly the case for sickle cell disease and thalassemia patients with mutations that disrupt hemoglobin formation. Harmful cellular oxidation events also occur due to elevated levels of reactive oxygen species (ROS).

Don Wojchowski, Ph.D.
Project Goal: Understand how to protect and repopulate red blood cells following anemia
Grant Support: National Institutes of Health

Don Wojchowski is a Principal Investigator with expertise in the development and function of blood cells. The Wojchowski laboratory has identified a novel way in which erythroblasts, the progenitors of red blood cells, are damaged by reactive oxygen species, and also discovered a candidate cytoprotective therapy. In particular, erythroblast lysosomes have proven to be the weakest link, and when damaged by reactive oxygen species, leach executioner cathepsins (especially cathepsins B and L). Normally, such cathepsins function within compartmentalized lysosomes to degrade and/or recycle proteins. When leached, these cathepsins can mis-identify target proteins, and become cytotoxic.

The Wojchowski group has discovered that the anti-anemia agent erythropoietin (EPO) induces the expression of a Spi2A serpin that inhibits leached cathepsins B and L. Beyond this, Spi2A's cytoprotective effects can be mimicked by a small molecule cathepsin inhibitor. Promise therefore exists for the clinical use of such inhibitors to increase the survival of red blood cell progenitors, lessen anemia among patients with (hemo)globin mutations, and potentially lessen high-cost EPO dosing in additional anemia contexts.

When wild-type erythroblasts are challenged by ROS (+ ROS), lysosomal Lamp1 staining is heightened (left panels). Spi2A-deficient erythroblasts in contrast are destroyed or highly damaged by ROS as indicated by their lysis, or markedly increased lysosomal Lamp1 staining (right panels). Images were obtained using the confocal microscopy facility at Maine Medical Center Research Institute, with consultation from Igor Prudovsky.
NEW APPOINTMENT IN 2014

Clifford Rosen, M.D.
Editor: New England Journal of Medicine

The New England Journal of Medicine (NEJM) is a prestigious journal covering the latest findings in medical research over a broad spectrum of specialties. The cutting edge science highlighted in this journal often appears in the New York Times, and NEJM is considered the “New York Times of Medicine”. In 2014, Clifford Rosen was appointed to the editorial board to help evaluate and decide which papers are published in the journal. The journal receives over 50,000 submissions a year and publishes about 200/year. The peer review and editorial process is in place to ensure the highest impact, rigorously performed science is brought to the community. Cliff discusses his editorial role.

Q: How are editors chosen for NEJM?
A: Editors are clinical scientists who are at the top of their field in a broad scope of medical areas. My specialty is endocrinology, and editors of the journal cover a wide spectrum of medicine, including oncology, cardiology, nephrology, epidemiology, biostatistics, and internal medicine.

Q: How does your editorial role help you as a researcher?
A: As an editor for NEJM, I see the best science in real time. I was surprised to see how much basic science is incorporated into the best clinical studies. New discoveries, new therapeutics, and cutting edge science come up on a weekly basis, and help me in my own research program.

Q: What advice do you have for researchers seeking to publish in top medical journals?
A: It is not enough to just have a good research finding – the science must have clinical impact, it must be groundbreaking science, and be adequately powered with an appropriate statistical approach. A common problem with clinical studies is that analysis of participant number isn’t performed prior to initiation, and the study ends up being underpowered and difficult to interpret.

A recent issue of NEJM covers diverse topics including therapies to reduce relapse in breast cancer patients and multiple myeloma patients, a vaccine for Dengue, certification of physicians, Ebola virus in West Africa, population health, and complications during pregnancy in kidney donors.
Bioenergetics during calvarial osteoblast differentiation reflect strain differences in bone mass. Guntur AR, Le PT, Farber CR, Rosen CJ. *Endocrinology*

Regulation of the follistatin gene by RSPO-LGR4 signaling via activation of the WNT/β-catenin pathway in skeletal myogenesis. Han XH, Jin YR, Tan L, Kosciuk T, Lee JS, Yoon JK. *Molecular and Cellular Biology*


The 51-Deiodinases are not essential for the fasting-induced decrease in circulating thyroid hormone levels in male mice: Possible roles for the type 3 deiodinase and tissue sequestration of hormone. Galton VA, Hernandez A, St Germain DL. *Endocrinology*

Regulatory interplay between microRNAs and Snail family proteins in cancer. Chen, Y., and Gridley T. *OA Biology*

Cryptic collagen IV promotes cell migration and adhesion in myeloid leukemia. Favreau AJ, Vary CP, Brooks PC, Sathyanarayana P. *Cancer Medicine*

miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Ufkin ML, Peterson S, Yang X, Driscoll H, Duarte C, Sathyanarayana P. *Leukemia Research*

Effect of sustained postnatal systemic inflammation on hippocampal volume and function in juvenile mice. Malaeb S, Davis J, Pinz I, Newman J, Dammann O, Rios M. *Pediatric Research*


Erythroid mRNA processing: a “splice of life”. Wojchowski D. *Blood*

Overview of genetic tools and techniques to study notch signaling in mice. Gridley T, Groves, AK. *Methods in Molecular Biology*

Lightening up a notch: Notch regulation of energy metabolism. Gridley T, Kajimura S. *Nature Medicine*


Marrow fat composition in anorexia nervosa. Bredella MA, Fazeli PK, Daley SM, Miller KK, Rosen CJ, Klubanski A, Torriani M. *Bone*

Life without the iodothyronine deiodinases. Anne Aalton V, de Waard E, Parlow AF, St. Germain DL, Hernandez A. *Endocrinology*

Elevated plasma levels of the pituitary hormone cthrc1 in individuals with red hair but not in patients with solid tumors. Duarte CW, Stohn JP, Wang Q, Emery IF, Prueser A, Lindner V. *PLoS One*


Inhibition of tumor-associated αvβ3 integrin regulates the angiogenic switch by enhancing expression of IGFBP-4 leading to reduced melanoma growth and angiogenesis in vivo. Contois LW, Akalu A, Caron JM, Tweedie E, Cretu A, Henderson T, Liaw L, Friesel R, Vary C, Brooks PC. *Angiogenesis*

Genomic imprinting variations in the mouse type 3 deiodinase gene between tissues and brain regions. Martinez ME, Charalambous M, Saferali A, Fiering SN, Naoumova A, St. Germain DL, Ferguson-Smith AC and Hernandez A. Molecular Endocrinology

Diet and gene interactions influence the skeletal response to polyunsaturated fatty acids. Bonnet N, Somm E, Rosen CJ. Bone

Computational biophysical, biochemical, and evolutionary signature of human R-spondin family proteins, the member of canonical Wnt/β-catenin signaling pathway. Sharma AR, Chakraborty C, Lee SS, Sharma G, Yoon JK, George Priya Doss C, Song DK, Nam JS. Biomedical Research International


Rationale and design of the vitamin D and type 2 diabetes (d2d) study: a diabetes prevention trial. Pittas AG, Dawson-Hughes B, Sheehan PR, Rosen CJ, Ware JH, Knowler WC, Staten MA; D2d Research Group. Diabetes Care


The Snail transcription factor regulates the numbers of neural precursor cells and newborn neurons throughout mammalian life. Zander MA, Cancino GI, Gridley T, Kaplan DR, Miller FD. PLoS One


Notch signal integration in the vasculature during remodeling. Rostama B, Peterson SM, Vary C, Liaw L. Vascular Pharmacology

3,5-Diiodo-L-Thyronine in Dietary Supplements: What are the Physiological Effects? Hernandez A. Endocrinology

Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. Cawthorn WP, Scheller EL, Learman BS, Pardee SD, Simon BR, Mori H, Ning X, Bree AJ, Schell B, Broome DT, Soliman SS, DelProposto JL, Lumeng CN, Mitra A, Pandit SV, Gallagher KA, Miller JD, Krishnan V, Hui SK, Bredella MA, Fazeli PK, Klibanski A, Horowitz MC, Rosen CJ, MacDougald OA. Cell Metabolism

Deficiency of Sef in mice is associated with increased postnatal cortical bone mass by regulating Runx2 activity. He Q, Yang X, Gong Y, Kovalenko D, Canalis E, Rosen CJ, Friesel RE. Journal of Bone and Mineral Research


Preadipocytes differentiated into adipocytes. Cells are stained with oil red O to reveal red lipid droplets. Experiment performed by Kevin Kennedy, an undergraduate research intern from the University of Southern Maine, and Igor Prudovsky.

Cthrc1 protein staining (brown) in the paraventricular nucleus of the hypothalamus. Experiment performed by Patrizia Stohn, postdoctoral fellow, and Volkhard Lindner.

Photo Gallery

Embryonic mouse kidney stained with the epithelial marker Troma-I (below). Left panel shows a confocal micrograph of the collecting duct tree. Right panel shows a volume rendering based on 3D reconstruction of serial confocal micrographs captured in multiple planes. Collecting duct tree structure determines the amount of functional kidney tissue at birth and using this method, changes in branching of the collecting duct tree during the embryonic period can be visualized and studied. Study performed by Sarah McCarthy, UMaine Ph.D. student in the Oxburgh lab.
“The MMCRI educational outreach programs are invaluable to teachers as they try to expose their students to medical and health related fields and make learning more relevant.”

~Judy Stanhope, Scarborough High School educator

Science Education

In 2014, Maine Medical Center Research Institute hosted:

- **8** job shadows from 4 high schools for a total of 77h
- **25** academic interns from 8 high schools and 8 universities for a total of 6,497h research training
- **2** international research scholars from medical schools in China, for a total of 1600h research

Our graduate students and postdoctoral fellows presented at a career day for high school students to discuss careers in the biomedical sciences.

Representatives from MMCRI attended the meeting of the Scarborough Council on Business and Education, and have become standing members of the group, which aims to connect students from Scarborough schools with Scarborough businesses to promote opportunities for the students to gain hands-on experience in career fields of interest to them.

Armie Mangoba, the Manager of our Histopathology Core Facility, demonstrates how tissue specimens from biopsies are processed and stained to allow for microscopic viewing of the tissue structure and analysis of tissue pathology.

High school student and teacher participants in our biomedical science program. This year, our program has hosted ten area high schools: Scarborough, Casco Bay, Deering, Portland, Cape Elizabeth, Greater Portland Christian, South Portland, Waynflete, Catherine McAuley, and Westbrook.