# Sanguine Manner

By Laura Stephenson Carter and Sion E. Rogers

**A better understanding of the process by which the body grows new blood vessels—known as angiogenesis—holds promise for treating a broad range of diseases. Dartmouth's multidisciplinary angiogenesis research group is optimistic about the future of the field. And the team has fun working together in the meantime.**

The members of the Angiogenesis Research Center don't just work together—they tango, hike, and joke around together. Director Mike Simons is in red in the middle.

"Feed me. Feed me. Feed me!" demands the man-eating plant in the Broadway musical *Little Shop of Horrors*.

"Feed me. Feed me. Feed me!" demands the malignant tumor—silently—in the body of a real-life cancer patient.

Both the plant and the tumor get what they want, but the tumor is more subtle in its approach. It tricks its host's body into growing new blood vessels, a process that's called angiogenesis, to keep it continuously fed with oxygen and nutrients.

Medical researchers and clinicians all over the world are discovering the secrets to controlling angiogenesis and hope to, one day, develop therapies to counteract the over- or under-stimulation of blood-vessel growth. In a healthy person, angiogenesis is a natural process whereby new blood vessels form to heal wounds, rebuild the uterine lining during a woman's menstrual cycle, or develop a circulatory system in a growing fetus.

But sometimes the body begins to build too many new blood vessels—such as in cancer, chronic and acute inflammatory diseases like rheumatoid arthritis, diabetic retinopathy, age-related macular degeneration, psoriasis, and countless other diseases. Or, in other cases, too few—such as in coronary artery disease, stroke, peripheral artery disease, and diabetic wound healing. (Some of these medical terms, plus many others in this article, are defined in a glossary on page 53.)

Dartmouth's Angiogenesis Research Center is led by one of the pioneers in the field—Michael Simons, M.D., who came to DHMC from Harvard in 2001 as chief of the Section of Cardiology. He brought with him a merry band of researchers and has added a few more since then. They are as apt to be teasing each other as they are to be teasing apart the mysteries of angiogenesis—but the lightheartedness is all in service of their work.

It's 9:00 a.m. on a Tuesday morning, and some 30 angiogenesis researchers have gathered in a conference room at DHMC for their weekly joint lab meeting. Each week, a different researcher presents a report on work in progress.

Before the presentation begins, people help themselves to coffee and bagels and chat noisily—about work, weekend activities, whatever else is on their minds. The proceedings are a little late getting under way because the PowerPoint projector is misbehaving—its bulb is flickering, causing the image on the screen to pulse. Someone jokes that the machine is having a seizure. Everyone laughs.

As the presenter fiddles nervously with the projector, Nicholas Shworak, M.D., Ph.D., walks over and places his hand on it. The machine calms down until he steps away. He places his hand on it again, and it behaves. The room rocks with laughter. Finally the projector is working properly and the presenter begins. But later it fails to produce a sound effect to accompany an animation. The presenter looks rattled. "What kind of sound do you want? Beeeep, beeeep?" Shworak jokes. Everyone laughs. The young researcher visibly relaxes and continues his presentation. People start asking questions—some serious and some not.

Shworak poses some gentle, probing questions and suggests that in the future the researcher might want to present his data in a graphical format, such as a histogram or bar chart. Simons wonders about the corkscrew-shaped blood vessels on one of the slides. Often, new blood vessels grow in a screw-like configuration. "Is the corkscrew always to the right?" he asks. Simons jokes around as much as anyone, but he wants to be sure the presenter knows he really is interested in an answer. "This is a serious question," he adds.

Later, when faculty member Armin Helisch, M.D., explains that an image on the screen shows a mouse hind leg where a double ligation had been done on a blood vessel, someone jokes, "Is that double-oh-seven?"

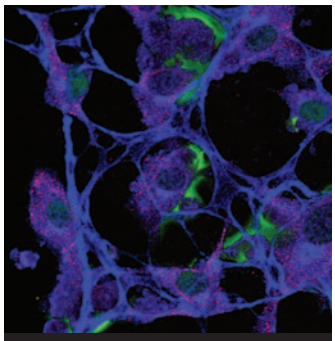
And so the presentation continues with similar give and take—good-natured teasing mixed in with serious questions and answers.

"Very nice," Simons says at the conclusion of the presentation.

"We're trying to understand how the vasculature grows and develops, with an idea that once you understand how blood vessels form, how they grow, you can influence the vascular process," explains Simons. "You can stimulate [angiogenesis] in people who have insufficient arterial growth"—people with coronary disease or peripheral vascular disease with occlusions in their major arteries. "So instead of doing bypass surgery, instead of doing angioplasty, maybe we can induce growth of the arteries."

While researchers from the cardiovascular section are looking for ways to stimulate angiogenesis, others want to learn how to inhibit the process. "If you inhibit the blood supply to the tumor, the hope

*Laura Carter has been* Dartmouth Medicine*'s associate editor for almost six years. One of her recent initiatives was starting a formal editorial internship, in which Sion Rogers is the second incumbent. He was graduated with a degree in biochemistry and genetics from Great Britain's University of Nottingham and then spent eight months with BBC Radio before coming to the U.S. to gain some print experience with* Dartmouth Medicine*. All of the photographs here are the work of Jon Gilbert Fox, while the micrographs in the upper righthand corner of each spread are courtesy of members of Dartmouth's Angiogenesis Research Center.*



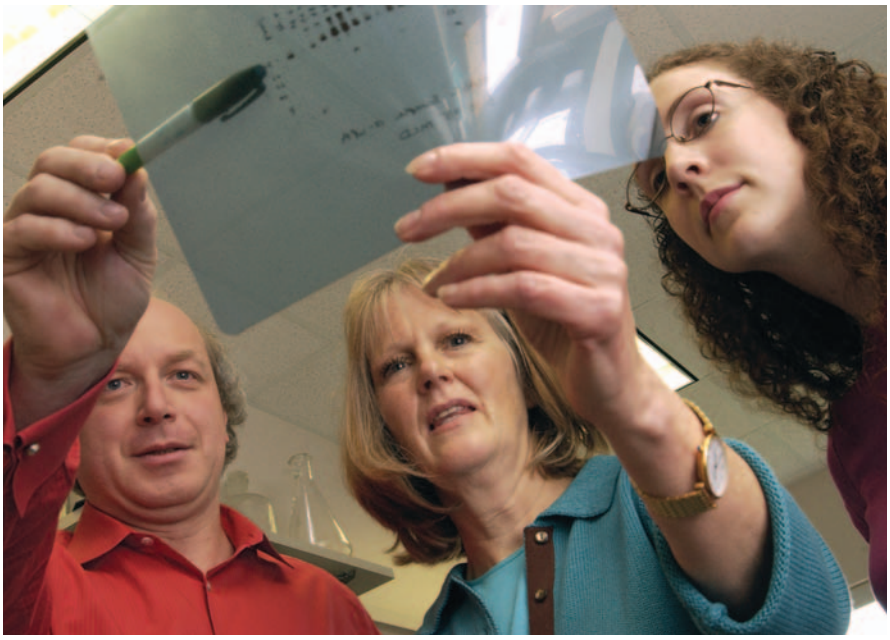
**These are endothelial cells—from the lining of blood vessels—that have been treated with rPAI-123.**

## The Angiogenesis Labs

Each of the labs associated with Dartmouth's Angiogenesis Research Center has its own focus. An army of graduate students, postdoctoral fellows, research assistants, and technicians help to carry out the labs' work, under the direction of the principal investigators listed here:

**Michael Simons, M.D.**
**Professor of Medicine; Cardiology Section Chief; Director, Angiogenesis Research Center**
Studies extracellular matrix and growth factor interactions with endothelial cells in the control of angiogenesis; role of syndecan-4 signaling in regulating endothelial cell adhesion and migration; control of FGF signaling; interactions with other proteins, including PDZ in the signaling cascade; function of a novel family of angiogenic peptides, PR39. His work on novel mechanisms of proteasome-dependent protein degradation has opened a new area of investigation of small molecule-dependent selective regulation of proteasome function.

**Kiflai Bein, Ph.D.**
**Assistant Professor of Medicine (Cardiology)**
Studies molecular basis of capillary-tube and blood-vessel formation; role of transforming growth factor-beta signaling.



Above, from the left: Mike Simons, Mary Jo Mulligan-Kehoe, and Jannine Walsh. Right: Armin Helisch, left, and Nicholas Shworak. Below: Lab conversation over lunch.

**Helisch and Simons aren't shy about disagreeing with one another in public. But all the group's scientific disagreements are in the same vein—cheerfully competitive and friendly.**

is the tumor will die," Simons continues. "If you're going to inhibit the active blood supply in an inflamed joint, then inflammation will go away and you will not have an inflamed joint with a destructive disease. The same applies in certain eye diseases as well—for example in macular degeneration, where you have excessive vascularity of the retina. It becomes permeable to all sorts of proteins and you have damage to the eye."

The process of angiogenesis begins when endothelial cells lining the inside of blood vessels are alerted that damaged or diseased tissue is in need of new blood vessels. The tissue releases angiogenic growth factors—VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), and others—that activate endothelial cells in nearby blood vessels. Those cells begin dividing, migrating through tiny openings in the blood vessel wall to adjacent tissue, then forming new blood vessels.

Scientists know that there are at least 20 angiogenic growth factors, about 30 natural angiogenesis inhibitors in the body, and more than 300 other angiogenesis inhibitors. There are many pieces to this puzzle. Once researchers have figured out what triggers and regulates angiogenesis, they can develop therapies to control it. Just last year, the Food and Drug Administration approved an angiogenesis inhibitor, bevacizumab (Avastin), for colorectal cancer, and it is expected to approve more angiogenesis-inhibiting cancer drugs in the near future. In 1997, the FDA approved the first angiogenesis-stimulating drug, becaplermin (Regranex) to treat diabetic foot ulcers. A few other angiogenesis-related therapies were approved in the late 1990s. But scientists have a long way to go before angiogenic therapies are commonplace.

Dartmouth's Angiogenesis Research Center includes basic scientists who are investigating cellular signaling mechanisms and other molecular processes; scientists who do preclinical research; clinicians who develop and test imaging technologies; and clinicians who conduct clinical trials.

"One of the real strengths of the entire group is that we all have different strengths and very different focuses," says Shworak, who works on molecules called heparan sulfate proteoglycans, which control cell signaling in angiogenesis; there are some 30 to 40 stimulators or inhibitors of angiogenesis that seem to be working through these molecules. He also helped to clone syndecan-4, a molecule that Simons's lab works on now. Syndecan is derived from the Greek work *syndein*, which means to bind. "We counterbalance each other's weaknesses," Shworak adds, "and we work a lot together on different projects. It makes for a much stronger approach because we can really be multidimensional in our analysis."

"Research in angiogenesis or collateral arteries really goes back," says Armin Helisch. "The first evidence actually goes back to the 17th century," when an English physician, Dr. Richard Lower, first described preexisting anastomosis, or the existence of connections, among the arteries in the human heart.

The term "angiogenesis" was coined in 1787 by an English surgeon, Dr. John Hunter, to describe new blood vessels growing in reindeer antlers. In 1935, a Boston pathologist described angiogenesis in the placentas of pregnant monkeys. In 1971, the *New England Journal of Medicine* published a theory by a Harvard surgeon, Dr. Judah Folkman, that angiogenesis enabled tumors to grow. And in 1975, Folkman and a colleague discovered the first angiogenesis inhibitor. In the 1980s, Folkman's lab identified a substance that stimulated capillary growth as well as a substance that inhibited it.

"Judah Folkman was like the founding father [of angiogenesis] in some ways," says Simons. "But he

concentrated on anti-angiogenesis for cancer." Research into the cardiovascular implications of angiogenesis started in the 1990s; Simons was then, and remains still, at the forefront of the field.

Simons has assembled a good team of people who are willing collaborators. "There are no proverbial walls between the labs," says cardiology researcher Arie Horowitz, D.Sc., whose lab focuses on intracellular signaling in endothelial cells. In particular, he studies the role of the PDZ protein synectin, the role of synectin-binding factors in regulating cell migration and endocytosis, and directional cues in endothelial cell migration. "There is a lot of collaboration and mutual help."

Researchers share reagents and other chemicals and often ask each other for advice on lab techniques. The lab meetings are meant for constructive criticism; they help keep everyone informed about each other's work. "It's good to be exposed to [other people's projects], both in order to expand my horizons [and to] not be completely channeled or confined in the walls of my own projects," he says.

Researchers were exposed recently to the details of Horowitz's work when he gave a presentation at a vascular biology seminar. This weekly seminar, held every Thursday afternoon, is another forum where researchers can practice their public-speaking skills and share ideas. Sometimes researchers lecture on journal articles and other times principal investigators present their own results when they're getting ready to submit a paper for publication. The tone of these meetings is more subdued than that of the joint lab meetings.

At the joint lab meetings, "the idea is to try to present unpolished, primary data. Then you can critique and evaluate it," says Kiflai Bein, Ph.D., who is investigating the molecular basis of blood cells growing under laboratory conditions. The vascular biology seminar "is supposed to be more for polished studies," he continues. "So when you share it with your colleagues, they serve as the primary critics. This is important, because if, for instance, they don't understand what I'm trying to say, then I can't expect much from outside reviewers."

Mary Jo Mulligan-Kehoe, Ph.D., a cancer researcher, not only understood what Horowitz was trying to say, but she was almost giddy with excitement. Horowitz had explained something that was relevant to her own research with a PAI-1 protein that inhibits angiogenesis *in vivo* for breast cancer. "We've been battling with the signaling cascades that are occurring as a result of endothelial cells being stimulated with my recombinant PAI-1 protein," she says. "But all of a sudden you're sitting there listening to this, and lights come on. . . . I had thought we were doing something to FGF. But it's really going through a different receptor."

With all the investigators concentrating on different aspects of the vascular process, and being so willing to share their knowledge, people are bound to learn from one another.

"It's like a moving kind of conglomerate. It's hard to explain the dynamic interactions which occur," says Armin Helisch, who is interested in the mechanisms of arteriogenesis, the growth of collateral arteries as opposed to capillaries. "I think it's inspiring because by people [being] in the different orientations, we can all learn from each other, expand our horizons, which is better than if everyone only thinks the same thoughts—as was somewhat true in my prior lab, where everything was only collateral growth with a certain focus."

It is to Simons's credit that he even recruited Helisch to join the Angiogenesis Research Center in the first place. Simons believes that new blood vessels form when endothelial cells migrate to an area in response to signals from damaged tissue. But Helisch argues that there are already preexisting blood vessels that remain invisible until they are needed. They are then called into action when a nearby vessel is blocked and the surrounding tissue needs an alternate blood supply.

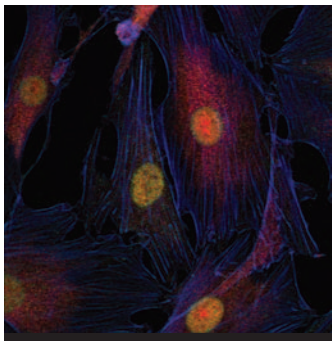
Helisch, an echocardiologist who spends part of his time seeing patients, jokes that he only became a researcher because the angel of research kissed him one day. Then he turns serious as he explains that angiogenesis scientists assumed that new blood vessels were being created in areas injured by cardiovascular disease, because a number of new capillaries formed. "It was kind of a shortcut-thinking approach that this is directly related," he says. "Because physiologically—if a big vessel is occluded—it doesn't really make any sense to assume that small capillaries, in the distal leg, for example, in humans or in an animal hind limb, would be able to have any effect on this restoration of perfusion."

Helisch and Simons aren't shy about disagreeing with one another in public. Once, at a joint lab meeting, Helisch inadvertently says something about collateral vessels having been formed.

Simons pounces. "Did you notice that he used the word collateral *formed*?" he asks gleefully. Everyone laughs. "Someone should mark the calendar," Simons teases.

Helisch is caught off guard but recovers quickly. "Even in arteriogenesis, vessels form based on preexisting collaterals," he insists. All the disagreements are in the same vein—cheerfully competitive and friendly.

"We are a very collegial group, both in the work-

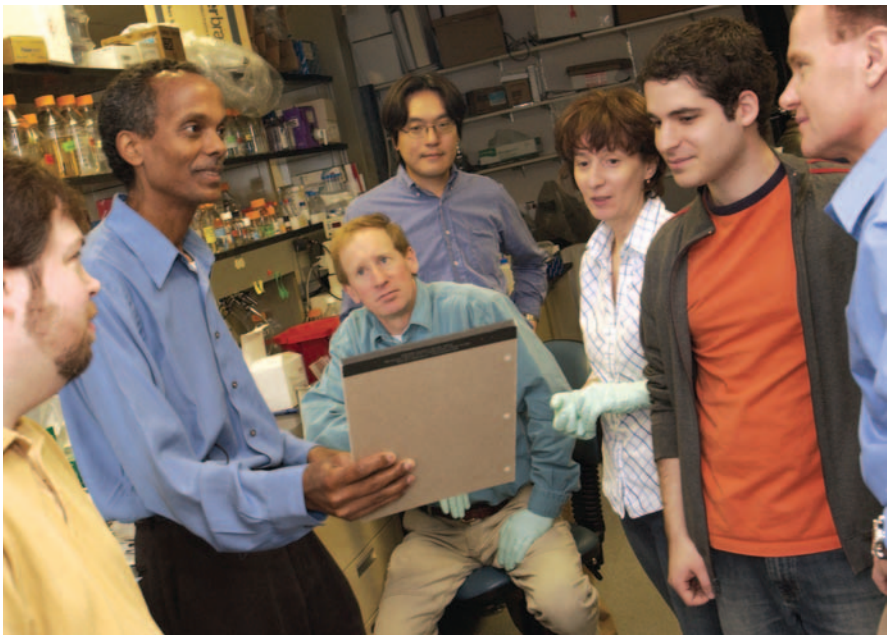


**These are mouse endothelial cells that have been stained for actin (blue), FGF (green), and FRS2 (red).**

**Ebo de Muinck, M.D.**
**Assistant Professor of Medicine (Cardiology) and Physiology; Director, Preclinical Research Labs**
Studies role of CD13, a cell-surface molecule, in activating angiogenic responses. He also works on the development of molecular imaging tools for angiogenesis research.

**Armin Helisch, M.D.**
**Assistant Professor of Medicine (Cardiology)**
Studies mechanisms in compensatory growth of collateral arteries; femoral artery ligation in mice, a technique he helped to establish as a postdoctoral fellow. His work has led to a realization of the importance of preexisting collateral vessels, and he did the first *in vivo* magnetic resonance images of growing collateral vessels in mice.

**Arie Horowitz, D.Sc.**
**Assistant Professor of Medicine (Cardiology)**
Studies intracellular signaling in endothelial cells during cell migration and angiogenesis; processes linking endocytosis and cell migration, including the role of the PDZ protein synectin; role of a synectin-binding growth factor in regulating cell migration and endocytosis; directional cues in endothelial cell migration delivered through the semaphorin pathway.



Above: Kiflai Bein, second from the left, gets input from other researchers on the progress of his lab's work. Right: Arie Horowitz. Below: Radu Stan, left, and Mike Simons.

**Just as plant roots grow toward water, laboratory-bred blood vessels grow toward other vessels in response to chemical signals, explains Kiflai Bein.**

place and out of the workplace," says Shworak. "We have a lot of [joint] outside activities. We have an occasional ski day, group barbecues in the summer. Mary Jo had a St. Patrick's Day party at her place."

Participants in the center also enjoy hiking and doing other activities together. Several take weekly tango lessons together. Helisch even tried to recruit a couple of Dartmouth Medicine staffers to come to a tango workshop.

"These social aspects oil the wheels of the research," says Horowitz solemnly. "They help it function easier. When things don't go well socially, or when there are tensions, people spend mental energy on this, and that always detracts from the research itself."

The socializing seems to work because the members of the group clearly expend plenty of energy on their research.

The projects are fascinating. Take Kiflai Bein, who coaxes endothelial cells to grow into blood vessels in culture dishes. The cells will form tubes like plant roots, as long as they are embedded in a fibrinogen matrix. Just as plant roots grow toward water, laboratory-bred blood vessels grow toward other vessels in response to chemical signals, explains Bein. Put two such matrixes side by side and the vessels will grow toward each other. Take away the barrier and the vessels will fuse.

"I think, for me, the beauty and the wonder in the life sciences is taking a small cell which then grows into a big thing," Bein says.

Pathologist Radu Stan, M.D., is also fascinated with how cells work, especially in angiogenesis. "What I do is I look at how molecules go from the blood to the tissues and vice versa—in normal tissues and in clinically significant settings, such as inflammation and tumors," he says. He's investigating the endothelial structures—particularly the openings known as caveolae, transendothelial channels, fenestrae, and vesiculo-vacuolar organelles—that are involved in vascular permeability. He also discovered PV-1, a protein that is upregulated in acute and chronic myocarditis.

Stan is a willing collaborator with the other researchers and often shares his knowledge of cell biology techniques, such as purification protocols and fractionation. "We have collaborations in which I have them set up experiments by which they could pursue their molecules—everybody has a favorite molecule," he says conspiratorially. "This goes both ways. I'm doing experiments that I was discussing with Nick Shworak. He helped me figure out something. That's how things work."

While the basic research on genes, molecules, and cells is important, there comes a point where the scientists have to see what's going on in living bodies. Ebo de Muinck, M.D., runs the Angiogenesis Center's preclinical research labs and is developing imaging techniques that will make it possible to see angiogenesis in live animals. He uses methods like echocardiography, Doppler technology, magnetic resonance imaging, electroparamagnetic resonance imaging, and 3-D imaging.

The preclinical labs rely on mice and other small mammals for thir research. Karen Moodie, D.V.M., a veterinarian and a member of the preclinical lab team, makes sure that precautions are taken to guard against pain and suffering in the animals used for research.

Mice are useful research models because they are very similar genetically to humans. Scientists can breed "knock-out" strains of mice to see whether blood-vessel growth is affected when certain genes or proteins are missing. But mice are small, their hearts beat 600 times a minute (compared to human hearts, which beat only about 70 times a

## VEIN ATTEMPT: A Glossary of Angiogenesis Terminology

**Angiogenesis:** The sprouting of new capillaries in response to ischemia.

**Angioplasty:** A catheter-based repair to unblock a vessel, such as a coronary artery.

**Arteriogenesis:** Maturation or new growth of collateral arteries; occurs outside an area affected by ischemia.

**Atherosclerosis:** A build-up of fatty material in the wall of a coronary artery; causes narrowing of the artery.

**Collateral artery:** A branch of an artery that runs parallel to the parent trunk.

**Coronary artery disease:** A condition that occurs when the arteries that supply blood to the heart become hardened and narrowed; this decreases the oxygen supply to the heart.

**Endocytosis:** A mechanism by which specific molecules are ingested into a cell.

**Endothelial cells:** Thin, flat cells that line the interior surface of blood vessels.

**Fibrin:** A protein essential for blood clotting.

**Fibroblast:** A connective-tissue cell that secretes collagen and other components of the extracellular matrix.

**FGF:** Fibroblast growth factor, a family of protein growth factors involved in new vessel formation, wound repair, and lung maturation.

**Growth factor:** A naturally occurring protein that stimulates cell division, differentiation, and proliferation.

**Hemangioma:** A cluster of abnormal but usually benign blood vessels; it can be internal or form an external birthmark.

***In vivo*:** Studies carried out in living organisms.

**Ischemia:** A decrease in the blood supply to an organ, tissue, or body part caused by constriction or obstruction of the blood vessels.

**Knock-out mouse:** A laboratory mouse in which researchers have inactivated, or "knocked out," an existing gene by replacing it or disrupting it with an artificial piece of DNA.

**Myocarditis:** An inflammation of the myocardium, the muscular part of the heart.

**Nanoparticle:** A microscopic particle measured in nanometers (one billionth of a meter); can be engineered to act as a drug carrier or an imaging agent.

**Occlusion:** Blockage of a blood vessel.

**PAI-1:** Plasminogen activator inhibitor protein, a protein that is required for endothelial cell migration; plasminogen is an inactive form of the blood enzyme plasmin.

**PDZ domain proteins:** Proteins that mediate interactions between proteins underlying the assembly of large protein complexes that are involved in signaling or subcellular transport.

**Peptide:** A short chain of two or more amino acids, the building blocks of proteins.

**Perfusion:** The passage of fluid, such as blood, through a tissue or organ.

**Peripheral artery disease:** A condition similar to coronary artery disease; it is most common in the arteries of the pelvis and legs.

**Proteasome:** A structure inside cells that breaks down proteins.

**Receptor:** A specialized protein on a cell's surface that binds to substances that affect the activities of the cell.

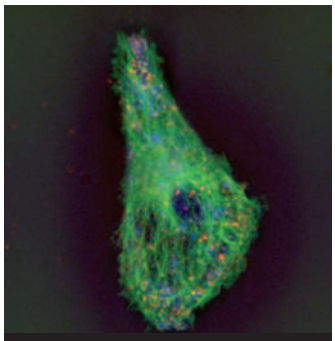
**Restenosis:** A renarrowing or reblockage of an artery at the same site where treatment, such as angioplasty, has already been performed.

**Syndecans:** A family of proteins that span the cell membrane; they are capable of carrying heparan sulfate and chondroitin sulfate chains (naturally occurring substances that inhibit enzymes that can degrade cartilage), and they perform a key role in fine-tuning transmembrane signaling and in influencing the processes of tissue repair, metabolism, and tumor formation.

**Synectin:** A PDZ-domain protein that is involved in syndecan complex formations.

**TGF-beta:** Transforming growth factor-beta, a cell-growth regulator; it reduces endothelial and smooth-muscle cell growth and migration.

**VEGF:** Vascular endothelial growth factor, a family of growth-factor genes that is critical in all aspects of angiogenesis.



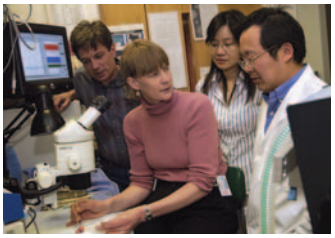
**These are rat endothelial cells stained for tubulin (green), syndecan-4 (red), and caveolin (blue).**

**Karen Moodie, D.V.M.**
**Research Assistant Professor of Medicine (Cardiology)**
Studies potential roles for cell and gene therapy in myocardial functional improvement in large- and small-animal studies.

**Mary Jo Mulligan-Kehoe, Ph.D.**
**Research Assistant Professor of Surgery (Vascular Surgery)**
Studies interactions with angiogenic molecules in the extracellular matrix of vascular endothelial cells using truncated recombinant PAI-1 proteins; signaling pathways and mechanisms contributing to the rPAI-123 protein (anti-angiogenic *in vivo* for breast cancer).

**Justin Pearlman, M.D.**
**Professor of Medicine and Radiology; Director, Advanced Imaging Center**
Studies imaging approaches to identification, mapping, and functional characterization of collateral beds in the ischemic myocardium; has pioneered novel MR-based methods. His lab is extending these techniques to mouse models of angiogenesis and is a core MR lab for several multicenter trials of therapeutic angiogenesis.

**Marcus Post, M.D., Ph.D.**
**Visiting Associate Professor of Medicine and Physiology**
Studies role of hepatocyte growth factor and platelet-



**Above: Justin Pearlman, who brings imaging expertise to the angiogenesis group. Right: Jannine Walsh. Below: Zhenwu Zhuang, right; Karen Moodie, in purple; and colleagues.**

**Dartmouth is one of the few medical centers anywhere that does angiogenesis imaging. "There are other places doing perfusion-sensitive imaging," a technique that is also done at DHMC, says Pearlman.**

minute), and blood moves through mice so quickly that it's difficult for imaging agents to illuminate their vessels unless very rapid scanners are used. That's because the blood vessels in mice are too small for the iodine-containing contrast agents used in humans to work. Zhenwu Zhuang, M.D., is trying to develop contrast agents that are safe and effective to use in small animals.

Meanwhile, de Muinck's lab is exploring ways to stimulate new blood-vessel growth in mice. The researchers first create a blockage by tying off part of a blood vessel in a hind leg or the heart. New vessels begin to grow in the border between the infarcted and the healthy tissue. The researchers can identify the new growth by way of targeting peptides that stick to molecules expressed only on actively growing blood vessels. De Muinck explains that he plans to "use nanoparticles that are decorated with these targeting peptides to deliver drugs to these new blood vessels, so we can stimulate their growth. [Later] we can come back again with targeted nanoparticles—but this time the particles carry an imaging molecule—so we can measure the extent to which we have increased the number of new blood vessels."

He points out that such imaging nanoparticles could also be useful in detecting new blood vessels in tumors. Of course, angiogenesis-stimulating drugs would only be tested on patients for whom cancer has been ruled out.

Justin Pearlman, M.D., the director of Dartmouth's Advanced Imaging Center, also shares his expertise with the Angiogenesis Research Center. Animals that need to be imaged are placed in a microinsert, which is a tube that goes inside the larger tubes of MRI or CT scanners. Dartmouth is one of the few medical centers anywhere that does angiogenesis imaging. "There are other places doing perfusion-sensitive imaging," a technique that is also done at DHMC, says Pearlman.

Nationwide, there are approximately 175 active angiogenesis clinical trials—testing angiogenic as well as anti-angiogenic agents—according to the NIH's *ClinicalTrials.gov* Web site. At least 15 of those trials are being conducted at Dartmouth—five by investigators affiliated with the Angiogenesis Research Center and at least 10 by cancer researchers.

One of the Dartmouth trials involves testing the blood of people with coronary artery disease to determine if there are genetic differences between people who have a natural ability to grow new blood vessels and those who don't. "Some patients are very efficient at making their own collateral arteries so are able, in the presence of narrowing of their coronary arteries, to open up new blood vessels that are really arteries that perfuse the ischemic territories," says de Muinck. "We already are noticing some differences between the genes that are switched on in the white blood cells from the patients with collateral, versus the white blood cells from the patients without collateral."

Mary Jo Mulligan-Kehoe is also helping to analyze the samples. "We have looked at those samples and asked the question, 'Can we see cleaved or truncated PAI-1 in those samples?' And the answer is 'yes' to varying degrees."

Doing science can be exciting yet tedious, satisfying yet frustrating, creative yet boring. But all of these researchers seem to love it.

"I love the science," enthuses Mulligan-Kehoe. "Everybody down in my lab is the same way. We have this high level of excitement over what the results are going to be. We discuss the mechanisms and where we're going with it, and they're as excited about it as I am."

"I feel it's exciting because it's fairly new research. . . . I sort of stumbled upon this, but it's cut-

ting edge," says Jannine Walsh, one of Mulligan-Kehoe's research assistants.

"Every day you come in you're finding out something new that nobody's looked at before," says Mary Drinane, a research assistant in the same lab. "I find it very exciting. And I especially like this area of research because of all the clinical applications to it. I feel that I'm contributing back, and that this will someday be helpful."

While not every day is exciting, most researchers don't seem to mind the routine tasks, the repetitive and tedious procedures. M.D.-Ph.D. student Arye Elfenbein, who works in Mike Simons's lab, listens to classical music while he gathers materials for his experiments. He gently removes culture dishes from an incubator that looks like a mini-fridge, mixes cells and reagents, places tiny vials into a centrifuge, scrutinizes cells under a microscope, and performs countless other tasks. The pace can vary. Sometimes his work has a slow, almost meditative, rhythm and other times it's like a quick, timer-driven race. Still, it's usually a creative process.

"For me, the science is almost an art form," says Mulligan-Kehoe. "You're able to express your creativity in the lab on a regular basis," whether by genetically engineering proteins, understanding molecular mechanisms, or writing the papers.

But some of the researchers do admit to feeling frustrated at times. "It's 95 percent frustrating. Absolutely," confesses Radu Stan. "An experiment that worked for years—last week somehow something happened." He laughs. "So we are trying to figure that out."

Yet he finds doing research to be very satisfying, too. "At the end of the day you know, if someone asks me 'What have you done for your field?' I say, 'Well, I found this marker and I found the function of this protein.' It's something that not many people can brag about. That's pure research."

But no matter how dedicated these scientists are, they still face a lot of challenges before they can translate successes in the lab and in preclinical trials into therapies for real patients.

"I think we have underestimated the complexity of the angiogenesis system," says Simons. "It turns out that when you have disease and when you have advanced age, all these agents don't work the same way as in young, healthy animals. So it's a challenge, even though there are a lot of ongoing clinical trials at the moment. But I don't really think they will be successful. It's just another step on the way."

Yet there's a sense of hope. And still more interesting research going on. One question being explored is whether it's possible to change the size of organs or even of entire organisms by changing the vascular system.

"We have a fascinating set of experiments where we have increased the vascularity of a normal heart in an adult mouse—the size of the heart has markedly increased," says Simons. "That implies that you can manipulate organ size by changing the vasculature size. So that's a very interesting fundamental biological question. Why is a mouse smaller than an elephant? It could be because it doesn't have as many vessels. And if you made a mouse with lots and lots of vessels, that will be a big mouse. So you can increase the organism size by increasing the size of the vasculature."

Simons worries, too, that a deeper understanding of angiogenesis might be misused to grow bigger muscles in athletes. "If this ever gets through professional sports, we will never see the end of doping," he says. He contends that there would be no test that could see whether increased muscle size was the result of stimulating angiogenesis. "Nobody would ever know, because there will be absolutely no way to check. There's no test for it. It's a natural thing."

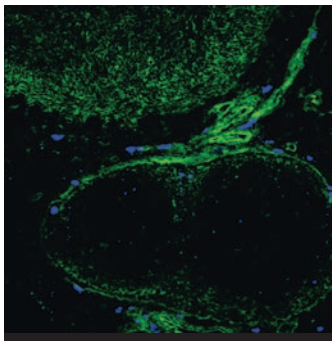
Ebo de Muinck chuckles. "Right now that's not been a worry because we haven't been terribly successful. What we're worried about now is creating normal blood vessels that are capable of transporting oxygen and that are not leaky," he points out. "Many of these new blood vessels that we've seen so far . . . are some kind of leaky tube-like structure. Some of them are also forming abnormal outgrowths—hemangioma-like structures—if you give too much growth factor."

Simons—who agrees that it will be a while before anyone has to worry about such a misuse of angiogenesis therapies—is active in an NIH initiative called the Trans-Institute Angiogenesis Research Program (TARP). Under TARP's auspices, several NIH agencies have partnered with each other and with other organizations to take a multidisciplinary approach to doing angiogenesis research.

So with funding organizations, agencies, and academic researchers banding together to try to understand the complexities of angiogenesis—how it underlies so many different diseases, how the proteins and genes that regulate it may also drive other processes, such as nerve growth—advances in one area may soon fuel advances in others.

"I guess the only bad thing is I wouldn't expect a cure of all the diseases tomorrow," Simons says. "Next week, maybe," he adds.

Then he laughs. ■



**These are, top, primary tumor and, bottom, metastatic cells, stained for vessels (green) and VEGF (blue).**

derived growth factor in coronary and peripheral angiogenesis. He is an internationally recognized expert in animal studies of vascular remodeling.

**Nicholas Shworak, M.D., Ph.D.**
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Studies molecules called heparan sulfate proteoglycans, which control cell signaling events; role of heparan sulfates in blood coagulation and atherosclerosis; biology of blood clotting; heart-valve disease.

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Studies endothelial structures involved in vascular permeability in normal and disease states; the regulation and function of components of endothelial microdomains, such as lipid rafts, caveolae, transendothelial channels, fenestrae, and vesiculo-vacuolar organelles. He discovered PV-1, the first marker for the endothelial stomatal and fenestral diaphragms.

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**Research Assistant Professor of Medicine and Radiology**
Studies molecular mechanisms of post-angioplasty restenosis at the genetic level in rats and transgenic mice; develops advanced imaging techniques in the Preclinical Research Labs.