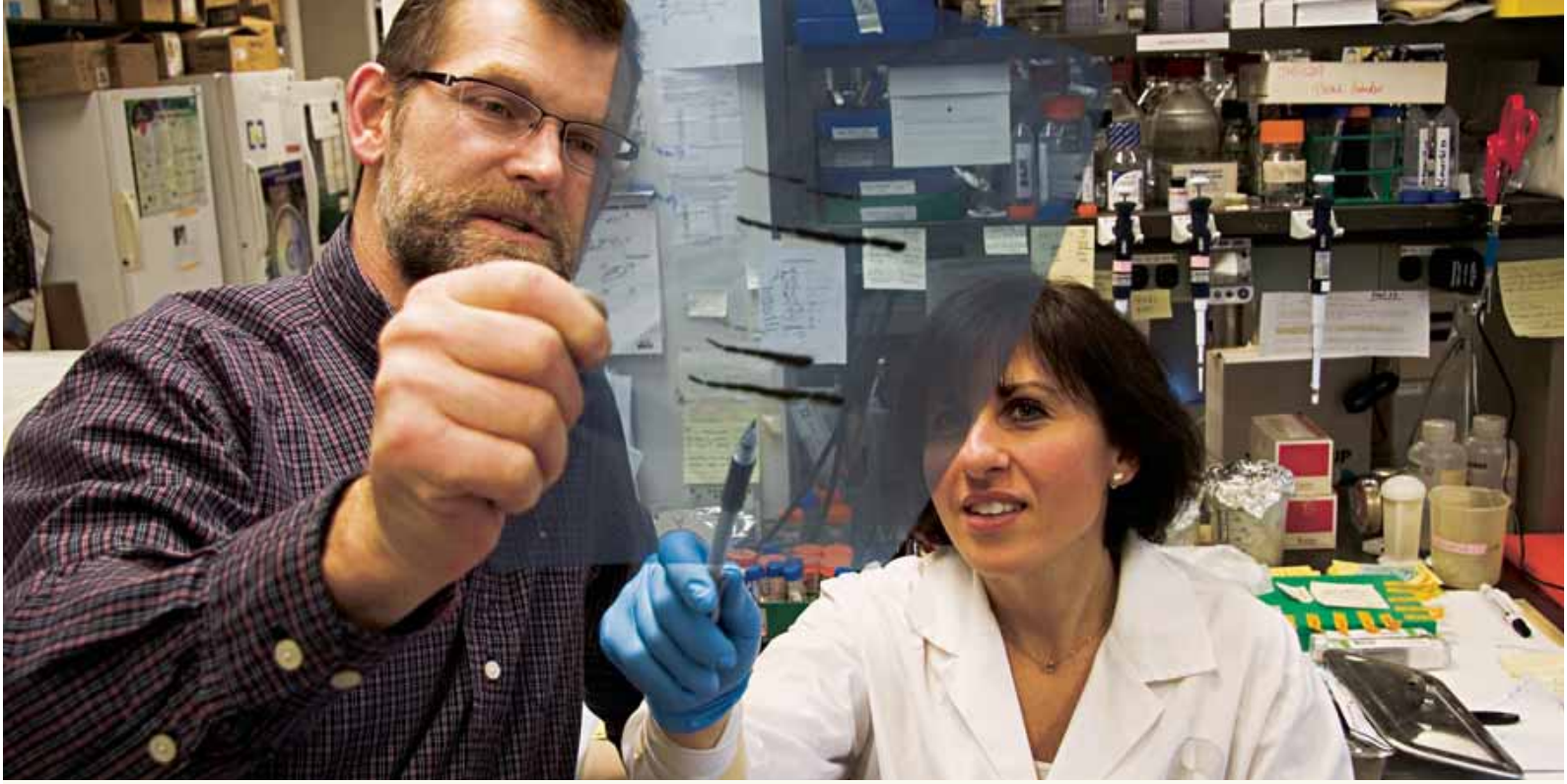

DRS. DAN DUMONT AND JENNIFER ALAMI

IN SICKNESS AND IN HEALTH
SRI SCIENTISTS LIFT UP THE VEIL ON THE ENIGMA OF ANGIOGENESIS

Innovation, says Dr. Robert Kerbel, a trailblazer in the field of antiangiogenic therapy, is "something that is off the beaten path, thinking outside the box. It is not just new, but unexpected. Counterintuitive."

Kerbel, along with Dr. Dan Dumont, a specialist in the genetic and molecular events that cause the vascular system to form, make up the vanguard of the science of angiogenesis at Sunnybrook Research Institute (SRI) and the translational applications it promises for people with cancer and other diseases. Together, these SRI senior scientists and Canada Research Chairs—Dumont in Angiogenic and Lymphangiogenic Signalling, and Kerbel in Tumour Angiogenesis and Antiangiogenic Therapy—are showing, time and again, what innovation looks like.

They co-lead the Toronto Angiogenesis Research Centre (TARC) at SRI, a unique-in-Canada virtual centre for the study of basic angiogenesis, antiangiogenic therapies and therapeutic angiogenesis. In their work there, they are striving toward a future in which cancer might be regarded as a chronic disease that can be managed, as diabetes is.

Each scientist brings his own œuvre of excellence to the enterprise. Dumont is celebrated for his advances studying the signalling processes controlling angiogenesis and lymphangiogenesis; Kerbel is renowned for his discoveries pertaining to novel antiangiogenic therapy strategies that are trained on making cancer therapy more effective and less arduous.

Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing ones. A natural process, it occurs in the healthy body for healing wounds and restoring blood flow to tissue after injury or insult, thanks to a series of "on" and "off" switches: angiogenesis-stimulating growth factors and angiogenesis inhibitors. The healthy body maintains a balance between the two.

Angiogenesis therapies are designed to control the switches—and, say some, are the next great wave in cancer care. Prior to the 1960s, there was barely a trickle. Indeed, researchers widely believed that blood supply reached tumours through the dilation of pre-existing blood vessels. Then, in 1971, a surgeon named Dr. Judah Folkman took a much-criticized stance with an article published in *The New England Journal of Medicine*, in which he hypothesized that tumour growth depends on angiogenesis. Angiogenesis-dependent diseases, he conjectured, result when new blood vessels grow either excessively or insufficiently; effectively, the body loses control over the process. Four years later, Folkman co-discovered the first of many angiogenesis inhibitors; and, in the years to follow, the role of angiogenesis in the spread of tumours increasingly became accepted.

Today, it is one of the hottest areas in medical oncology, with three antiangiogenic drugs having been recently approved in the U.S., Canada and many other countries.

Dumont is director of molecular and cellular biology research at Sunnybrook. He was the first to identify, clone and map the Tie2 gene on the human and preclinical model genomes, work he started in 1988 as a postdoctoral fellow. "It's been very much a niche for me because it's been a difficult receptor and ligand pair to work with," he says.

Inhabiting this niche has paid off. A recent success came when he created a mouse model with virtually all of the hallmarks of the human disease psoriasis—an ailment of the immune system involving interaction among skin, immune and blood vessel cells—by overexpressing the receptor Tie2 in the skin of genetically engineered mice. That they responded to immune system suppressor cyclosporine A, a classic human psoriasis treatment, paved the way for researchers to test other combination therapies prior to human clinical trials. The breakthrough was printed in March 2005 in *The American Journal of Pathology*.

Key was the identification of the interplay of angiogenesis, the immune system and a particular proliferation of the skin—three interdependent arms, each contributing to the disease. Dumont's work revealed that if the expression of the angiogenic response is turned off, the disease—the skin phenotype (basically, how it presents)—goes away. And if the drug is withdrawn, it returns.

Also in 2005, Dumont discovered that Dok-R, a protein that his lab was one of the first to describe, had a crucial mediating role to play with the recruitment of other proteins, findings he published in *Molecular and Cellular Biology*. "It was one of the early papers showing [that] this class of sort of signalling or scaffolding does play a role in down-regulating signalling cascades from receptors," he says. Several other groups then went on to show that knocking-out both Dok-R and Dok1 in mice leads to mild proliferative disease. "So, in essence, you knock out these negative regulators and you actually get a cancer."

"This paper says we might be able to test angiogenesis by drawing a little bit of blood from the patients."

While both Dumont and Kerbel do fundamental work in molecular and cell biology, Kerbel's work is closer to clinical application, by virtue of its translational nature. Kerbel is considered to be a pioneer in the study of antiangiogenic "metronomic" chemotherapy. This strategy—regular, long-term administration of low doses of cytotoxic drugs, with few or no breaks—is a complete departure from the way chemotherapy is usually given: at the highest doses possible, separated by long rest periods to recover from the toxic effects of this way of giving chemotherapy. Metronomic chemotherapy is thought to have an angiogenic basis.

Kerbel's work focuses on testing modified metronomic chemotherapy regimens in an effort to delay the relapses that he has seen in preclinical models. He and his lab have shown that repeated administration of cyclophosphamide every three or six weeks, combined with a daily, oral, low-dose metronomic regimen, improved efficacy and postponed relapses. Antiangiogenic activity, measured by reduction in circulating endothelial precursor cells in the bloodstream, revealed that the greatest degree of suppression occurred using the combination.

"For reasons that we still don't fully understand, low doses of chemotherapy in mice seem to have a powerful effect on these circulating endothelial progenitor cells, and even on the normal differentiated dividing endothelial cells in the tumour vascular bed. In other words, you're making chemotherapy a de facto antiangiogenic treatment strategy when you give it repetitively at close regular dosing, with no prolonged breaks."

Kerbel has long been interested in this concept, he says, because of the possibility of reduced acute toxicity, lowered costs, increased convenience for patients when using oral drugs, and its possible role as an adjuvant therapy for early-stage disease.

This work has given rise to clinical trials worldwide. The results so far of some of these are "very encouraging," he says.

In later work, Kerbel and postdoctoral fellow Dr. Yuval Shaked, who chose to work in Kerbel's lab because of the former's reputation and publishing record, attempted to zero in on on the optimum biologic dose (OBD) for this treatment strategy.

The absence of OBD information, Kerbel says, is a major problem with the metronomic method. "Once you get away from the maximum tolerated dose concept, it becomes much fuzzier, much harder, to determine what is an optimal therapeutic dose."

But they made some headway when they discovered that levels of circulating endothelial progenitor cells (CEPs) in peripheral blood can be used as surrogate biomarkers to help determine the best dose of antiangiogenic therapies. Shaked was the lead author of a study in which Kerbel and an A-list clutch of international colleagues found that levels of circulating endothelial cells (CECs) and CEPs vary, depending on the genetic background of a preclinical model. But within a particular strain, levels of these cells correlate with the ability to induce tumour blood vessel growth and the response to antiangiogenic therapy. He found that treatment with a drug that interferes with the major signalling receptor for a key regulator of blood vessel development caused a dose-dependent reduction in CEPs. This reduction closely reflected the previously established anti-tumour activity of this regulator, and the optimal decline in CECs and CEPs was reached at the optimal dose.

This article was published in January 2005 in *Cancer Cell*. In it, Shaked, the lead author, and colleagues, including Kerbel and Dumont, concluded that measuring peripheral blood cells could reliably indicate how well a therapy inhibits angiogenesis. "This paper says we might be able to test angiogenesis by drawing a little bit of blood from the patients," explains Shaked.

Clinical application, if it comes to that, is a ways off, but clinical trials based on this work are in full swing. Shaked is doing testing for centres across North America, including six for clinical trials, because SRI is where the expertise is. The centres send their blood samples to Shaked, and he teaches others the technique. While he hasn't yet come full circle, he's starting to close in on it. He leaves in 2007 to start his own lab. He is, says Kerbel, one of the brightest. On his experience, Shaked is effusive: "I got much more than I wanted," he says. "In my best dream, I never thought I would get this much."

Angiogenesis is still a field in development, with relevance to cancer, other diseases and regenerative medicine. In this field, Dumont and Kerbel and their lab teams find themselves leading the chase, a pursuit that not so long ago would have been dismissed as folly, but today is increasingly recognized for what it is—a new way of thinking with unexpected impact: innovation.

TARC is funded by the Canada Foundation for Innovation and Ontario Innovation Trust. Other funding for this research was provided by the Canada Research Chairs Program; Canadian Cancer Society; Canadian Institutes of Health Research; Heart and Stroke Foundation of Canada; National Cancer Institute of Canada; National Heart, Lung and Blood Institute; and National Institutes of Health (U.S.).

DRS. YUVAL SHAKED AND BOB KERBEL