Volume 1, No. 2 | 2007

National Cancer Institute

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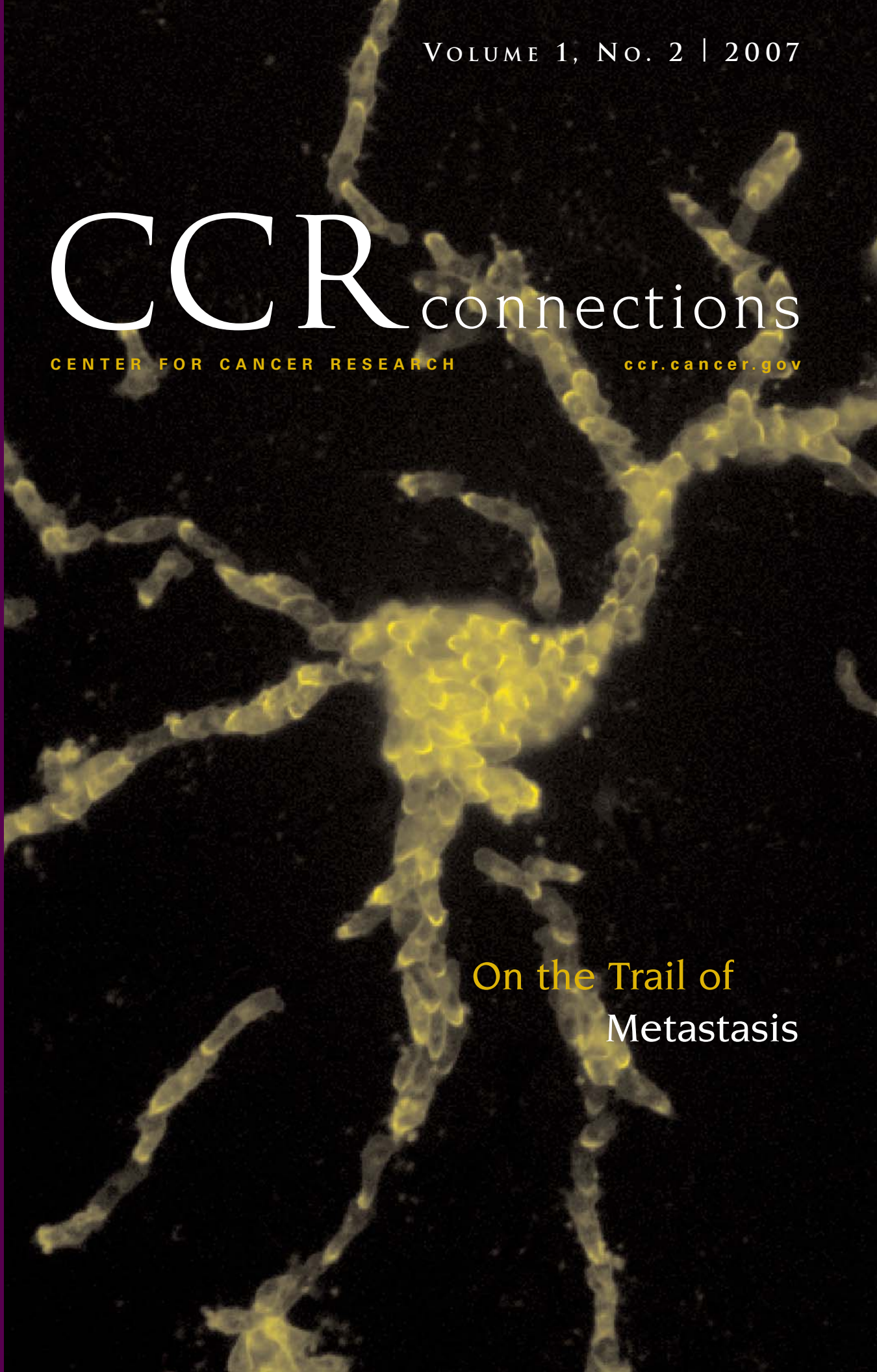

On the Trail of Metastasis

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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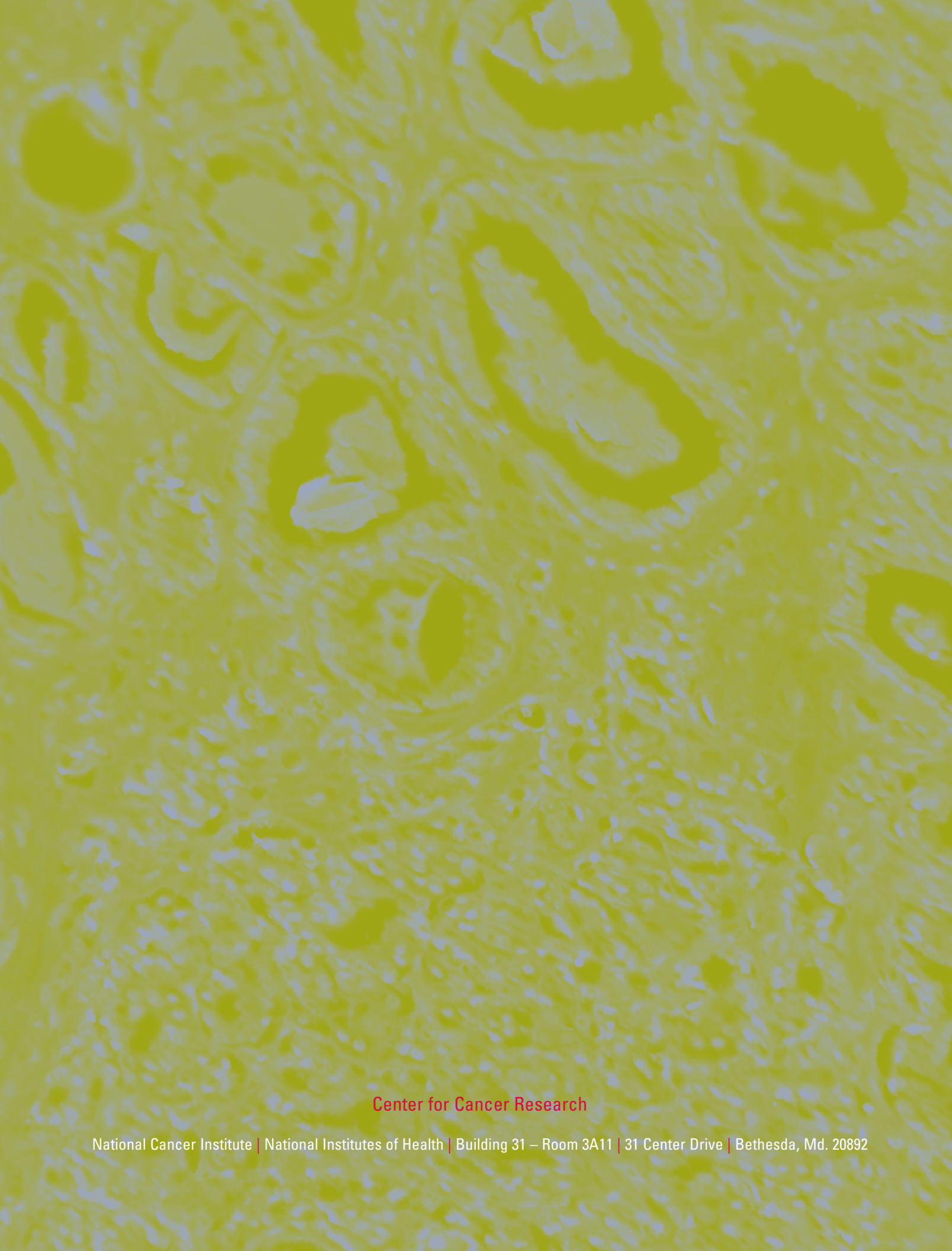

Center for Cancer Research

National Cancer Institute | National Institutes of Health | Building 31 – Room 3A11 | 31 Center Drive | Bethesda, Md. 20892

Web Sites with More Information about CCR

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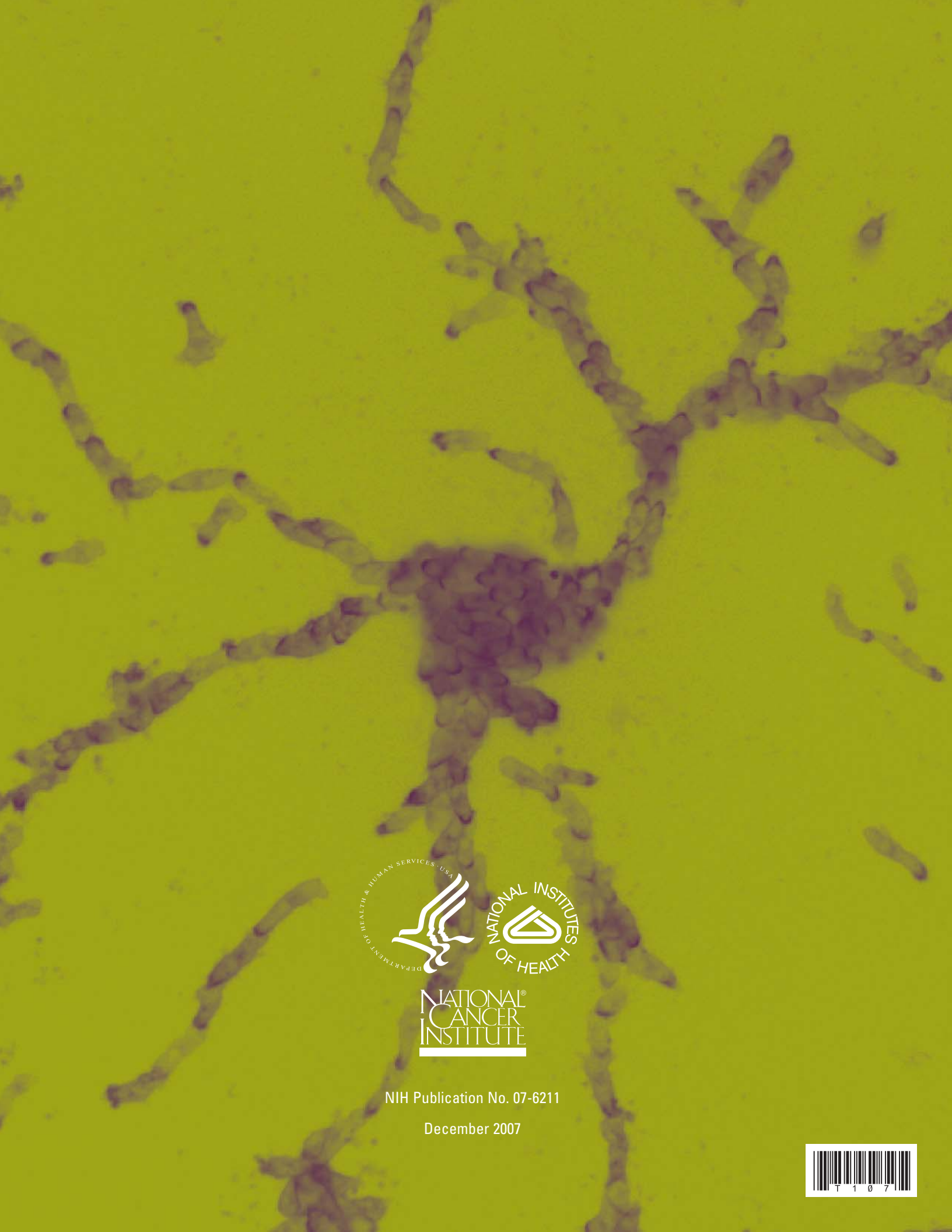

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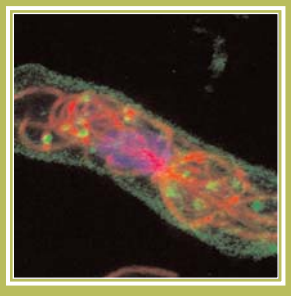

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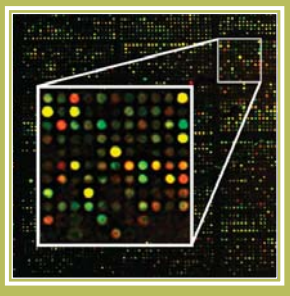

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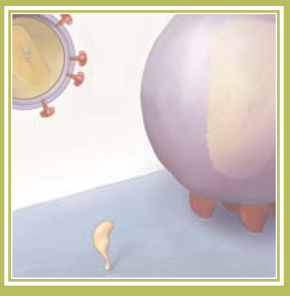

Seeing the Invisible

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Shrinking Prostate Tumors

CENTER FOR CANCER RESEARCH

The mission of the CCR is:

To inform and empower the entire cancer research community by making breakthrough discoveries in basic and clinical cancer research and by developing them into novel therapeutic interventions for adults and children afflicted with cancer or infected with HIV.

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Weaving the Future of Cancer and HIV/AIDS Medicine

Our lives are connected by a thousand invisible threads, and along these sympathetic fibers, our actions run as causes and return to us as results. – Herman Melville

When a scientist gives a talk or writes an article that describes where a specific cellular protein is located in relation to other proteins it interacts with, he or she almost always relies on a graphic depicting the protein as a labeled oval with simple lines or arrows connecting it to its protein partners in a "transduction cascade." As helpful as these graphics can be in understanding the immediate connections, they cannot begin to capture the complexity of the actual physiology, which can include "a thousand invisible threads" of protein and gene interactions in space and time. This complexity is true of both normal and disease physiology, and it is at the root of the challenges facing us in detecting, treating, and even preventing cancer and HIV/AIDS.

(Photo: Bill Branson)

Research aimed at unraveling such complex diseases requires significant resources, patience, and the ability to rapidly change direction as the scientific understanding evolves. Thus, in weaving together our strategic plan, the Center for Cancer Research (CCR) faced the formidable task of articulating strategic objectives to guide our work while, at the same time, making those objectives flexible enough to allow our individual researchers and their collaborators both within and outside NCI to nimbly pursue new leads in even the most unexpected directions.

At the heart of our strategic plan are dual foci: understanding the causes and mechanisms of cancer and HIV/AIDS, and intervening in the earliest possible stages in the disease processes. Just as important as the strategic objectives that we have put into place to support these aggressive goals are the approaches we pursue that provide the critical connectedness that will maximize our chances of success. These include:

- Strongly supporting basic, translation, and clinical research, not as separate entities but as a seamlessly integrated set of specialties
- Building meaningful collaborations among investigators both within NCI and across the research community and establishing incentives that reward such work
- Training and mentoring the next generation of investigators, including active recruitment at all levels, as well as providing significant laboratory experience for promising young students
- Empowering our researchers and their collaborators with access to world-class scientific resources and technical expertise, which allows them to answer the questions that *need* to be addressed, not just those that *can* be addressed with more limited resources

Perhaps the greatest challenge facing us—apart from the formidable challenges raised by cancer and HIV/AIDS themselves—is making sure that everything we do under our strategic plan is connected to all other parts of CCR and NCI, to the cancer research and cancer patient communities, and to the wider scientific and global community so that all can benefit from our efforts and we, in turn, can benefit from theirs. We know that, like cellular proteins, we are connected by "a thousand invisible threads" to the entire cancer community and beyond, and that everything we do can have an impact elsewhere. To that end, we are committed to making our work toward attaining our stated objectives as transparent and accessible as possible through scientific publications and meetings, through online databases and other electronic means, and through public communication vehicles like *CCR Connections* and our Web site.

Finally, we recognize that any strategic plan, including ours, is only as good as the talent, dedication, and passion of those who implement it. On that count, I have no doubts: Everyone at CCR, scientists and non-scientists alike, is a critical thread in a rich tapestry of expertise and commitment to curing—or at least managing effectively—cancer and HIV/AIDS for patients everywhere.

The CCR Strategic Plan is available at our Web site, http://ccr.cancer.gov/news/ccr-strategic-plan.pdf

Robert H. Wiltrout, Ph.D.
Director, Center for Cancer Research

The Devil Is in the Differences:

Highlighting Discrepancies between Tumor and Normal Angiogenesis

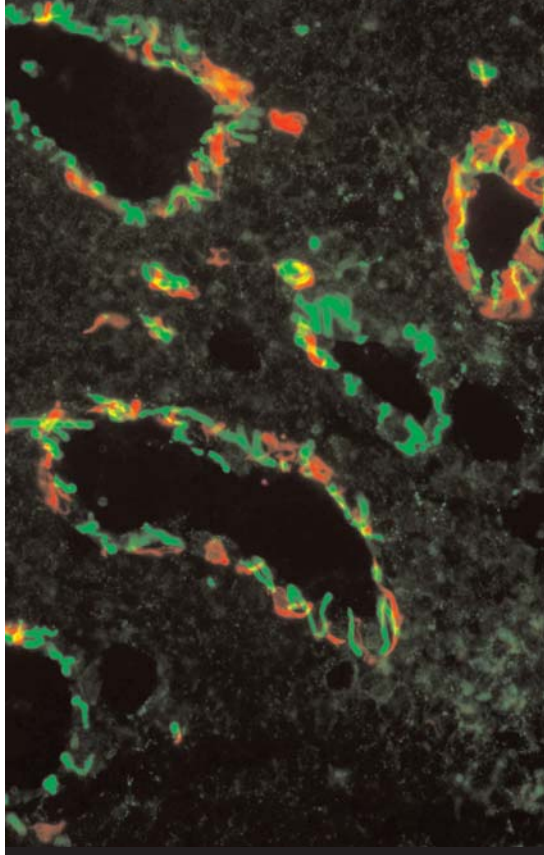
A young microtumor, the beginnings of a malignancy, will divide and grow until it outstrips its supply of oxygen and nutrients. But microtumors can adjust quickly, sending out chemical messages that fuel the growth of new blood vessels and help to feed their growing needs. This process of blood vessel growth—called angiogenesis—is a target of interest for cancer researchers because strangling a tumor's nutrient supply could both cause it to shrink and also increase its susceptibility to cancer drugs.

However, angiogenesis also ensures that our healthy organs and tissues maintain a proper blood supply, particularly in the context of organ growth and repair. Specifically targeting tumor blood vessels without compromising normal biological activities that rely on angiogenesis (e.g., menstruation, pregnancy, wound healing) has proven challenging.

To better understand the molecular nature of angiogenesis in tumors, a team led by CCR's Brad St. Croix, Ph.D., Head of the Tumor Angiogenesis Section in the Mouse Cancer Genetics Program, compared the transcriptomes (the full complement of expressed genes) of endothelial cells from eight different resting tissues, regenerating liver, and five different tumor types in mice.

In a June 2007, *Cancer Cell* paper authored by St. Croix lab member Steven Seaman and colleagues, the team reported its data on the differences in gene expression between endothelial cells (which line the interior of blood vessels) from regenerating liver and those from tumor-bearing liver. The liver is a popular model for angiogenesis research because it readily regenerates and revascularizes after damage or surgical resection.

The group's experiments resulted in a list of 13 genes that were overexpressed in the malignant liver compared to regenerating liver. The largest expression difference was seen in a gene called *CD276*, a member of the B7 family of immunoregulating genes known to encode a cell-surface protein. When the St. Croix team looked at *CD276* expression patterns in endothelial cells from cancer patients, they found that the gene was overexpressed in blood vessels from colon, lung, breast, esophageal, and bladder cancers. In addition, they found that the gene was overexpressed on the surfaces of the tumor cells themselves. This overexpression makes *CD276* an attractive therapeutic target, as drugs engineered against it could interfere with the cancer directly while simultaneously preventing the flow of blood to the tumor cells.



(*Image: Brad St. Croix, CCR*)

Endothelial cells (green) define the walls of blood vessels within a lung cancer tumor. Research to identify the molecular differences between normal (physiologic) angiogenesis and tumor angiogenesis could help improve the effectiveness of anti-angiogenic therapies aimed at strangling tumors' oxygen and nutrient supply.

Fostering International Outreach

The World Health Organization's World Cancer Report claims that cancer rates are set to increase at an alarming rate globally. CCR is committed to stemming this increase through outreach that promotes research and training and encourages cancer control in at-risk countries.

(Photo: Courtesy of Steven Pavletic, CCR)

CCR's Steven Pavletic, M.D. (second from right), and NIH Director Elias Zerhouni, M.D. (center), joined Croatian researchers, clinicians, and health officials to discuss strategies for cancer control in nations, like Croatia, that are transitioning to free-market economies.

Cancer Care in Croatia

The overall incidence of cancer in Croatia—a nation that is rapidly transitioning to a free-market society—differs little from that of the United States or Western Europe. Cancer mortality, however, is much higher. Cancer accounts for 23 percent of deaths in Croatia, second only to heart disease. Overall five-year survival rates are 57 percent for women and 40 percent for men. (Compare these to the five-year survival rate of 66 percent for both men and women in the U.S.)

Five hundred million people live in transitioning nations like Croatia, mostly in Eastern Europe or former Soviet republics. "That indicates that more can be done in prevention, early detection, and cancer care," said Steven Pavletic, M.D., Head of the Graft-Versus-Host and Autoimmunity Unit in CCR's Experimental Transplantation and Immunology Branch and a Croatian native.

In May 2007, CCR clinicians flew overseas to join a three-day meeting focused on the strategies for cancer control in Croatia. "We approached Croatia as being a model country, hoping that the results could have an impact on other countries," said Pavletic. The meeting, held in the Croatian capital of Zagreb, was organized by NCI, the Croatian Medical Association, the University of Zagreb School of Medicine, the Croatian Ministry of Health and Social Welfare, and the Croatian Ministry of Science, Education, and Sports. U.S. attendees included CCR Clinical Scientific Director Lee Helman, M.D.; NIH Director Elias Zerhouni, M.D.; the President of the Oncology Nursing Society, Georgia Decker, M.S., R.N., and two former presidents of the American Society of Clinical Oncology. Oncologists, nurses, patient advocacy groups, health educators, and government officials from Croatia were all represented as well. "Within a year or two, we would like to see a national cancer control plan for Croatia as a palpable outcome of this effort," said Pavletic. "This is not all that common; not many countries have one."

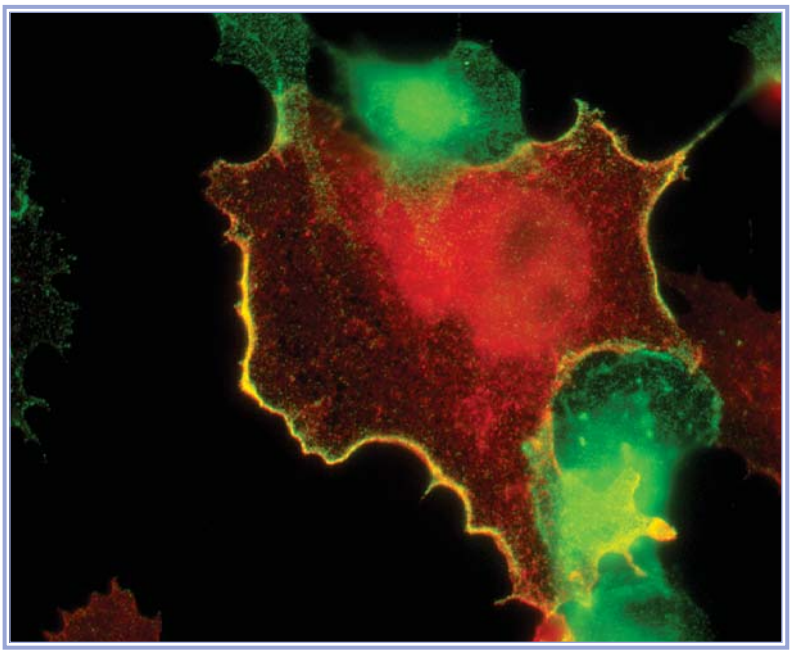
Training in Morocco

Rajae El Aouad, M.D., the Director of the Moroccan National Institute of Hygiene, visited the NIH on April 24–27, 2007, to develop international cooperation in research training. During her visit, El Aouad met with Staff Scientist Helen Sabzevari, Ph.D., and Lab Chief Jeffrey Schlom, Ph.D., both from the Laboratory of Tumor Immunology and Biology. The trio addressed the importance of providing female researchers in Morocco in particular, and those from North Africa and the Middle East in general, with additional opportunities for training.

El Aouad and Sabzevari conceived the idea of establishing annual training workshops in Morocco for such female scientists. Sabzevari, who has worked for several years for the advancement of women in science, hopes to lead the first workshop (focused on tumor immunology), with possible funding from the State Department's Office of Science and Technology Cooperation.

Sabzevari said, "Bringing in more cutting-edge science will encourage women to participate in scientific research and obtain research positions in their countries."

Bringing down Cancer's Edifice



(*Image: Deborah Morrison*, CCR)

KSR1 (green) coordinates the assembly of ERK (red) and numerous other proteins into a potent signaling complex (represented here by the colocalization of ERK and KSR1 in yellow) that activates ERK and promotes cell survival. In a paper highlighted by the *Journal of Biological Chemistry* as one of their top one percent of papers submitted in 2007, the Morrison laboratory discovered that cleavage of KSR1 promotes apoptosis through two distinct mechanisms.

Cell survival is a molecular balancing act. The trick is to have the right number of cells at all points in the cellular life cycle—from early development through full differentiation—survive in any given tissue. To accomplish this feat, each cell carries within it a program known as "apoptosis," a cellular self-destruct mechanism that activates when needed to eliminate unwanted or even diseased cells from an organism. But cancer cells have learned to ignore this program and proliferate, even at the expense of their host organism.

In a paper published on July 5, 2007, in the online edition of the *Journal of Biological Chemistry (JBC)*, Deborah Morrison, Ph.D., Head of the Cellular Growth Mechanisms Section of the Laboratory of Cell and Developmental Signaling at CCR, and Postdoctoral Fellow Melissa McKay, Ph.D., describe a unique molecular mechanism that lies at the heart of the apoptotic program. The paper has been selected by *JBC's* editors as a *JBC* Paper of the Week, placing it in the top one percent of papers submitted to the journal each year. This recognition underlines the significant implications these findings carry for cancer research.

When successful, chemotherapeutic drugs can activate a cancer cell's apoptotic machinery, shutting down tumor growth. However, cancer cells may gain resistance to chemotherapy, at least in part, by blocking chemotherapy-induced apoptosis.

In their studies of the molecular mechanisms that control apoptosis, Morrison and McKay focused on a central player called kinase suppressor of Ras 1 (KSR1). KSR1 is a unique molecule whose job it is to organize other molecules in the cell, much as a scaffold acts as a temporary structure for holding workers and materials during their work on a building, and thereby promote pro-survival signaling within the cell.

Through studies in test tubes and mammary tissue in mice, Morrison's team learned that KSR1 normally sits inside the cell's cytosol, part of a "survival team" of cell proteins. A healthy cell sends out a molecular signal that activates an enzyme known as Ras GTPase. Ras activation causes KSR1 to migrate from the cytosol to the cellular membrane. Once near the surface, KSR1 performs its "scaffolding" job, coordinating the assembly of many other proteins into a potent signaling complex. Other key members of the complex include a protein called MEK, its activator Raf, and MEK's molecular target, ERK. Together, these proteins activate ERK by attaching a phosphate group to it, empowering it to function as a critical cell survival agent.

But the researchers found that when a cell is in trouble, it uses a caspase, a kind of molecular scissors protein, to cleave a fragment off the tail end of KSR1. Once in pieces, KSR1 loses its scaffolding function, and the entire cell survival complex falls apart, pushing the cell towards apoptosis. In addition, one of KSR1's fragments directly interferes with ERK activation. These observations suggest that KSR1 cleavage is a key step in apoptosis and is another promising approach to finding novel therapies that reactivate the apoptotic program in cancer cells.

(Photo: Rebecca Brown)

Frank Cuttitta, Ph.D. (seated), and Steven Libutti, M.D. (standing)

TARP and ACF: Allies in Angiogenesis Research

Angiogenesis, the process by which the body grows new blood vessels, is important in growth, development, and wound healing. It is also an integral step in the progression of cancer and has been implicated in a host of other diseases. Controlling this process could benefit cancer patients, and it might also help those with heart disease, diabetes, and eye diseases like macular degeneration.

Until recently, though, progress in angiogenesis research suffered from a lack of standardization among cell lines, assays, and methods used to quantify neovascularization (new blood vessel formation). This lack of standardization made it hard for scientists to share and compare data from one lab to another.

To address these challenges in a systematic way and to help stimulate and support multidisciplinary angiogenesis research, NCI and the Juvenile Diabetes Research Foundation came together in 2003 to create the NIH Trans-Institute Angiogenesis Research Program (TARP). The program, which now includes scientists and clinicians from 6 of the 27 institutes within NIH, aims to accelerate the discovery of new interventions for a variety of diseases and conditions in which blood vessel development plays a major role. "By creating a multidisciplinary forum where researchers can learn from each other," explained Steven Libutti, M.D., Head of the Surgery Branch's Tumor Angiogenesis Section and one of the leaders of TARP, "advances from one disease area can fuel progress in others."

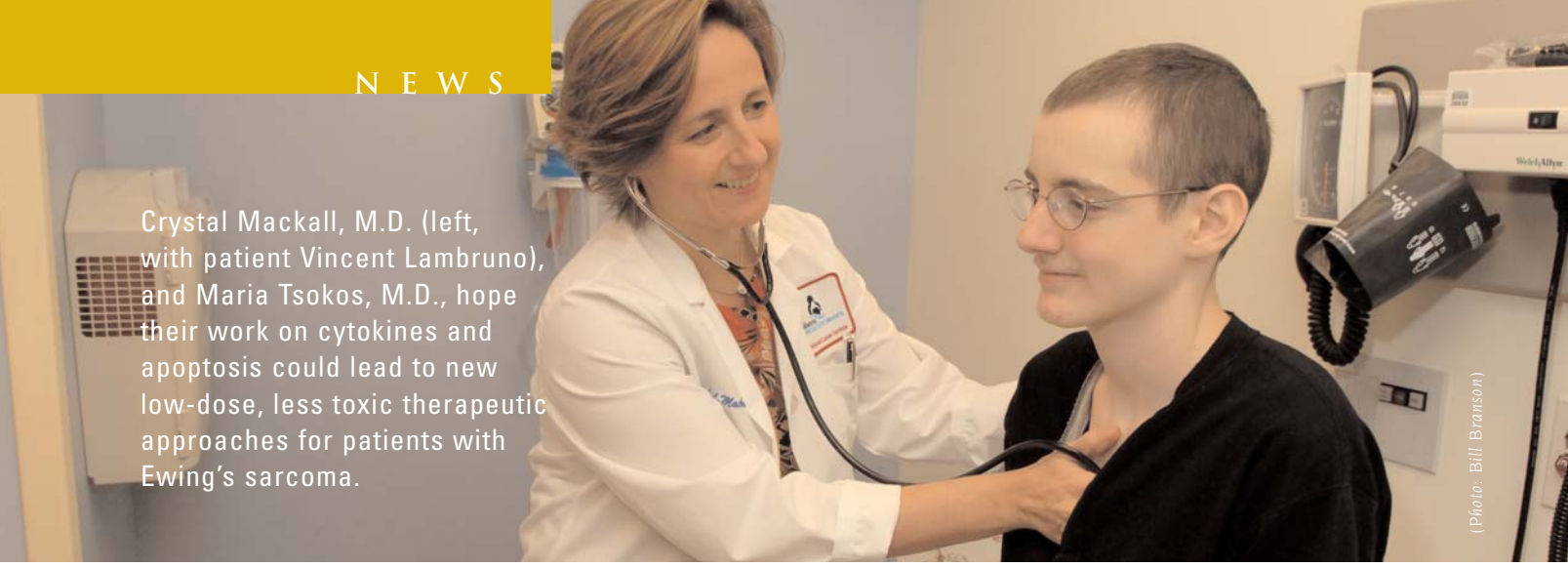
CCR houses and supports a critical component of the TARP program: the Angiogenesis Core Facility (ACF). Established in 2006, the ACF "helps angiogenesis researchers speak the same language," said Frank Cuttitta, Ph.D., the facility's Director. "The ACF is producing the standards that were lacking." The Core has found ways to provide reliable cell lines, reagents, and methods for data analysis and comparison. It has developed best practices for maintaining the purity of commercially available human primary microvascular endothelial cells (hPMEC) from different vendors and for minimizing cell drift—a process that causes primary cells in culture to alter their physical appearance and gene expression with the passage of time.

The ACF continues to advance and "standardize" angiogenesis research in several ways, such as:

- Finding a method to enrich cell populations of hPMEC
- Encouraging vendors who sell angiogenesis-research products to now meet ACF purification standards
- Advancing the development of relevant cell lines by using the enzyme telomerase (which helps control cellular lifespan) to immortalize hPMECs without compromising their genetic makeup
- Developing software that standardizes the measurement of vessel growth in the matrigel/endothelial cell tube formation assay and the chick chorioallantoic membrane assay (CAM)
- Developing assays to better mimic the tumor microenvironment in order to study the ways it influences angiogenesis

"Taken together, all of the ACF milestones," said Dr. Cuttitta, "will help scientists to advance the field of angiogenesis research."

To learn more about the TARP and ACF, please visit www.tarp.nih.gov.



Crystal Mackall, M.D. (left, with patient Vincent Lambruno), and Maria Tsokos, M.D., hope their work on cytokines and apoptosis could lead to new low-dose, less toxic therapeutic approaches for patients with Ewing's sarcoma.

(Photo: Bill Branson)

Jump-Starting Treatment for Relapsed Ewing's Sarcoma

The outlook for patients with Ewing's sarcoma—the second most common bone tumor in children and adolescents—has improved markedly over the past several years. Now an international team of clinicians, including CCR physicians, hopes to extend that improved status to the children who relapse with this sarcoma.

Successful treatment for Ewing's sarcoma is aggressive, combining dose-intensive chemotherapy with surgery, radiation, or both. With these tools, clinicians are able to attain cure rates approaching 70 percent. But survival itself comes at a price. Current standard therapies are highly toxic and leave many cured patients with lifelong therapy-related effects.

Intensive research is under way to identify new and less toxic approaches to treating Ewing's sarcoma. Researchers at Germany's University of Freiburg are working with CCR clinicians to pursue a promising new low-dose therapy that they hope will prove effective for both primary and relapsed tumors. The international collaborators' optimism is reflected in experimental findings reported in the June, 2007, issue of the *American Journal of Pathology*.

A protein called tumor necrosis factor apoptosis-inducing ligand (TRAIL, also called Apo-2L) stimulates cancer cells to self-destruct via apoptosis, or programmed cell death, but leaves normal cells untouched. Maria Tsokos, M.D., of CCR's Laboratory of Pathology, and Crystal L. Mackall, M.D., of CCR's Pediatric Oncology Branch, teamed up with German colleagues to study the activity of TRAIL in tumor samples from 47 Ewing's sarcoma patients. They discovered that when TRAIL successfully triggers apoptosis in a cancer cell, it does so in the presence of high levels of an enzyme called caspase-8. Interestingly, Ewing's sarcoma cells from patients who relapse or resist therapy often have unusually low levels of caspase-8.

This unresponsiveness to TRAIL is not permanent, however. Tsokos, Mackall, and colleagues found that treating resistant Ewing's sarcoma cells with interferon-gamma, a protein normally produced by the body's immune system, can stimulate them into producing more caspase-8, re-sensitizing them to TRAIL's apoptotic influence. A Phase I clinical trial to assess the safety and effectiveness of a TRAIL-receptor agonist—a compound functionally and structurally similar to TRAIL—is currently under way in CCR's Pediatric Oncology Branch. With her young patients in mind, Dr. Mackall explains, "Once we complete the safety testing, we hope to combine our new TRAIL-like agonist with interferon-gamma and attempt to clinically induce cell suicide in resistant or relapsed Ewing's sarcoma. These studies suggest that such an approach may help even more children."

Progress in Two Types of Skin Cancer

Recent work by CCR scientists has led to important discoveries that help to better define the genes and proteins involved in two distinct types of skin cancer, squamous cell carcinoma (SCC), and melanoma.

(*Image: Timothy Triche, NCI*)

The Yuspa and Merlino laboratories are probing the molecular features of two different forms of skin cancer, melanoma (above, as viewed using a scanning electron microscope) and squamous cell carcinoma.

A Genetic Signature for SCC

The formation of a precancerous lesion typically precedes the development of skin cancer. What is unclear is whether all precancerous lesions have a similar likelihood to becoming cancerous or if some are genetically predisposed to cause disease. A team of scientists co-led by Stuart H. Yuspa, M.D., Co-Chief of the Laboratory of Cancer Biology and Genetics at CCR, and Adam Glick, Ph.D., at Pennsylvania State University, set out to determine whether a genetic signature might reveal whether a lesion has a low or high risk of becoming cancerous.

The research team found that a relatively small subset of genes appears to delineate low- and high-risk lesions and that 90 percent of high-risk lesions had expression patterns similar to those seen in SCC (a common non-melanoma skin cancer), even though the lesions themselves were still premalignant. These results were published in the May, 2007, issue of *Oncogene* in a paper by lead author Nadine Darwiche, Ph.D., associate professor at the American University of Beirut, who worked with Yuspa and Glick on this research while on a sabbatical at NCI. This work could help physicians preemptively identify lesions that are likely to become cancerous, a critical step in cancer prevention.

A New Player in Melanoma

It has been known for some time that the protein p53 acts as a tumor suppressor in many cell types; researchers have recently shown that a protein that regulates p53—not p53 itself—is involved in advanced melanoma.

Cutaneous malignant melanoma is an extremely aggressive skin cancer, with few successful treatment options. The disease has become increasingly prevalent, emphasizing the need to understand its biology and find better treatment options.

Recent evidence has shown that a specific chromosomal region known to be altered in most melanomas harbors the gene for a tumor suppressor protein called ARF. This protein was originally characterized as a regulator of the tumor suppressor p53, which is mutated or deleted in many forms of cancer but typically not in melanoma. More recent studies of ARF indicate that it functions in numerous ways beyond its role as a p53 regulator.

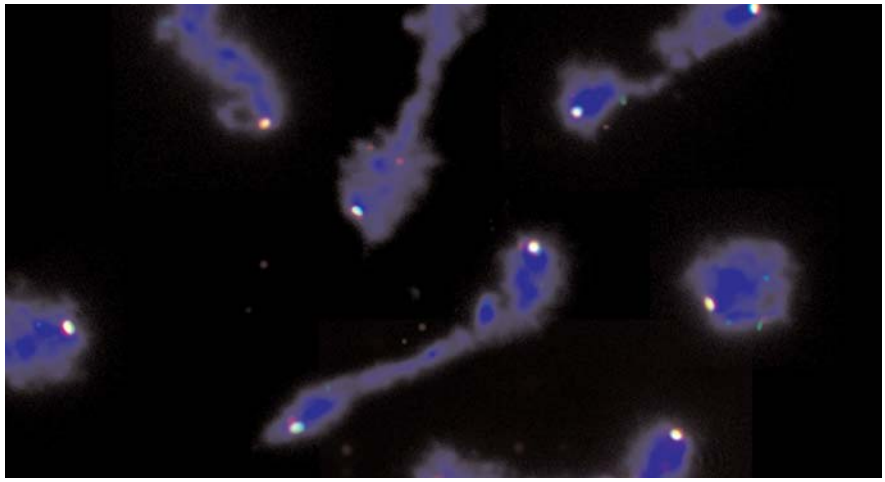
These findings led CCR scientist Glenn Merlino, Ph.D., Co-Chief of the Laboratory of Cancer Biology and Genetics, and his staff to explore the role of ARF in melanocyte carcinogenesis. The results of their work were published in the June, 2007, issue of *Proceedings of the National Academy of Sciences*, in a paper by lead author Linan Ha, Ph.D, a Postdoctoral Fellow in Merlino's group. Using a mouse model of skin cancer, the scientists analyzed the specific roles of p53 and ARF in skin carcinogenesis. They showed that while mice that did not express p53 did not develop melanomas, those lacking ARF did, indicating the importance of ARF expression in normal skin cells.

The investigators also analyzed cellular senescence, a permanent halt to cell division that can prevent cancer formation. They found that ARF can induce senescence, and thus suppress the early formation of skin cancer, while p53 cannot. These studies verify the importance of ARF in skin cancer in a manner that is completely independent of p53.

These results might explain why ARF is absent or altered in human melanoma and also shed light on the ineffectiveness of some of the current targeted cancer therapies. The work could also provide promising leads on how to improve the analysis and treatment of melanoma.

Safeguards to Ensure Genetic Fidelity

Accurate maintenance of a cell's genetic material during cell division is crucial to ensuring that genetic defects capable of fueling abnormal cell growth are not passed on to daughter cells. CCR scientists are studying the proteins necessary for ensuring proper DNA content.



(*Image: Ian Wisnieski and Carl Wu, CCR*)

Binding of a novel protein, Scm3 (green), to the histone Cse4 (red) in the cell nucleus helps chromosomes properly segregate during cell division. Errors in segregation can promote cancer or, during development, congenital birth defects. White appears where the two proteins bind together.

Separating Chromosomes during Cell Division

In eukaryotes, failures in proper chromosome duplication and segregation during cell division can result in cancer or congenital birth defects. So a DNA-protein superstructure, called a kinetochore, assembles just in time to separate chromosome pairs and link each to microtubules that move them away from one another. Understanding the complex mechanisms that guarantee accurate chromosomal separation may lead to potential new therapeutic interventions.

Carl Wu, Ph.D., Head of the Chromosome Structure and Gene Regulation Section of the Laboratory of Biochemistry and Molecular Biology at CCR, leads a team of scientists who study kinetochore assembly. A paper published in the June, 2007, issue of *Cell* by lead authors Gaku Mizuguchi, Ph.D., and Hua Xiao, Ph.D., senior scientists in Wu's laboratory, describes a novel protein, Scm3, that is required for kinetochore formation in yeast. Usually proteins called histones keep DNA neatly coiled around them when cells are not dividing, but when the time for separation arrives, the nonhistone Scm3 displaces some histones so it can bind directly to one called Cse4. This dynamic action sets the stage for the kinetochore's assembly and allows chromosome segregating activity to begin to occur.

Although the protein sequence of Scm3 is evolutionarily conserved only in fungi, a human equivalent to Scm3 may exist, which could be an exciting new clinical target for interfering with the highly aberrant cell division seen in many forms of cancer.

Reining in Broken DNA

The same method of gene rearrangements that allows lymphocytes to create an arsenal of immune responses can potentially lead to aberrant DNA breakage and genetic instability. Andre Nussenzweig, Ph.D., of the Experimental Immunology Branch at CCR, and his brother Michel Nussenzweig, M.D., Ph.D., of the Laboratory of Molecular Immunology at Rockefeller University, investigate how lymphocytes maintain this delicate balance.

A recent paper published in the July, 2007, issue of *Cell* by lead author Elsa Callén, Ph.D., a visiting fellow in Andre Nussenzweig's laboratory, demonstrates that a protein called ATM kinase—which, when mutated, is responsible for the genetic syndrome ataxia telangiectasia (AT)—normally functions to prevent broken DNA strands from joining inappropriately in lymphocytes. ATM kinase also keeps damaged cells from dividing, thus ensuring that genetic stability is maintained. When ATM kinase is absent, cells with broken DNA continue to divide, propagating genetic instability that could lead to cancer.

Considering that hematologic malignancies are found in AT patients, and that ATM kinase is nonfunctional in various cancers, this research holds the promise of helping to unravel the underlying biology of AT and its accompanying blood cancers.

Staff News at CCR

new recruits

(Photo: Scientific Publications, Graphics and Media)

Terry Van Dyke, Ph.D.

Van Dyke has been appointed Director of the Mouse Cancer Genetics Program (MCGP) in Frederick, Md. She is affiliated with the Lineberger Comprehensive Cancer Center and the Departments of Genetics and Biochemistry at the University of North Carolina, Chapel Hill. She received her Ph.D. from the University of Florida and pursued postdoctoral research at the State University of New York, Stony Brook. Van Dyke is a leader in the field of genetically engineered mouse models (GEMMs) and has played an active role in the MMHCC (Mouse Models of Human Cancers Consortium). She has been the organizer of several meetings on mouse models and serves on the external advisory boards of a number of cancer centers. At NCI, Van Dyke will provide leadership for both Institute- and Division-level scientific initiatives focused on development of GEMMs of cancer and their application to the production of more effective therapeutics and earlier diagnostics for the detection and treatment of human cancer.

(Photo: Bill Branson)

Christopher B. Buck, Ph.D.

Buck received his Ph.D. from the Johns Hopkins School of Medicine. His graduate research on the translation and immunogenicity of the HIV-1 capsid protein Gag earned him the Alicia Showalter Reynolds Award. His postdoctoral research in CCR's Laboratory of Cellular Oncology ranged from basic studies of HPV virion structure and morphogenesis to translational research that identified compounds capable of blocking HPV infection. For his HPV work, Buck and his CCR mentors shared the 2006 Norman P. Salzman Award. Dr. Buck joins the CCR faculty as an Investigator.

(Photo: Bill Branson)

Deborah E. Citrin, M.D.

Citrin is a graduate of Duke University School of Medicine. She completed her residency training at NCI and the National Capital Consortium (Walter Reed Army Medical Center and National Naval Medical Center). Citrin's laboratory research interests include normal tissue radiobiology, malignancies of the gastrointestinal tract, and novel molecular therapeutics combined with radiation. She conducts her translational research in the Radiation Oncology Branch.

Newly Tenured CCR Scientists

Michael R. Bishop, M.D.
Experimental Transplantation and Immunology Branch

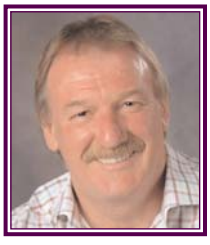
Kent Hunter, Ph.D.
Laboratory of Cancer Biology and Genetics

announcements

(Photo: Courtesy of Stephen Hughes, CCR)

Stephen H. Hughes, Ph.D.

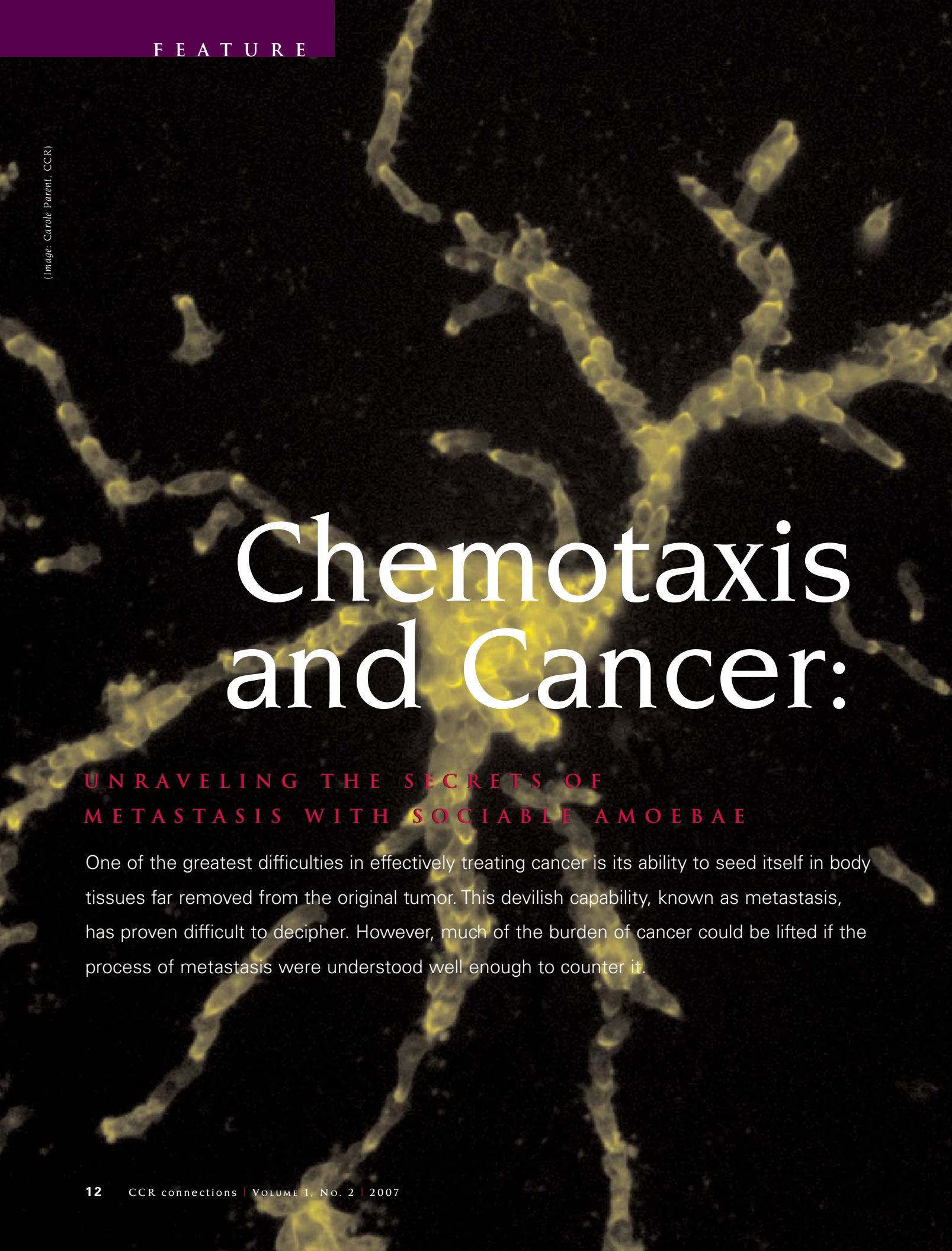
Hughes has recently been appointed Director of the HIV Drug Resistance Program (DRP) located on NCI's Frederick campus. He is also serving as Chief of the DRP's newly merged Resistance Mechanisms and Retroviral Replication Laboratories. His career at NCI began in 1984, when he established the Gene Expression in Eukaryotes Section within the Applied Bioscience Laboratories–Basic Research Program at NCI-Frederick. He became Deputy Director of that program in 1988 and Director of the Molecular Basis of Carcinogenesis Laboratory in 1995. He joined the DRP as Chief of the Retroviral Replication Laboratory in 1999.



(Photo: Scientific Publications, Graphics and Media)

Stuart Le Grice, Ph.D.

Le Grice has been appointed Head of the newly formed Center of Excellence in HIV/AIDS and Cancer Virology, where his extensive HIV experience will help integrate the strengths of scientists from the Frederick and Bethesda campuses of NCI. After postdoctoral training in the U.K., Germany, and the United States, Le Grice joined Hoffmann-La Roche in Basel, Switzerland as a senior scientist. In 1990, he moved to Case Western Reserve University (CWRU) in Cleveland, Ohio, serving as Director of the NIH-funded CWRU Center for AIDS Research from 1994 to 1999. Le Grice joined NCI's HIV Drug Resistance Program in 1999 as Chief of the Resistance Mechanisms Laboratory.



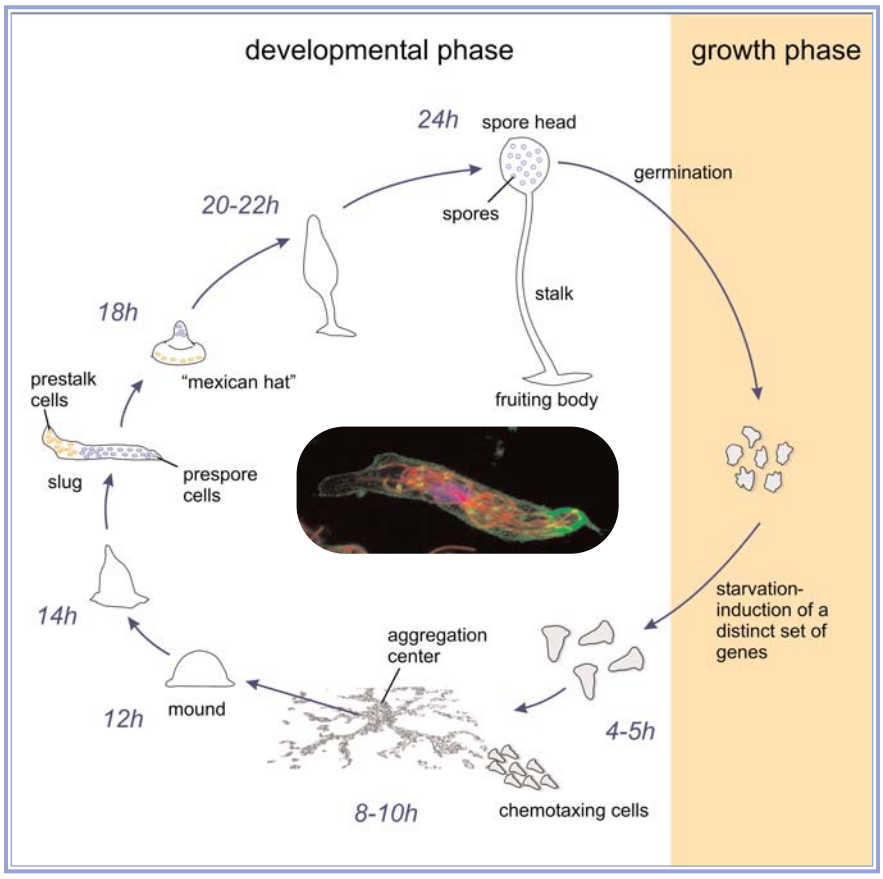
(*Image: Carole Parent, CCR*)

Chemotaxis and Cancer:

UNRAVELING THE SECRETS OF METASTASIS WITH SOCIABLE AMOEBAE

One of the greatest difficulties in effectively treating cancer is its ability to seed itself in body tissues far removed from the original tumor. This devilish capability, known as metastasis, has proven difficult to decipher. However, much of the burden of cancer could be lifted if the process of metastasis were understood well enough to counter it.

"When you look at the number of deaths from cancer, almost all of them are related to metastatic spread of the disease," said **Carole Parent, *Ph.D.***, *Senior Investigator in CCR's Laboratory of Cellular and Molecular Biology. "It is critical that we figure out how this phenomenon happens so that we can figure out how to stop it."*



(*Image: Carole Parent, CCR*)

Over the course of its life cycle, *Dictyostelium discoideum* (or "Dicty") cells use chemotaxis to move en masse in the search for friendly environments.

The itch to travel is not limited to cancer cells or even human cells, nor is it limited to our recent evolutionary past. Cellular mobility has proven a fundamental evolutionary trait of many organisms, living and extinct, for millions of years. Indeed, the key to unraveling metastasis may lie in understanding not only what makes all cells decide to pull up stakes, but also how they choose a location and a means of getting there. To that end, a team of CCR scientists led by Parent is taking advantage of evolutionary conservation to uncover the signaling pathways that give cells their wanderlust.

One phenomenon by which cells move about is chemotaxis—the ability to sense and gravitate toward external chemical signals called chemoattractants. The intracellular pathways a cell uses to translate external signals into movement are remarkably well conserved up and down the evolutionary ladder, from human cells like neutrophils to simple organisms like the heavily-studied slime mold *Dictyostelium discoideum*.

"When you look at the number of deaths from cancer, almost all of them are related to metastatic spread of the disease."

Affectionately called "Dicty," this mold is the perfect model organism for unraveling the mechanics of cell migration, and it gives Parent and her colleagues a unique way to begin probing the fundamentals of cancer metastasis. "We come at metastasis from a cell biology perspective rather than a cancer perspective," said Parent, "which I think allows us to learn things that we would miss if we studied only cancer cells.

There is a subset of cancer cells that, like *Dictyostelium*, expresses chemokine receptors and responds to chemokine gradients," Parent continued. "These chemotactic abilities have been shown to govern where certain cancer cells metastasize, thereby providing an explanation for the preferential metastatic destination of certain tumor types."

The Signal to Move

The cascade of events from the time that a cell first detects an external signal until it moves in response to that signal is amazingly complex, yet amazingly efficient. It is also highly conserved across millions of years of evolution. For example, Dicty's molecular mechanisms for chemotaxis are remarkably similar to those mammalian neutrophils use to detect and pursue bacteria and call for assistance from other immune cells. And it is possible that the same mechanisms underlie metastasis.

Regardless of the organism, chemotaxis requires several physical and chemical components:

- Chemoattractants to trigger cell changes
- Receptors to detect and amplify the chemical signal
- Cytoskeletal redistribution to change the cell's internal architecture and promotecellular polarity
- Enzymes and other chemicals to facilitate dynamic signaling between the front and back of the cell
- Molecules devoted to regulating movement

Loss of or damage to any one of these components can impair the movement of a cell or groups of cells. For example, studies have found that blocking the receptors or other enzymes necessary for signal amplification can prevent a cell from receiving signals, moving toward the source of those signals, or recruiting other cells in their trek.

In eukaryotes, the urge to move on begins when a chemoattractant signal activates a G protein-coupled receptor (GPCR) on the cell surface. While mammalian GPCRs can be stimulated by a wide range of chemoattractants, the major chemoattractant in Dicty is cyclic AMP (cAMP). When bound to specific GPCRs called cARs, cAMP causes the cell's internal architecture to rearrange itself. This dramatic restructuring is only beginning to be understood, thanks in large part to the huge strides made by Parent and her colleagues in the use of innovative imaging techniques like Fluorescence Resonance Energy Transfer (FRET) and Fluorescence Recovery After Photobleaching (FRAP) to dissect Dicty's signaling and amplification pathways. By fluorescently tagging specific proteins with almost every color of the rainbow, these tools let researchers visualize processes in migrating cells in real time and quickly gain insights into the cellular mechanics of cancer and other diseases (see figure on previous page).

> The cascade of events from the time that a cell first detects an external signal until it moves in response to that signal is amazingly complex, yet amazingly efficient.

Follow the Leader: Regulating Movement through Cellular Cross-Talk

Once a signal has been received and amplified, successful chemotaxis requires dynamic signaling, or "cross-talk," between the front and back of the cell. In Dicty, proteins like CRAC (cytosolic regulator of adenylyl cyclase) form the backbone of this relay loop.

As a Postdoctoral Fellow, Parent showed that CRAC is found at the front of chemotaxing cells. Using real-time fluorescent microscopy, Parent and her colleagues have since shown that adenylyl cyclase (ACA)—CRAC's target and the producer of cAMP—accumulates at the back of chemotaxing cells. They propose that from here, cAMP is released and recruits neighboring cells.

Thus a cycle is formed: CRAC at Dicty's front end stimulates ACA at the back; ACA releases cAMP; cARs at the front of the next cell detect the cAMP, activating CRAC, and so on. Eventually, neighboring cells line up front to back as they move toward the source of the original signal, much like ants heading toward a picnic (see "From Sociable Amoebae to Man").

Other labs, extending Parent's work, have found that this very same mechanism is at play during neutrophil chemotaxis, illuminating potential targets for chemotaxis-manipulating therapies. And remarkably, as cancer cells metastasize, they transition from clusters to single, amoeboid-like cells, often moving in a head-to-tail fashion and forming ranks of cells that move along paths of least resistance. Metastatic cancer cells, therefore, appear to revert to a very primitive and efficient mode of migration shared by leukocytes and Dicty cells.

Moving to Humans

Although neutrophils and other human cells respond to a wider range of chemoattractants and have more complex signaling systems than Dicty, the lessons learned from this little mold have greatly expanded scientists' understanding of chemotaxis and metastasis. In addition, researchers may be able to target anti-metastasis therapies to specific physical components of cellular migration, keeping cancer cells from receiving or acting on signals to move. Like the organisms they study, Parent and her colleagues hope that their research remains nomadic, continually moving from the bench to the bedside.

From Sociable Amoebae to Man

Dictyostelium discoideum is a great model for studying cancer metastasis because chemotaxis and migration are essential for the slime mold's survival, and the signaling pathways that control its migration are highly conserved with mammalian cells. Yet "Dicty" is much simpler, making the process of dissecting the migration pathways relatively easy. In the laboratory, researchers can easily grow and manipulate *Dictyostelium* cells, observe their behavior under the microscope, and determine the role of specific gene products during migration.

During the *growth* portion of its life cycle, Dicty's autonomous, free-living amoeboid cells divide through binary fission and hunt bacteria by following their folic acid excretions. However, adverse conditions like starvation cause Dicty cells to enter their *developmental* phase. "Starving" cells produce and release cAMP, which acts as a chemoattractant and draws neighboring Dicty cells together into a migrating mass called a "slug." The slug can include 100,000 cells and behaves like a multicellular organism, responding to heat, light, and other environmental factors in its search for a new, more welcoming home.

Attraction to Cancer Research

Carole Parent, Ph.D.

(Photo: Rhoda Baer Photography)

Carole Parent is a Senior Investigator in CCR's Laboratory of Molecular and Cellular Biology. Throughout her scientific career, she has focused on understanding how cells translate signals into complex cellular behaviors, especially chemotaxis.

Although the idea that *Dictyostelium* and metastatic cancer cells harbor similarities may seem far-fetched at first, Parent and her colleagues have made it clear that evolution has carefully protected the basic cellular mechanisms across the years. "Our work allows us to think in a new way about the unity of biology," said Parent. "We find that we can go back and forth quickly between human cells and Dicty, which, for a variety of reasons, allows us to probe chemotactic mechanisms at a deeper level."

Parent earned a Ph.D. from the University of Illinois at Chicago in 1992. She then joined the laboratory of Peter N. Devreotes, Ph.D., in the Department of Biological Chemistry of the Johns Hopkins University School of Medicine for postdoctoral training, where she was promoted to the rank of Instructor in 1996. She joined CCR in May 2000.

Gene Garcia

(Photo: Rhoda Baer Photography)

Gene Garcia is a doctoral student in Parent's laboratory through NIH's Graduate Partnerships Program (GPP) with Johns Hopkins University. In the three years that he has been part of the laboratory, he has focused on the role of regulated degradation of chemoattractants in chemotactic signaling.

Garcia attributes his interest in science to his wife: "After having been out of high school for nine years, the woman who was to be my wife and I decided to take a summer class in human biology at Keene State College in Keene, NH. We found it so fascinating that the following fall we both enrolled in the undergraduate biology program there. The lab work appealed to me, but what I found most interesting were the connections between the sciences, how the understanding of one field of science was largely dependent on the understanding of others," he said.

Although Garcia plans to finish his degree and pursue postdoctoral work soon, he notes that the collaborative environment of CCR and the interesting work he has been doing will be tough to give up. "The truth is that the only things that really motivate me to leave work are that I have a wonderful wife and children to come home to, and sleep," he says.

Annarita (Anna) Bagorda, Ph.D.

(Photo: Rhoda Baer Photography)

Anna Bagorda, a visiting fellow in Parent's lab, was drawn to research out of "curiosity." "In a laboratory experience during my last year of college, I realized that for every solved question, there are many more coming," said Bagorda. "That was it: I couldn't stop!"

Bagorda received her Ph.D. in Physiology in 2002 from Italy's University of Bari and joined the CCR lab in 2004 from the Venetian Institute of Molecular Medicine in Padua, Italy. She is currently doing research to unravel the spatio-temporal dynamics of cAMP during chemotaxis in both *Dictyostelium* and neutrophils.

Bagorda plans to return to her home country after her work at CCR. "I surely hope to bring back some technical experience, but more than that I wish to be able to bring back the approach to science that I developed during these years. The communication and help among scientists is a key aspect in our daily job, and here at CCR, I have been exposed every day to this experience."

Paul Kriebel

(Photo: Rhoda Baer Photography)

Paul Kriebel has been in the Parent lab since 2000, where he started as a biologist with a master's degree. His work with his NCI colleagues, and "enthusiastic support" from Parent, inspired him to pursue a Ph.D. through the NIH's GPP program with The George Washington University, which he will complete in May 2008. "I have worked in both the private sector and NCI, and because of the wonderful environment and experience I have had at NCI, I have decided to devote my life to scientific research," he said.

Kriebel's research focus is on understanding how chemoattractant signals are transmitted between cells during chemotaxis. But the application of these discoveries to metastasis or normal physiological processes (e.g., lymphocyte responses or embryonic development) is his ultimate goal.

Although Kriebel will complete his degree next spring, his immediate plans are to stay with the Parent lab "at least a few more years, because the research is so interesting and exciting. I love to discover new things about what cells can do, and how that knowledge can contribute to the fight against cancer and other diseases."

Making Sense of Lymphoma:
The Definition Makes a Difference

A partnership among three scientists is meticulously dissecting and revealing the many faces of lymphoma that have stymied clinicians for years. For decades, doctors could not explain why one patient was cured by treatment while another—who appeared to have the same cancer under a microscope—would succumb to the disease. Today scientists at CCR are finding molecular distinctions among various forms of lymphoma that help them predict a patient's outcome, and they are beginning to use that information to tailor therapy to an individual's cancer.

Lymphoma is the name for a cancer that originates in the lymphocytes, the white blood cells that help defend the body against disease. Once classified into two broad categories, Hodgkin's and non-Hodgkin's types, lymphoma is now known as much more complex, according to Elaine Jaffe, M.D., of CCR's Laboratory of Pathology. "Twenty years ago, clinicians didn't understand the complexity of lymphoma," she said. "It's not one disease; it's 40 diseases."

Jaffe, Louis M. Staudt, M.D., Ph.D., and Wyndham Wilson, M.D., Ph.D., have joined forces to find the abnormal molecular pathways inside cancer cells that are controlling their proliferation and survival. "If we can understand those pathways and interfere with them, then we can kill the cancer cell," said Staudt of the Metabolism Branch.

To find those pathways, Staudt has developed DNA microarrays that simultaneously measure the activity of thousands of genes expressed in a single tissue sample. The researchers use the resulting molecular profiles to validate pathology findings, define important pathways in cancer, and devise better treatments.

"The partnership is very strong because we're very different," said Staudt. "I'm a dyed-in-the-wool molecular biologist and genomicist. A decade ago, I started reading about lymphoma and saw these cancers were described largely by how the cells looked and whether they were clumped or not clumped. It seemed to be crying out for some molecular insights."

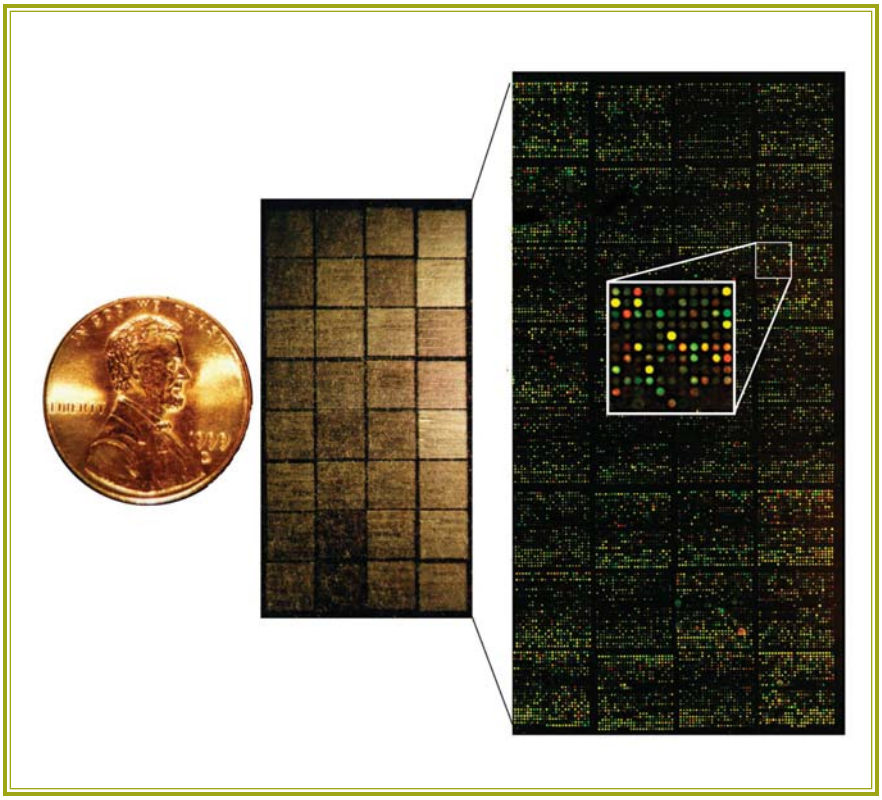
> "Twenty years ago, clinicians didn't understand the complexity of lymphoma."

"Elaine and Wyndham (Lymphoma Therapeutics Section, Metabolism Branch) bring very different and important perspectives," he continued. "Elaine is arguably the most admired hematopathologist in the world. She's made a career out of very careful examination by microscope of these various lymphomas and, through some very astute observations of patterns, she reliably teased out diagnoses that our new molecular tools confirm are real subdivisions."

Jaffe played a leadership role in developing worldwide standards for the diagnosis of lymphomas and leukemias, culminating in the 2001 World Health Organization (WHO) classification of lymphomas. "Before the WHO classification, there was no international consensus on the diagnosis of lymphoma and leukemia," Jaffe explained. That made it difficult to compare results of clinical trials, validate molecular studies, or do epidemiologic studies. "You have to know that a diagnosis made in Washington, D.C., means the same as that made in Munich, Germany. Developing consensus that leads to international standardization for diagnosis is really critical for cancer research."

For example, said Jaffe, "Twenty years ago, mantle cell lymphoma (a very aggressive form of cancer) was lumped in with the other lymphomas. Now, no clinician in the world would not see it as a separate disease."

The third member of the triad, Wilson, is a clinician who runs clinical trials to improve treatments for patients with lymphoma and other cancers. "Gene expression profiling is all relatively new. It has to be tested in prospective clinical trials," Wilson said. He has several studies at CCR that target pathways revealed by molecular profiling or that vary the timing of treatment to hit the cancer when it's most vulnerable.



(*Image: Louis Staudt, CCR*)

With DNA microarrays, researchers are beginning to unravel the complexities and highlight the unique molecular features of the 30+ types of lymphoma.

In a pilot molecular profiling study, Staudt and his collaborators found that a form of lymphoma called diffuse large B-cell lymphoma (DLBCL), based on its genetic signatures, is multiple diseases, with different clinical outcomes. With that exciting finding, NCI launched the Lymphoma/Leukemia Molecular Profiling Project (LLMPP), with Staudt as co-leader and John Chan, M.D., from the University of Nebraska as a collaborator. This international consortium of eight groups has frozen tissue samples and clinical data that is pooled for pathologic and genomic analysis. Several pathologists in the group review each case to come up with a consensus diagnosis. Then Staudt's group runs molecular profiling on the same sample, using DNA microarrays, and compares the results.

Subsequent studies showed that DLBCL is actually three diseases. One subgroup, germinal center B-cell-like DLBCL, has a good prognosis. A second subgroup, activated B-cell-like DLBCL, has a poorer prognosis and expresses different genes. The third, called primary mediastinal B-cell lymphoma (PMBL) has distinct expression of hundreds of genes that are most closely related to Hodgkin's lymphoma. Many important findings have come from the LLMPP, including a deeper understanding of DLBCL, Burkitt's lymphoma, mantle cell lymphoma, and follicular lymphoma.

In June 2006, the scientists published the molecular distinctions between Burkitt's lymphoma and DLBCL in *The New England Journal of Medicine*. Both are highly curable, Staudt said, but only with the correct treatment regimen. "If Burkitt's patients, for example, are treated with intensive therapy, there is roughly an 80 percent survival rate. However, if they are misdiagnosed and treated with the lower-intensity regimen recommended for DLBCL, the survival rate is reduced to 20 percent or even less," Staudt said. In their study, one-sixth of the cases were Burkitt's by gene expression signatures but had been diagnosed as DLBCL using conventional pathology. "This was a proof of principle of the added value of molecular diagnosis," he said.

In follicular lymphoma, the researchers have used molecular profiling to describe the pace of the disease. Three-quarters of patients have extraordinarily slow-growing disease with a median survival of 11 years, according to Staudt. But one quarter have aggressive disease, with a median survival of 3.9 years. "If I were given a diagnosis of follicular lymphoma, I'd want to know which category I'm in," Staudt said. It would help a patient decide whether to watch and wait—and feel confident in that decision—or undergo treatment right away.

They did the same for mantle cell lymphoma, finding molecular markers that indicate whether a patient has the most aggressive tumor, with median survival less than a year, or slow-growing, with median survival of 6.7 years. If a patient is diagnosed with the aggressive type of mantle cell, they could consider one of the many clinical trials that are testing new agents against mantle cell lymphoma.

> The scientists also found a molecular signal in follicular lymphoma that predicts long or short survival time, but it is not from the malignant cells; it is from cells of the immune system that attack the tumor.

The scientists also found a molecular signal in follicular lymphoma that predicts long or short survival time, but it is not from the malignant cells; it is from cells of the immune system that attack the tumor. They found a favorable type of immune cell and an unfavorable type. "It tells us how the tumor is interfacing with the immune response," Staudt said, and brings them closer to knowing the critical factors that are responsible for a good or bad prognosis.

From Prognosis to Treatment

Wilson's clinical trials incorporate molecular analysis of biopsies to help choose the right treatment for each patient and shed light on the patient's response to treatment.

"We get clues from microarrays that suggest that certain pathways are very important," Wilson said. For example, Staudt's group found that in activated B-cell DLBCL, levels of a transcription factor called NF-kappaB are abnormally high. Cancer cells use NF-kappaB to turn on a number of different genes to block cell suicide and remain immortal.

"Based on Lou's work, we combined an inhibitor of NF-kappaB with chemotherapy and tested it in patients," Wilson said. Preliminary results show that pairing this drug called bortezomib with chemotherapy worked much better in the activated B-cell group, which often does not do well with standard therapy.

"The hope is that we can understand some common pathways that seem to be active in patients who don't have a good outcome and identify targets within those pathways to interrupt them," Wilson said.

In several lymphomas, a protein called bcl-2, which also blocks cell suicide, is overactive. The CCR team collaborated with Abbott Laboratories to test its newly designed bcl-2 inhibitor, ABT-263. In lab tests, all of the cell lines that had high levels of bcl-2 were killed with this drug. Wilson is now testing the experimental drug in clinical trials for patients with follicular lymphoma and other lymphomas. "That's the goal," Wilson said, "to have very thoughtfully chosen, targeted, designer drugs."

Wilson is also adjusting the timing of the chemotherapy to hit the cancer when it is weakest. He has developed a regimen called DA-EPOCH-R for patients whose lymphoma has a high proliferation signature, meaning the cancer cells are growing rapidly, which leads to a bad outcome for the patients. Instead of giving chemotherapy in the standard way, which is a 30-minute infusion, they changed the scheduling to a 4-day continuous infusion to overcome the proliferation effect. "If the drugs are in the body for four days, they can trigger cell death when the tumor is duplicating its DNA and is more sensitive to cell suicide signals. "

Finding the Achilles Heel

The Staudt team has added RNA interference (RNAi) to their toolbox, with exciting results. RNAi is a technology for silencing specific genes to observe the impact on cancer cells. It is helping the researchers find genes that keep cancer cells active and could be potential targets for anti-cancer therapies. They published a paper in the May, 2006, issue of N*ature* describing how they used RNAi to find three proteins critical to turning on the NF-kappaB pathway in activated B-cell-like DLBCL cells, but not germinal center B-cell-like DLBCL cells. The proteins, Card11, Malt1, and BCL-10, when interfered with, turn off the NF-kappaB pathway. This gives the researchers many new ways for attacking the pathway with small molecules.

"Once you find the right pathway genetically, it's impressive how easy it is to kill the cancer cells," Staudt said. "These tumors are addicted to these signaling pathways in ways normal cells are not.

RNAi is what I spend most of my days thinking about," explained Staudt. "It's teaching us things we didn't know about cancer cells."

Elaine S. Jaffe, M.D.

(*Photo: Rhoda Baer Photography*)

Louis M. Staudt, M.D., Ph.D.

(*Photo: Rhoda Baer Photography*)

Wyndham Wilson, M.D., Ph.D.

(*Photo: Rhoda Baer Photography*)

> "These tumors are addicted to these signaling pathways in ways normal cells are not."

Molecular profiling is still a research tool, but Staudt hopes to move profiling into widespread clinical practice, so he is partnering with other members of the LLMPP and Roche Molecular Systems to do just that. Working together, this public-private partnership will study 2,000 to 4,000 lymphoma samples over the next four years via whole genome expression profiling. They aim to create a clinical diagnostic tool that can be used beyond the experimental context. "It's a very ambitious effort that will have to be approved by the FDA to show it is accurate and reproducible," Staudt said. "But it's the logical outcome of all the work we've been doing."

Jaffe agrees. "More and more diseases are being defined at the molecular level," Jaffe said. "The pathologist will ideally base the diagnosis not just on morphology seen through a microscope, but on information revealed by immunohistochemical tools (which can identify cells by the characteristic markers on the cell surface) and eventually molecular tools, which are becoming more and more a part of the diagnostic pathology lab."

Kimberly McAllister knows the importance of a correct diagnosis. She was told she had non-Hodgkin's lymphoma (NHL) in January 2001—three months after the birth of her second son. The traditional therapy for NHL, a four-drug combination referred to as CHOP, was not doing the job. "Initially I seemed to be responding, but in March, my tumors started growing again," said McAllister. "It was obviously an aggressive tumor and they weren't sure what the next step would be."

She started doing research on her own and talking to lymphoma experts around the country. McAllister works at the NIH's National Institute of Environmental Health Sciences in Research Triangle Park, N.C., and she knows the power of research—asking questions and getting as much information as possible.

She and her husband Robert went to Bethesda and met with CCR's Wyndham Wilson, M.D., Ph.D., to talk about enrolling in a clinical trial. He brought in Elaine Jaffe, M.D., to read the slides from a new biopsy. Rather than NHL, Jaffe determined that the 35-year-old had Hodgkin's lymphoma. With the new diagnosis, Wilson recommended McAllister switch to a different set of four drugs called ABVD. She returned home and began the new regimen at the end of April. By the third week of June, new scans revealed the cancer was gone.

"It was a good thing I went on my own to NCI," she said. "If the diagnosis hadn't been cleared up, I would've continued to be treated for the wrong thing," said McAllister, who switched from being a lab scientist to managing grants in genetic epidemiology and genetics so she could spend more time with her boys Ryan and Evan, who are now ages six and nine. Charting her experience in annual Christmas cards to family and friends, McAllister wrote in 2006 that, for the first time since her diagnosis, she finally believes she will live to see her sons grow up.

McAllister wanted the CCR team to feel her appreciation. She wrote to Jaffe: "Do you know what you were doing five years ago? You were saving my life."

(*Photo: Courtesy of Kim McAllister*)

Kimberly McAllister (right, with husband Robert and sons Evan and Ryan) credits CCR's lymphoma team with saving her life with the right diagnosis and the right treatment.

Seeing the Invisible:

Understanding Structure to Understand Disease

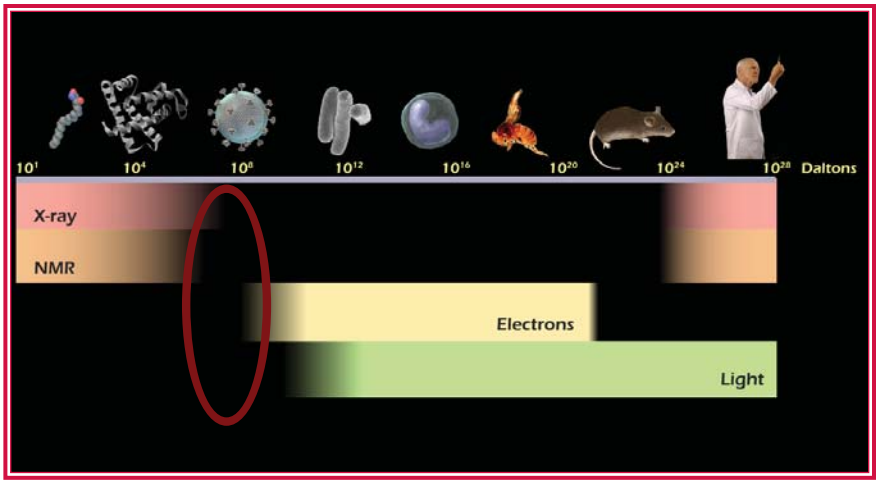
Vision is the art of seeing what is invisible to others. – Jonathan Swift

The submicroscopic world is yielding its secrets as new technologies probe further into its depths. "Seeing" this world is more than an exercise in intellectual curiosity: it is critical to understanding basic biology and the diseases that are rooted in subcellular space. Scientists in the Biophysics Section and Unit, in CCR's Laboratory of Cell Biology, are developing technologies with ever keener eyesight, capable of offering amazing glimpses into the nanometer-sized world of macromolecular complexes. These efforts go beyond simple technological advances, drawing on the collaborative expertise of biochemists, geneticists, physiologists, and computational modelers both at NCI and in the external scientific community.

Bridging the Gap

"Although genetics, physiology, and biochemistry are important tools for understanding and predicting the behavior of living cells and small organisms, we need to understand structure to complete the picture," said Sriram Subramaniam, Ph.D., Head of the Biophysics Section. "Our goal is to interpret cells at the level of molecular resolution."

Ever since Anton von Leeuwenhoek first saw bacteria through his finely ground glass lenses in 1676, visual technologies have steadily improved to the point of seeing even small viruses and cellular organelles. At the same time, X-ray diffraction (aka X-ray crystallography) and nuclear magnetic resonance have allowed us to "see" the structure of individual molecules, even ones with fairly complex structures. Yet there remains a significant—and critical—visual gap between individual molecules and subcellular organelles and organisms (Figure 1). This gap is where the action happens (i.e., the space where viruses perform their destructive function or where disease breaks down normal biological processes). And it is precisely on this gap where Subramaniam and his colleagues have trained their technological sights.



(*Image: Sriram Subramaniam*, CCR)

Figure 1: The Subramaniam laboratory aims to illuminate the gap between X-rays and electrons, where chemistry becomes biology.

The laboratory has woven studies in three interrelated, yet diverse areas—bacterial chemotaxis, HIV structure and infection, and development of new technologies for cancer imaging—in "a combination which you could only do at CCR," said Subramaniam. These studies are providing the basis for pursuing an entirely new way to understand and treat many different diseases, including HIV and cancer.

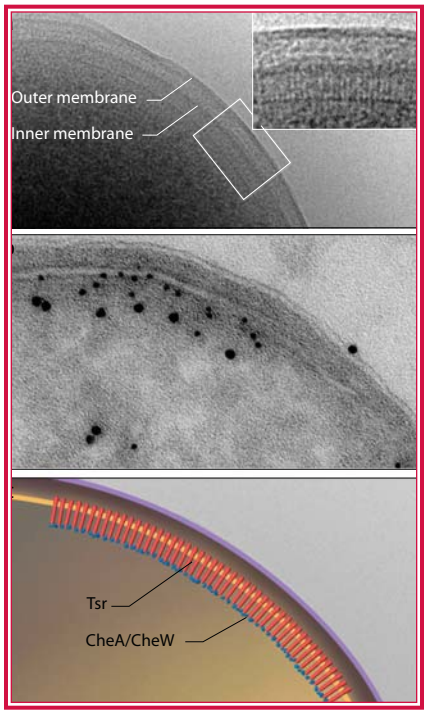
What the three share in common is a combination of powerful new software algorithms married to ever more precise electron microscopy (EM) techniques (see "Penetrating Vision: The Next Generation of Observational Tools").

Bacterial Chemotaxis: How Do Cells Respond to Their Environment?

Despite their small size, bacteria are not passive participants in their environmental niche. Motility is essential to their survival, as they need to sense both the good stuff (e.g., food) and the bad stuff (e.g., harmful chemicals) and move appropriately, a behavioral trait known as chemotaxis. This process is mediated by the binding of external molecules to so-called bacterial "chemotaxis receptors," which in turn set off an internal signaling pathway of molecules that ultimately revs the motors that drive the bacterial flagella. Understanding the spatial and temporal structure of chemotaxis is important not just for the sake of appreciating bacterial movement, but also because cellular sensing of environmental cues is a common evolutionary trait, from bacteria through human immune cells. Joining structural information to what is known about the genetics, physiology, and biochemistry of bacterial chemotaxis apparati should reveal basic principles about the machinery of cell signaling.

(*Photo: Feinstein Kean Healthcare*)

Collaborations like those between Subramaniam and Jacqueline Milne, Ph.D. (left), are helping researchers develop the complex visualization and computation tools to solve "simple" structural problems like how surface receptors physically transduce external signals to a cell's chemotactic machinery.



(*Image: Sriram Subramaniam*, CCR)

Figure 2: The physical relationships of the *E. coli* proteins CheA and CheW—which help drive the bacterium's chemotactic response—were only recently brought to light through the use of electron microscopy techniques developed in the Subramaniam laboratory. Tsr is an additional component of the *E. coli* chemotaxis mechanisim.

A great deal of work has been done in understanding the structures of individual components of the chemotaxis machine in the common bacteria *Escherichia coli* (*E. coli*), but how they assemble and work has been a mystery. Subramaniam and his colleagues have employed the visualization technology they have developed to make amazing progress in revealing the chemotactic machinery's structure in intact *E. coli*. A series of manuscripts over the past two years, the most recent published in the March 6, 2007, issue of the *Proceedings of the National Academy of Sciences*, describes a cluster of receptors in an extended lattice that is dependent on the interactions of two critical *E. coli* signaling proteins, CheA and CheW (Figure 2).

Working together with Jacqueline Milne, Ph.D., in CCR's Laboratory of Cell Biology, the scientists are now focusing their attention on looking at how these complexes transduce environmental signals at the level of molecular structures (i.e., how the individual receptors work in the context of their location within the entire chemotaxis structure). This "simple" structural problem requires continual technological improvement in visualization and computational tools, as well as ongoing collaboration of the computational modelers and technologists with the biochemists and physiologists within and outside of the laboratory.

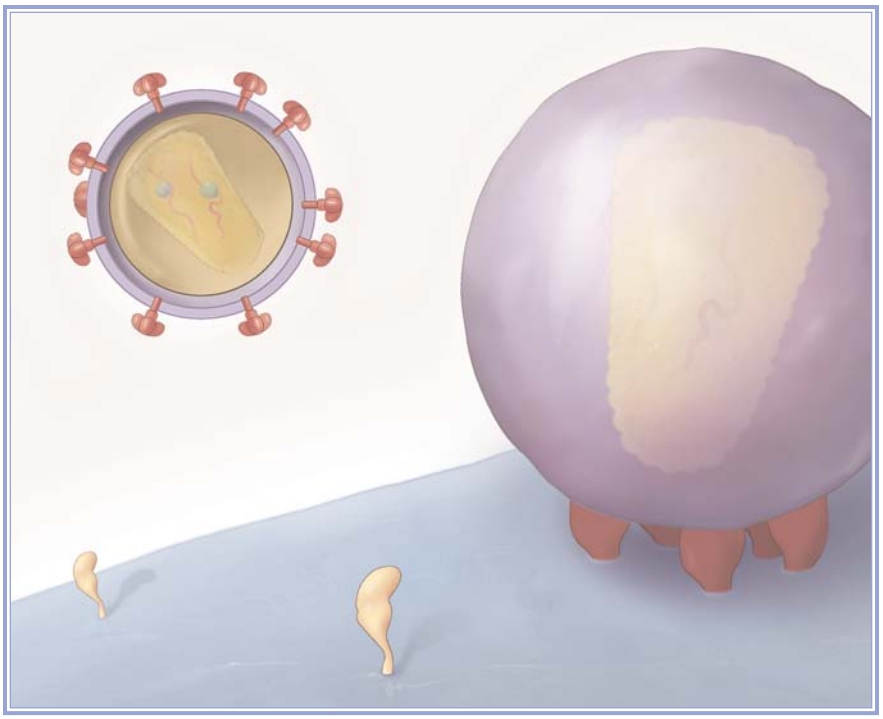
> Joining structural information to what is known about the genetics, physiology, and biochemistry of bacterial chemotaxis apparati should reveal basic principles about the machinery of cell signaling.

A Good Look at a Viral Foe

HIV's devastating ability to evade the immune system's attempts to control or remove it is primarily linked to its structure and how it interacts with its host's cells. This trait is largely a function of one specific HIV protein (gp120) interacting with a specific target cell receptor (CD4), with subsequent viral and host proteins getting involved to allow infection following the initial binding. It is known that some antibodies and certain drugs can interrupt this binding, but not always effectively. Understanding the actual structure of this interaction (and the subsequent series of events) could lead to more effective ways to prevent it.

In the fall of 2004, the CCR scientists began research aimed at visualizing the series of structures involved in HIV infection. Their interests included understanding the actual conformation and distribution of the gp120 proteins on the viral surface, the interaction of different kinds of antibodies with those proteins, the binding of gp120 to CD4, and the functional structure of the infection machinery. Over the past year, research into each of these issues has begun to yield new—and surprising—information about HIV and its molecular interchange with its host target cell.

One particularly striking recent finding is the actual physical structure of the viral coat proteins. Although there are atomic models of gp120 in both its CD4-bound and -unbound states, there is still significant uncertainty about what the actual surface proteins look like in their native state and how they are distributed around the viral coat. It is thought that the gp120 spikes on the viral coat are composed of three gp120 molecules in a "trimer" formation. However, retroviruses such as HIV are more heterogeneous in their structure than other virus types, which undoubtedly contributes to their success at eluding immune responses. Thus, it is critical to uncover the actual structure, both for the sake of developing potential new and effective drugs and for understanding the immune responses to HIV that need to be elicited by a vaccine.



(*Image: Sriram Subramaniam*, CCR)

Figure 3: The HIV "entry claw," a unique structure the virus uses to grasp cells it seeks to infect. The claw was discovered using 3D software tools for electron tomography developed in the Subramaniam lab.

To address this question, Subramaniam and his colleagues first developed new computational tools that can rigorously classify three-dimensional structure data derived from electron cryo-tomography techniques. Bringing these tools to bear on the viral coat proteins, the scientists have found a unique feature—an "entry claw"—that HIV uses to grasp onto and infect its host (Figure 3). These findings, published in the May, 2007, issue of PLoS *Pathogens*, open up new pathways to understanding HIV and thus will have a major impact on future drug and vaccine discovery efforts.

Ongoing work on HIV-host interactions is focused on the molecular machinery by which HIV actually gains access to the cell, how neutralizing antibodies exert their effects, and the structural mechanisms of action of current experimental HIV drugs that are believed to inhibit viral entry.

HIV's devastating ability to evade the immune system's attempts to control or remove it is primarily linked to its structure and how it interacts with its host's cells.

Building on the Foundation

Although the Subramaniam laboratory chose bacterial chemotaxis and HIV structure as the initial problems to focus on in concert with developing the necessary tools,

the goal is the refinement and application of these powerful new techniques to many areas of biology and disease research. Current projects in the lab include cancer cell imaging at resolutions approaching 20 nm (about 15 times better than can be achieved with current confocal techniques), structural analysis of the mechanism of melanosome transfer in the skin (which is related to the development of skin cancer), nanoparticle detection and 3D tissue localization, building further automation into EM workflow (thereby allowing relatively high-throughput of samples and collection and interpretation of data), and the adaptation of these techniques for use in pre-clinical and clinical diagnostics. In one exciting recent advance, the lab has developed techniques for imaging entire cancer cells and visualizing how various internal organelles such as mitochondria are organized—a major step toward defining some of the structural hallmarks of cancer at the cellular level.

Although the implications for clinical application of these technological advances are astounding, at heart this is still a basic life sciences laboratory interested in answering fundamental questions about biology and disease. To that end, the Subramaniam lab is comprised of about 15 scientists—about half of whom are students—with a diversity of experience and expertise, including electrical engineering, physics, and biochemistry (see "Laboratory Visionaries"). These fields would not interact regularly in any other setting, but within Subramaniam's domain, they work well together. "I believe that integrating all these expertises is the only way to do this kind of research successfully," said Subramaniam, who himself has experience in very different scientific areas. "Only at a place like CCR can you build something like this group of people and give them the freedom to produce great things."

For more information and astounding peeks at the submicroscopic world, visit the lab's Web site: http://electron.nci.nih.gov.

Penetrating Vision:

The Next Generation of Observational Tools

Electron Cryo-Tomography

Although the first electron microscope (EM) was developed in 1931 by Max Knoll and Ernst Ruska at the Technical College in Berlin, it was not until 1968 that it became feasible to generate three-dimensional (EM) images, a technique known now as electron tomography. Such images are created by taking a single EM image of the specimen being studied, and then tilting it slightly to take another image. A series of these images can then be recombined into a three-dimensional structure using software designed specifically to handle such image data.

Initial attempts to image native biological specimens, uncompromised by fixation procedures, were not successful. This was largely because the harsh environment of the EM chamber (high vacuum and electron beam bombardment) was too much for fragile cells, which were usually torn apart before they could be imaged. Improvements in cryo-preservation techniques in the 1980s finally allowed some cells to survive the EM chamber long enough to provide useful images. This technique, known as electron cryo-tomography, was a breakthrough in imaging cellular structures in their native form.

Constant improvements in both the technology (cryo-preservation methods, low-dose electron beams, and more sensitive electron detectors/cameras) and in the analytical software that reconstructs the images into a 3-D model are pushing the resolving capabilities of electron cryo-tomography ever smaller, filling the gap between biophysical methods and more traditional electron microscopy.

Ion-Abrasion Scanning Electron Microscopy ("Dual Beam")

The National Library of Medicine's "Visible Human Project" is a computer-assisted virtual 3-D atlas of the human body. The Project makes it possible to examine a real body, either male or female, from almost any angle, in three dimensions. The data that feed this tool are a series of images taken of a donor body that was sliced from the top, a millimeter at a time (or less, for the female body), with a picture taken at each slice.

Ion-abrasion scanning electron microscopy (IA-SEM), called "dual beam" in laboratory shorthand, is based on the same basic principle as the Visible Human Project, though on a much smaller scale: In this case, the slicing is done by an electron beam followed by a scanning electron photograph after each slice. Described last July by Subramaniam and his colleagues in the *Journal of Structural Biology*, IA-SEM offers the resolution of subcellular organelles an order of magnitude greater than can be achieved by other techniques. In addition, it works on whole specimens, eliminating the need for generating thin sections of the specimen to use traditional EM approaches.

The FEI Company, a long-time maker and supplier of electron microscopy and related products, is collaborating with the Subramaniam lab in developing this new technology.

Laboratory Visionaries

(Photo: Rhoda Baer Photography)

Sriram Subramaniam, Ph.D., Senior Investigator

When Sriram Subramaniam came to CCR in 2000, NCI was actively looking to build its capabilities in high resolution imaging at scales that had not yet been attainable. Although he had experience in studying the structure of membrane proteins, including rhodopsin, Subramaniam did not have a great deal of experience in electron microscopy. "CCR took a huge risk," he said. "I was learning even as I was building these capabilities." By all accounts, that risk is paying off.

Subramaniam completed his doctorate at Stanford University in 1987, followed by postdoctoral work in the laboratory of H. Ghobind Khorana, Ph.D., at MIT. He then joined the faculty at the Johns Hopkins School of Medicine (where he is still a visiting associate professor) prior to coming to CCR—and back to bench work. "I never wanted to be a manager; I need to stay close to the science," said Subramaniam. "Other places I was writing grants while everyone else did the science. Here I can be at the bench, as well as mentor some amazingly talented students who will drive the future applications of this work."

Subramaniam also notes that the work in his laboratory has benefited immensely from the close collaboration with Jacqueline Milne, Ph.D., who has pioneered work on studying large multiprotein assemblies using electron cryo-tomography. "CCR has been very supportive of this type of team science approach that has allowed us to focus intensively on difficult problems requiring interdisciplinary approaches," he said.

(Photo: Feinstein Kean Healthcare)

Adam Bennett, Graduate Student

Adam Bennett joined CCR's Biophysics Section in the fall of 2005 as an Oxford-Cambridge student in the NIH-University of Cambridge Graduate Partnerships Program (gpp.nih.gov). He earned a B.S. in chemistry from the University of Florida in 2004, and then went to the University of Cambridge (UK) on a Churchill Scholarship.

"From the age of ten, I was going to be an organic chemist," said Bennett, and his chemistry work focused on making optically pure drugs using enzymatic biological systems. However, he grew excited by the potential for the techniques being developed in the Subramaniam laboratory to define the molecular architecture and mechanisms of such systems.

Among his current projects is visualizing the mechanistic and structural basis of endosomal HIV-1 budding from macrophages.

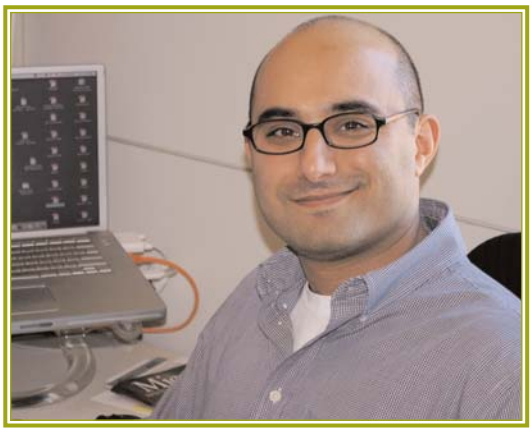
(Photo: Feinstein Kean Healthcare)

Sang Kim, Post-Baccalaureate Student

Sang Kim also joined CCR in the fall of 2005, after receiving a B.S. from the University of Maryland. His ultimate goal is medical school, but he decided to enroll in the two-year NIH post-baccalaureate program to gain greater insight into scientific method. "Medicine is not a static field," said Kim. "There are constant advances. I believe what I learn here will prepare me to understand and 'keep up' in the future."

Kim has been happily surprised by the amount and kind of work he is doing. He was particularly excited to get regular access to the new dual-beam electron microscope to perform important cancer-cell imaging studies. "They train you to become independent pretty quickly and encourage you to take the initiative," he said. "I think I've received a pretty good glimpse of how science works at its best."

Although leaving for medical school in the fall of 2007, Kim decided to forego the usual practice of taking the summer off before starting medical school: He stayed in the lab to get as much done as possible.



(Photo: Feinstein Kean Healthcare)

Cezar Khursigara, Ph.D., Postdoctoral Fellow

Cezar Khursigara did his doctoral work on *E. coli* membrane proteins using 3-D crystallography and biophysical techniques at McGill University in Montreal, Canada, and came to CCR looking for a different approach to bacterial proteins for his postdoctoral training. He had met Subramaniam at a Federation of American Societies for Experimental Biology (FASEB) meeting about eight months before he finished his degree and recalls being excited about the potential of electron cryo-tomography to elucidate the bacterial chemotaxis machinery. He contacted Subramaniam once he began his hunt for post-doctoral positions. "He remembered me, invited me to CCR to give a talk, and the rest is history," he said.

Since joining CCR, Khursigara has worked on a diverse collection of projects concerned with the structure of chemotaxis receptors and signaling complexes, including more biochemical and molecular approaches in addition to the cryo-tomography work. He is particularly struck by the diverse experience and expertise of the lab. "Sriram isn't big on position and title," said Khursigara. "If you have drive and ability, and he can provide the tools and environment to match it, then his attitude is, 'let's do this.'"

A Cancer Research Renaissance

Possibly the oldest reference to cancer can be found in a papyrus document dating from about 1600 B.C.E., *describing eight cases of breast tumors that were treated, but not cured, by cauterization. The 15th century witnessed the evolution of modern scientific investigation and its application to the study of disease, with autopsies and anatomical studies greatly improving our understanding of what goes wrong in cancer. Another three centuries passed before it was realized that some cancers might be effectively cured with surgery. In the mid-20th century, chemical methods were developed to control or reverse the tumor growth. Former CCR* Deputy Director ***H. Richard Alexander, M.D.****, reflects on the past and future role of surgery in cancer treatment.*

After 3,600 years of medical history, it is only in the last two decades that we have begun to turn the tide against cancer. I feel privileged to have witnessed many of the seminal advances that have made this progress possible while serving 17 years at CCR. The Center has been instrumental in developing anti-angiogenic and immunomodulatory therapy, and it has catalyzed a major shift in medical thought, from an anatomical to a molecular conception and definition of cancer. The technologies of molecular characterization (e.g., gene expression profiling), which have their most fundamental roots in research conducted at CCR, are increasingly used in hospitals around the world.

The impact of CCR's research is widespread, and I can even say that it has affected me personally. During my first three years at CCR, my two youngest daughters were born. Now, 15 years later, they are being vaccinated against cervical cancer because of the pioneering work in cancer prevention that was done by my CCR colleagues such as Douglas Lowy, M.D., and John Schiller, Ph.D. It is a remarkable experience both to witness and to participate in advances that ultimately support the health of your own family.

When I first joined CCR in 1990, I took a position in the Surgery Branch with limited responsibilities, mostly consisting of implanting long-term venous access devices for patients in the clinical center. When not performing procedures, I worked in the laboratory of Jeffrey Norton, M.D., who was then the Head of the Surgery Branch's Surgical Metabolism Section. Over the years, I moved into positions of increasing responsibility, eventually taking over for Jeff in the Surgical Metabolism Section and becoming Deputy Director of CCR.

> Surgery has coevolved with molecular biology to enable effective treatments, personalized care, and preventive strategies.

While the treatment of cancer using molecular biology was advancing rapidly, we have continued to develop innovative surgical approaches. For instance, at CCR my colleagues and I developed novel techniques for isolated perfusion—a surgical method of isolating the blood circulation of regions of the body using an extracorporeal bypass circuit (similar to the way a heart-lung machine shunts the flow of blood during heart surgery) and perfusing the isolated region with a high dose of cancer drug.

The advantages of isolated perfusion are several-fold. By completely separating the blood supply of an organ or region that you are treating from rest of the body, you can intensify the dose of cancer agents within the region until the normal tissues cannot tolerate any more. For instance, one of our more exciting lines of work involved the use of tumor neurosis factor (TNF) as a therapeutic agent in conjunction with isolated perfusion. TNF was known to be a very potent agent in laboratory animals; in the 1980s and 1990s, there were high expectations that it would be a successful anti-cancer agent in humans as well. The problem was that humans are exceedingly sensitive to the toxic effects of TNF, and the high doses of the drug required to make the cancer regress often have debilitating effects.

We were struck by the preliminary findings reported by a group of European investigators who used TNF with melphalan in isolated lymph perfusion. They saw extraordinary results in their isolated lymph perfusion studies: a 90 percent complete response rate for patients who had extremity in-transit melanomas or inoperable high grade extremity sarcomas. At CCR, we wanted to confirm and expand on those observations, hopefully

applying them to different isolated regions of the body. We first conducted animal studies to see if TNF in isolated liver perfusion could treat cancers confined to the liver, and we found it to be very effective. We also performed the procedure in several hundred patients and found that it could cause regression of very advanced cancers.

By focusing TNF delivery to one organ or region of the body that needs it, we were able to use TNF at therapeutic levels—in fact, at levels far greater than what could be tolerated if TNF were given as a systemic agent—while circumventing the cytokine's toxicity and making some other discoveries about its mechanism of action. We found that TNF was not directly effecting on the cancer, but rather had some dramatic effects in inhibiting a tumor's ability to stimulate blood vessel growth (thus preserving its supply of oxygen).

Isolated liver perfusion can have a significant impact on patients, because many different types of cancers can lead to liver metastases. For example, of the 150,000 new colorectal cancer diagnoses in the U.S. each year, up to 30,000 will develop metastases in the liver. I worked with a number of surgeons at CCR to help them develop isolation perfusion in other organs such as the lungs and the kidney, or within the peritoneum.

This method requires further development and remains a complex experimental procedure, but other centers are beginning to try it, including the University of Maryland. The progress that has been made with the procedure thus far, though, was only possible because of the resources available at CCR. Critical to our success were state-of-the-art animal laboratories in which we could develop the isolation perfusion method. We were able to collaborate with other CCR investigators to conduct necessary pharmacokinetic studies, and we employed novel imaging methods to track tumor response. Investigators from the Laboratory of Pathology helped us analyze the tumors using tissue protein arrays (a means of measuring the levels of a large number of proteins directly from the tumors), which allowed us to predict the treatment responses that we would see many months later in the clinic. It was a significant effort with shared commitment from so many different experts and services at CCR.

The field of cancer surgery has evolved in directions that I would not have anticipated 17 years ago. Surgery was the mainstay of cancer treatment for many years, before the development of radiation, chemotherapy, biologic therapy, and anti-angiogenic therapy. Today, surgery is used as part of an integrated treatment program for individuals with solid organ cancers. Surgery has coevolved with molecular biology to enable effective treatments, personalized care, and preventive strategies. Like other surgeons, I find myself in a position of having not only to evaluate patients for a surgical procedure and conduct that procedure safely, but also to understand the greater biological context of their tumors.

With more effective cancer drugs and chemotherapeutics, we can combine the strengths of two different approaches: surgery to remove the bulk of the visible tumor, and chemotherapeutics to eliminate microscopic remnants or metastases. Until recently, if a cancer reached the stage of metastasis, the window of opportunity for surgical intervention was basically closed. Now, we are beginning to put together innovative combinations of surgical procedures and chemotherapy that may translate to better outcomes for the individual patient.

Increasingly, the tissues that surgeons remove are being used for genetic and molecular profiling to understand what types of specific agents or treatment strategies would be most effective for that particular tumor. We are well on our way toward the goal of personalized medicine, in which patients are given tailored therapy based upon the molecular characteristics of their tumors.

Using genetic and molecular profiling, we can also identify individuals who are at high risk of developing certain cancers before they arise and perform preventative operations, removing either the ovaries, uterus, colon, thyroid, or breast. Most recently, there have been reports of families who have undergone preventative gastrectomy because of known genetic mutations that predispose them to gastric cancer. The concept of preventive cancer surgery was unheard of two decades ago, made possible only with the advent of molecular profiling.

As I carry on my work at the University of Maryland Greenebaum Cancer Center, there are many aspects of CCR that I have brought with me. These include a strong emphasis on evidence-based medicine, compassionate care for the cancer patient, an environment of respect and open collaboration among colleagues, and an openness to doing things differently and with a commitment to progress. The "CCR approach" has already helped to transform much of our work at UMD in terms of the productivity of our clinical and laboratory research programs.

I continue to collaborate on new initiatives with many of my colleagues in the Surgery Branch and the Laboratory of Pathology at CCR, while also continuing some of the existing initiatives that we had started several years ago, particularly in the area of isolation perfusion and proteomic tumor profiling. We are also exploring ways to foster collaboration between clinical investigators here at UMD and their appropriate counterparts at CCR. The UMD Medical Center is a large facility and sees a very large number of patients, who come in with a variety of diseases that represent opportunities to answer important questions. CCR certainly has the tools to help answer those questions and continue to contribute to the treatment and prevention of cancer in significant ways.

(*Photo: Bill Branson*)

H. Richard Alexander, Jr., M.D.
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Shrinking Prostate Tumors by Starvation

William L. Dahut, M.D., has always straddled both the clinic and the lab. He completed his clinical training in internal medicine at the National Naval Medical Center in Bethesda, Md., and in hematology and medical oncology at the Bethesda Naval Hospital and the Medicine Branch of NCI. When offered the opportunity to join CCR's Medical Oncology Branch and a clinical program in prostate cancer research, Dahut leapt at the chance. The draw was CCR's unique intramural program, in which the science drives the design and implementation of clinical trials.

When a man develops prostate cancer, he can expect any of a number of possibilities. The good news is that fewer than 10 percent of the estimated 230,000 men diagnosed in the U.S. each year succumb to the disease. In essence, there is life after a prostate cancer diagnosis.

Unfortunately, researchers have not yet found a cure. At CCR, we are working on the cutting edge to develop those cures. In the interim, there is treatment. It can delay the progression of disease, sometimes for a lifetime. Treatment also helps relieve pain and other complications that arise as the disease progresses.

Prostate tumors can grow slowly or more aggressively. They can remain contained in the glandular region, where the tumor originates. Or they can spread (metastasize) to other locations in the body, mainly the bones.

Long before patients arrive at NCI, their local physician probably detected the first signs of a problem—a continued rise in the blood levels of prostate specific antigen (PSA), a marker for replicating prostate cells (see "PSA and Its Vaccine Potential?"). A biopsy would have confirmed the diagnosis of cancer. The next step would have been either surgery, radiation therapy, or, if the tumor was growing very slowly, "watchful waiting" (simply monitoring the tumor over the course of time).

In about a third of these cases, however, the disease recurs or progresses, as signaled by a rise in PSA. At this stage, the malignancy is still not life-threatening. But once it spreads outside the prostate, as detected by a bone scan, the situation becomes more serious. At this stage, we say that the patient has metastatic prostate cancer and give him a choice: drugs that block the action of the male sex hormone testosterone (which can fuel prostate cancer growth), or orchiectomy (surgical castration).

If PSA levels begin to rise again, we consider the patient "castrate resistant." He may benefit from second- or third-line agents that block testosterone or its receptor on prostate cancer cells. But not everyone responds well to these therapies. At this point, patients come to us at NCI, where we are working with men whose tumors have metastasized to discover unique treatments and strategies that can delay the progression of disease.

At the Cutting Edge

Outside NCI, patients with metastatic prostate cancer are usually prescribed a regimen of chemotherapy, primarily a combination of an agent called docetaxel and the steroid prednisone. Patients receive this treatment every 21 days. But chemotherapy does not cure the disease; in some cases, it does not even prolong survival.

Our lab at the Medical Oncology Branch of CCR, in collaboration with others such as William Figg, Sr., Pharm.D., Senior Scientist and Head of the Molecular Pharmacology Section, is looking for other options. We have chosen to let the molecular discoveries made in our laboratories guide the design of our clinical trials, an approach that is made possible at CCR by its close connection (both in location and collaboration) to the clinics at NIH's Clinical Center.

Blocking Blood Vessels—Starving Tumors

A decade ago, we became intrigued by the concept of angiogenesis, or blood vessel growth and development. In 1971, Judah Folkman, M.D., at Children's Hospital Boston (CHB), published a seminal paper proposing that solid tumors need a supply of blood vessels to sustain their growth. Tumor cells create these blood vessel networks by producing so-called angiogenic proteins, molecules such as vascular endothelial growth factor (VEGF) that promote the shaping and sprouting of new blood vessels. Folkman hypothesized that if oncologists could somehow block

The Dahut team (clockwise from center front): Dahut, Lea Lathan, R.N.; Jackie Jones, R.N.; Phil Arlen, M.D.; James Gulley, M.D.; Yanh-Min Ning, M.D.; Kim Scott, R.N.; Marica Mulquin, R.N.

angiogenesis, they could starve tumors, and so shrink them. His laboratory went on to purify the first angiogenic tumor protein, discover the first molecules that could inhibit angiogenesis, and initiate clinical trials of anti-angiogenic therapies.

In 1994, Robert D'Amato, M.D., Ph.D., then in Folkman's CHB laboratory, demonstrated that the drug thalidomide inhibited angiogenesis by blocking fibroblast growth factor, a molecule that stimulates cell reproduction. While thalidomide was withdrawn from the market 30 years ago after it was linked to birth defects, researchers in the last two decades started looking at thalidomide as a potential anti-cancer drug, thinking that if thalidomide could prevent new blood growth to prostate tumors, it might provide a means to shrink them, and so help patients achieve remission.

We decided to apply these concepts to prostate cancer, designing a "hypothesis driven" clinical trial that used the basic science on thalidomide's putative anti-angiogenic capabilities to make predictions as to how it might act on prostate tumors in patients.

In our first trials, we learned that thalidomide alone is not enough by itself to stop prostate tumor growth. But we found that when we combined thalidomide with standard chemotherapy (docetaxel), more than half of patients experienced a 50 percent or greater drop in PSA levels after 26 months of treatment, compared to slightly more than a third of those treated with docetaxel alone. Even more promising, the combination of docetaxel and thalidomide prolonged overall survival.

> We have chosen to let the design of our clinical trials be guided by the molecular discoveries made in our laboratories, an approach that is made possible at CCR by its close connection (both in location and collaboration) to the clinics at NCI's Clinical Center.

While this research was a step forward in improving pain management and survival, the treatment still was not a cure. Thus, we opened our third and current metastatic prostate cancer trial in 2004, this one combining two anti-angiogenic compounds (each with a different mechanism of action) with docetaxel and prednisone. While we know that thalidomide can hinder blood vessel growth, its precise tactics for doing so are still unclear. Thus, we hypothesized that we might improve our metastatic prostate cancer treatment even further by adding another anti-angiogenic drug, bevacizumab (Avastin®, Genentech), which works through a different biochemical pathway.

Our preliminary results show clearly that this combination is our most active yet. After receiving the combination in 21-day cycles, nearly every patient enrolled in the trial has experienced a drop in PSA levels of at least 50 percent. Typically, patients at this stage of disease survive about 18 months when treated with chemotherapy alone. Thus far, three-fourths of trial participants have passed the 18-month mark. As we go

forward with the analysis, we are accruing the data to support innitiation of a larger randomized trial including thousands of patients. And while patients do experience side effects, which are to be expected as we add more drugs to a regimen, most find that the benefits of therapy outweigh the side effects.

Another Vein of Trials

One caveat is that patients enrolling in these anti-angiogenic trials cannot have undergone chemotherapy before enrolling, as previous treatment would confound the results. Many patients who come to NCI do not fit that eligibility criterion, having exhausted their chemotherapeutic options beforehand. For these men, we have another clinical trial, this one focused on a small molecule called AZD2171 (Recentin™, AstraZeneca). It acts similarly to bevacizumab, which targets VEGF, except that AZD2171 actually targets the receptor for VEGF. One major benefit of AZD2171 is that patients can take it at home as a pill once daily for 28 days; with bevacizumab, they have to travel to NCI every three weeks for infusions.

Early data shows that AZD2171 can shrink tumors in patients' lymph nodes, another common site for metastasis. This activity gives us an opportunity to see whether we can use measurements of blood flow as a surrogate marker for blood vessel growth and, by extension, anti-tumor activity. If we can correlate changes in blood flow to stalled blood vessel growth to anti-tumor activity, we will have a way to better monitor patients' progress as well as a better understanding of disease progression and drug action.

The Road Ahead

In the future, we hope to personalize our prostate tumor research by studying the unique biology of each patient's tumor and the possible genetic differences that not only cause each tumor to grow at different rates, but also cause each person to respond differently to therapy. Researchers are genetically comparing tumor and normal tissue and are looking for differences in gene expression and markers of metabolism of various drugs. This tailored approach to medicine is just over the horizon in other types of cancer.

Prostate cancer research is often hindered by the difficulty in obtaining tumor cells from biopsies, which in our case have to come from bone since most of our patients have previously undergone surgery to remove the prostate (prostatectomy). We are working with collaborators on a method to capture cancer cells that have escaped the tumor and are circulating throughout the blood stream. Such an advance would improve our ability to conduct the kinds of molecular studies that will let us match the biology of the tumor to the age, cancer stage, and health of individual patients.

As we move forward, we have a clear goal in mind: to develop treatments that are beneficial for the patient and, at the same time, advance the field of cancer research. At CCR, we have the unique ability to determine not only if the drugs are working in patients, but also why they are working (or not), thanks to our close connection to the lab. We can only achieve this feat because of the heroes—the patients who volunteer to join our trials (see "Patient Perspectives").

PSA and Its Vaccine Potential?

At the most basic level, cancer starts as healthy cells gone awry. In many cases, these cells are still able to produce the proteins that they produced as normal cells, but in higher amounts. This difference is what has made prostate-specific antigen (PSA) a valuable tool for the last 20 years. PSA is normally produced by healthy prostate cells. However, as prostate cells turn cancerous and begin to increase in number, so does the level of PSA; this rise can be measured in the blood with the PSA test.

In addition to its value as a biomarker, researchers like Jeffrey Schlom, Ph.D., Head of the Immunotherapeutics Group in the Laboratory of Tumor Immunology and Biology at CCR, look at PSA as a means of creating prostate cancer vaccines. Unlike vaccines for influenza or chickenpox, though, these vaccines are therapeutic, not preventative. Schlom's group has come up with eight vaccines by inserting the PSA gene into large poxviruses (e.g., vaccinia, fowlpox), which are able to deliver considerable amounts of genetic material. When injected into patients, the viruses carry the gene into the body and trigger an immune response against the PSA-carrying prostate cancer cells. Other strategies include combining vaccines with hormonal therapy, radiation, chemotherapy, or, most recently, molecules that take the brakes off the immune response.

Schlom and his collaborators, including Clinical Immunotherapy Group directors Philip Arlen, M.D., and James Gulley, M.D., Ph.D., are targeting men who are castrate resistant (no longer respond to hormonal therapy) but whose tumors have not yet metastasized. The vaccine project also involves the design and development of novel immunoassays to analyze patients' immune responses both pre- and post-vaccination.

Patient Perspectives

Lenny Renner

For Lenny Renner, 63, it all began with a cholesterol test. In 2003, he went to his doctor's office in Minneapolis, Minn, simply to get blood drawn in a typical wellness check.

"And while you're at it, why don't you take my PSA?" Renner said, referring to the protein that signals whether a man may have prostate cancer.

The test came back with a high value, a sign that a tumor might be growing. His doctor felt Renner's prostate and found a lump. A local urologist confirmed the suspicion of cancer. But the definitive answer came after a local oncologist took a biopsy and made a positive diagnosis.

When given the options, Renner chose to have his cancerous prostate removed. During the procedure, however, his oncologist noted that the cancer had spread to Renner's bladder. That brought the more serious diagnosis of "metastatic prostate cancer" and the suggestion that Renner travel to Bethesda to join a prostate cancer trial at NCI.

Renner made his first trip in June 2005, expecting that because he was entering a research hospital, "people would be cold, aloof, clinical." But he experienced the opposite. Physicians, nurses, and staff were "the nicest, friendliest caretakers." A nurse gave him a hug. "It feels more like they are on your side," he said, "not like they are looking at me as if I am a guinea pig."

Renner chose to enter a trial of a cancer vaccine (see "PSA and Its Vaccine Potential?"). Given that he is the kind of person who "hated even taking aspirin for a headache," he liked the idea of using his body's own immune system to "fend off" the cancer. But after four months, his PSA levels began to rise again.

Thus, Renner joined William Dahut's combination clinical trial (see main text), taking two anti-angiogenic drugs, chemotherapy, and a steroid.

So far, the signs are good. Renner's PSA has "fluctuated a bit" but stayed within a healthy range. A pain in his hip—caused by the spread of his tumor cells—has now dissipated. And while he has experienced some side effects, he takes it all in stride. He knows that while he will not be "cured" with today's level of medical technology, he is not "terminal." Thus, he accepts that treatment at CCR, which he calls "the best in the country, if not the world," is now "a part of my life."

That acceptance has made him more philosophical. "I am hoping that regardless of my outcome," he said, "others will get some benefit out of my participation in this important research."

His advice to others considering clinical trials at CCR: "If there is any way to swing it, including the travel, I would highly recommend it."

David Thorpe

For David Thorpe, 69, a diagnosis of prostate cancer was the beginning of a journey, full of highs and lows, triumphs and disappointments.

His first sign of trouble came in 1992, with a PSA reading of 12 nanograms per milliliter (the healthy range is 0-4 ng/ml). A urologist confirmed the diagnosis through a biopsy. Thorpe, living in Connecticut, traveled to Yale, in New Haven, for surgery to remove his prostate.

Six years later, his PSA levels rose again, a sure sign that renegade cancer cells remained in his body despite the surgery. After seven weeks of radiation treatment, Thorpe's PSA levels dropped to zero, only to climb again after another two years. This time, treatment was hormonal therapy, which blocks the production of testosterone. His PSA levels went back down.

But again, the fix was temporary. Within seven years, Thorpe's PSA climbed to 4, even while taking the anti-hormonal drug. Doctors added a second anti-hormonal drug, one that blocks an additional source of testosterone in the adrenal glands. After six months, the second therapy stopped working, too.

Thorpe, now retired and living with his wife in Vero Beach, Fla., watched helplessly as his PSA levels rose; "It was not a fun situation," he recalled. He sought help from an oncologist in Vero Beach. There was nothing to do but wait and see, checking bone and CAT scans for signs that the cancer had spread. Five months later, the bad news came: Thorpe's tumor had metastasized into the lymph nodes in his pelvic area.

That was when Thorpe's oncologist in Vero Beach introduced him to William Dahut. After getting a second opinion at the Fox Chase Cancer Center in Philadelphia, Thorpe traveled to NCI in December 2006 and joined the same Phase II clinical trial as Renner.

When he started the trial, Thorpe's PSA was up to 17.6. Today, with the three-week cycles of therapy, it has dropped to 0.4. CAT scans show that his formerly enlarged, cancer-laden lymph nodes are either back to normal or near normal. His bone scan is stable. And his side effects are all manageable with drugs and vitamins. "Even though I am dealing with reduced energy and stamina levels," Thorpe noted, "I have been able to pursue normal activities."

While no one can make predictions about his specific outcome, 65 percent of the patients in Thorpe's trial are still in the protocol 18 months later, and some have been in it as long as 30 months.

"I'll take it," said Thorpe, who is now traveling to visit his children, grandchildren, and friends. "I know that I had better live today," he said, "because, at some point, there won't be a tomorrow. If you are dealing with a limited time horizon like I am, it is encouraging when you can realize a quality life for an additional 18 or 30 months, or longer."

"I have been very pleased," Thorpe said, "with the care and service of the dedicated professionals at NCI."

A Molecular View of Prostate Cancer Therapy

(Photo: Courtesy of Douglas Figg, CCR)

William Douglas Figg, Sr., Pharm.D.

Traditional chemotherapy has long produced disappointing results in prostate cancer patients. But William Douglas Figg Sr., Pharm.D., Head of the Molecular Pharmacology Section at CCR, is trying to change that reality. Figg's group is not only taking a molecular view of cancer—drilling down into the ways that small molecules might slow tumor growth—but is also describing how the body metabolizes new anti-cancer drugs.

Before introducing a promising new drug into patients, researchers first determine two parameters: the drug's pharmacodynamics (where in the body the drug will travel and how it will behave when it reaches its target) and pharmacokinetics (how long it will stay there before being broken down and eliminated). For instance, predicting liver enzyme metabolism is "huge for cancer," Figg said, because many drugs can interact with each other. Further, many anti-cancer drugs have a very narrow time frame in which to work. Therefore, a drug that does not reach a tumor by a specific time could be essentially useless.

Figg and his team are leading CCR's efforts to address these complexities, working with analytical chemists to develop assays to measure many different aspects of drug metabolism before a drug ever enters a patient's body.

For instance, Figg's team has developed assays that determine what concentration of drug builds up in different "compartments" of the body (e.g., the bloodstream, liver, kidneys). Liver enzyme tests they have developed can give an idea of how a drug is metabolized, information that can help pinpoint whether a person is likely to be a "slow" or "fast" metabolizer, which in turn affects how much drug they need to achieve a certain effect. And they have also created tests to determine how well a drug binds to a class of blood proteins called AAG plasma proteins; such binding can increase the time a drug remains intact and active (dubbed its "half-life"), but leaves less drug available to do its job.

Figg's group is also part of an international team that is synthesizing and screening 120 variants of the anti-angiogenic drug thalidomide (see main text). They have already flagged seven for additional study. The CCR team is able to test these and other anti-angiogenic agents—some provided by companies such as Pfizer, Novartis, and Aventis—in at least four model systems of blood vessel growth and development.

The key to this whole drug development system is collaboration—both outside CCR and within. Figg has teamed up with William Dahut, M.D., who conducts patient studies of drugs later in the development process. In this pairing, Figg focuses on pharmacokinetics and pharmacogenomics—the study of the unique genetic variations in each person's enzymes that determine how they metabolize drugs.

"Pharmacokinetics and pharmacogenomics are key to the drug development enterprise at CCR," Figg said. "Ours is a model for what most of CCR is moving to: tying a translational lab like mine to a clinician such as Bill Dahut."

Patient Perspectives (continued)

Jimmie Smith

For Jimmie Smith, 73, prostate cancer has been an odyssey—of doctors, institutions, and more than one clinical trial. His journey began July 3, 2002, with a PSA score of 17 and a biopsy confirming a diagnosis of metastatic prostate cancer.

Smith lives in the small town of Rocky Mount, N.C., where everybody knows everybody; his general practitioner and urologist are close friends. But the town held limited treatment options for him. Thus, Smith traveled to Duke University in Durham, then to University of North Carolina's Memorial Hospital in Chapel Hill. At UNC, he received a battery of treatments, including two chemotherapy agents and a steroid. By February 2003, his PSA had dropped to 0.3.

When his PSA rose again, a friend suggested a trip to MD Anderson Cancer Center. The oncologist there told Smith that he had six months to live. He made a trip to an oncology/hematology clinic in Los Angeles, Calif., that boasted alternative treatments, but none that Smith wanted to try. He called The Johns Hopkins Hospital; unfortunately, they had no prostate cancer clinical trials at the time.

Smith began to lose hope, until another friend told him about a family in Rocky Mount whose son who worked at the National Institutes of Health (NIH). The son told him about a clinical trial there for men with metastatic prostate cancer.

By March 2005, he had undergone castration surgery, his cancer had spread to a lymph node, and his PSA was 11. In short, he was running out of options. Thus, on April 18, 2005, he became the first patient to enter William Dahut's combination therapy trial, the same that Renner and Thorpe would later join.

Twenty-eight months later, Smith is still in the trial. His PSA initially dropped to 0.8 and the cancer seems to be at bay, a far cry from the six month pronouncement made previously. But more importantly, Smith is ebullient about his experience with the NIH doctors and staff.

"Everybody up there is just so intelligent and so caring, even the security personnel," he said. "And I can't think of anything to say that wouldn't be a real honor to them."

To this day, Smith believes if not for the friend and the tip about the NIH, he would have died sometime in 2005. Now, whenever he meets a person in his town with cancer, he tells them about NIH.

"I tell them, 'Go one time. One time. I'll even pay for your trip,'" he said. "I feel that strongly about NIH."