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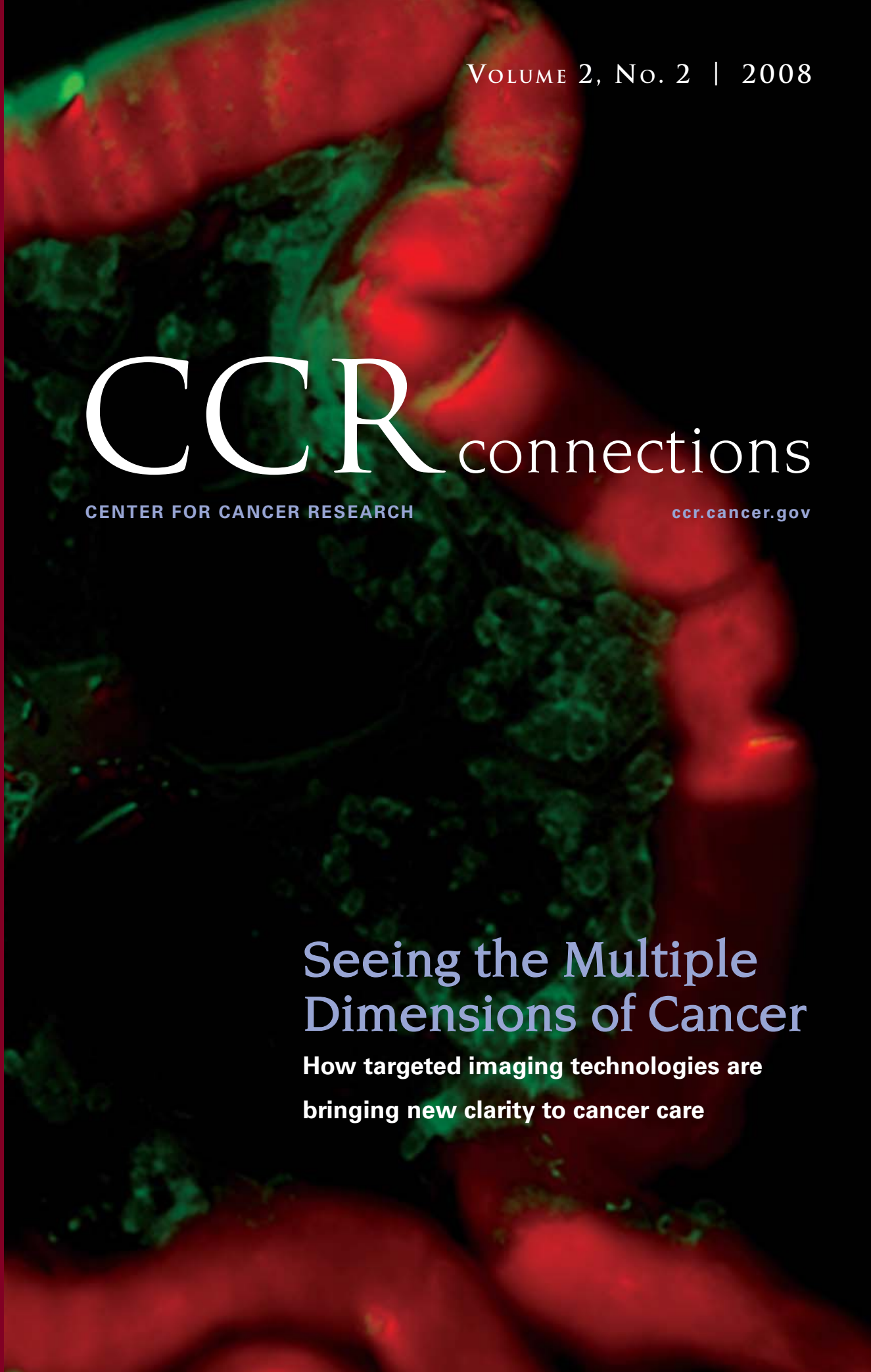

CCR connections

CENTER FOR CANCER RESEARCH

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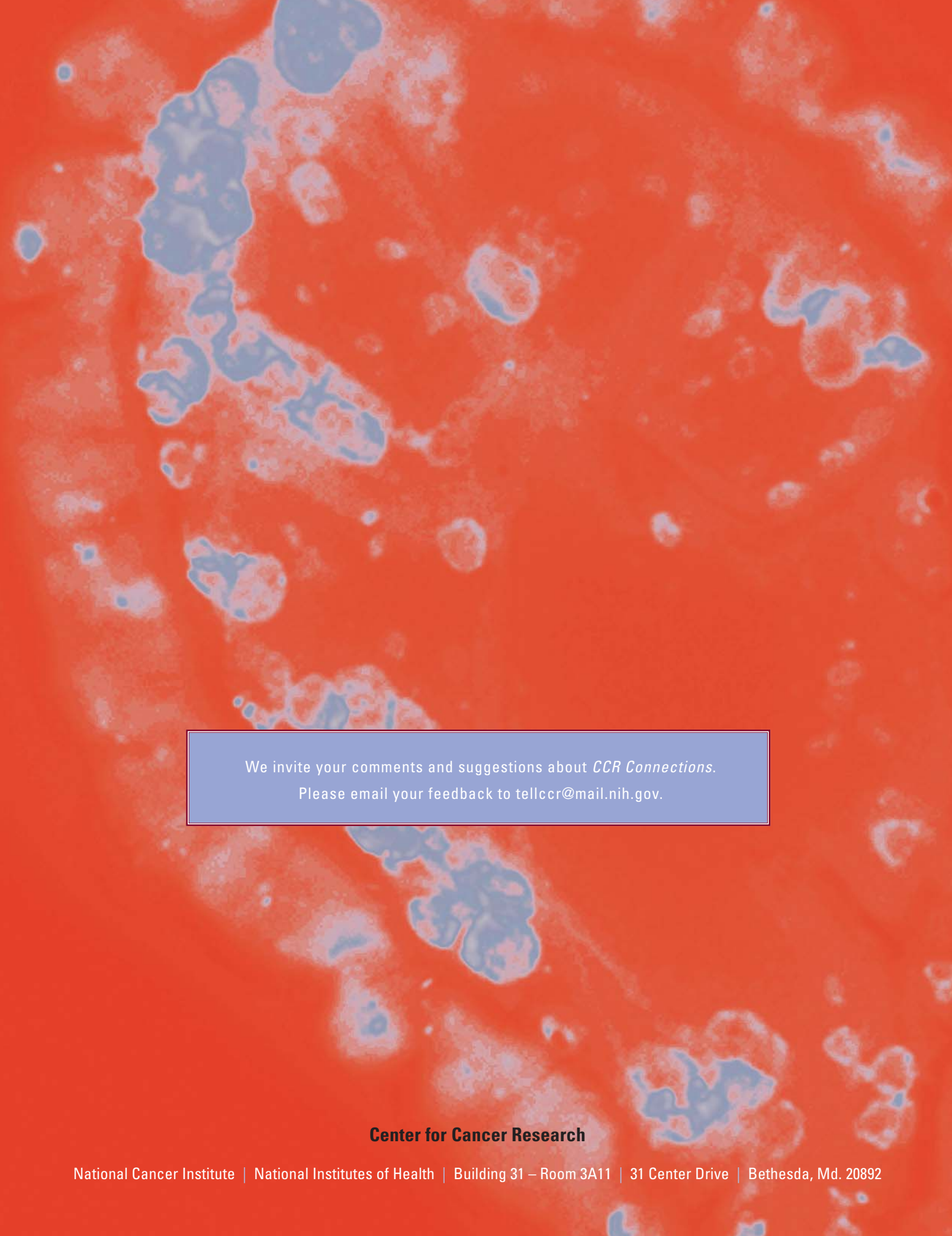
Seeing the Multiple Dimensions of Cancer

How targeted imaging technologies are bringing new clarity to cancer care

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health



We invite your comments and suggestions about *CCR Connections*.
Please email your feedback to tellccr@mail.nih.gov.

Center for Cancer Research

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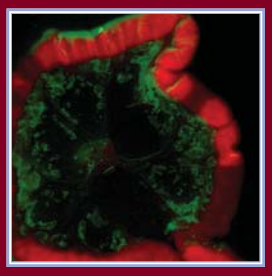
VOLUME 2, NO. 2 | 2008

Table of Contents

12 FEATURE

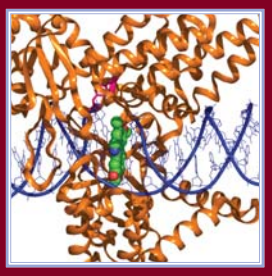
Medical Oncology Redefined: A Conversation with the New Chief of the Medical Oncology Branch at CCR

16 FEATURE



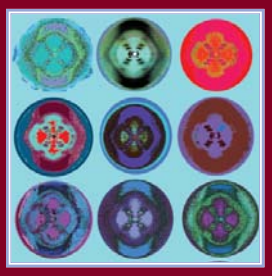
Seeing the Multiple Dimensions of Cancer: How Targeted Imaging Technologies Are Bringing New Clarity to Cancer Care

22 FEATURE



The DNA of Drug Discovery

28 IN THE CLINIC



Ovarian Cancer: A Silent Killer "Speaks" through Proteins

CENTER FOR CANCER RESEARCH

The mission of 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.

http://home.ccr.cancer.gov/connections/

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Insight, in Sight

Basic, translational, and clinical research, the core activities of the Center for Cancer Research (CCR), together form the backbone of an integrated enterprise aimed at making cancer preventable, curable, or chronically manageable. Achieving this goal requires that we address the complexities of tumor biology at every level: in scale (genes, proteins, cells, systems, populations), activity (expression, translation, function, interaction), structure (cancer cell, stroma, vasculature), and biological models (cell cultures, rodents, primates, people).

Every patient we see or each tumor we investigate raises critical questions:

- Which tumors will progress?
- How can we identify targets and develop therapies to interfere with or prevent progression?
- Is a treatment hitting the right target?
- Are we giving it to the right patients?
- Is there a mutation, pathway, or physiological state that impedes its effects?
- What approaches should be combined?

Molecular oncology—combining non-invasive imaging techniques (e.g., PET, MRI, EPRI) with "-omic" -based molecular profiling, biomarker discovery, and chemical biology—lets us peer into the deepest biological characteristics of cancer, providing the data and insight needed to inform strategies for therapeutic development. Such research is facilitated and enriched through innovative and productive partnerships, ones that leverage the contributions of each of these fields of expertise and apply both clinical observation and laboratory insight to define and refine new therapeutic techniques and approaches.

CCR is a translational research center where researchers and clinicians can routinely gather comprehensive molecular and imaging data on the tumor of every individual who participates in one of our clinical protocols. By integrating the work of CCR's experts across multiple fields, we can take these data and find novel solutions to seemingly intractable problems, such as discovering markers for:

- Diagnosis (What tumor does this patient have?)
- Prognosis (How will they fare?)
- Therapeutic efficacy and safety (Will they respond to drug X? Will they experience off-target effects? Would they respond to a combination of treatments?)
- Resistance or recurrence (Will the tumor compensate? What other molecular opportunities exist?)
- Biological comparison (Do our laboratory and preclinical models accurately reflect human biology? Are there biological alterations in our models that we have not yet discovered in nature and vice versa?)

The systematic integration of these data with our existing biological knowledge and understanding of how tumors behave clinically reveals to us the interplay of complexities. Such observations and patterns help us at CCR, and help our international collaborators design better clinical trials, develop better techniques and approaches to drug discovery and development, advance the linkage of targeted therapies and targeted diagnostics, abandon treatments destined to fail more quickly, and—ultimately—accelerate the success of those treatments that will succeed.

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

Lee Helman, M.D.
Scientific Director for Clinical Research, Center for Cancer Research

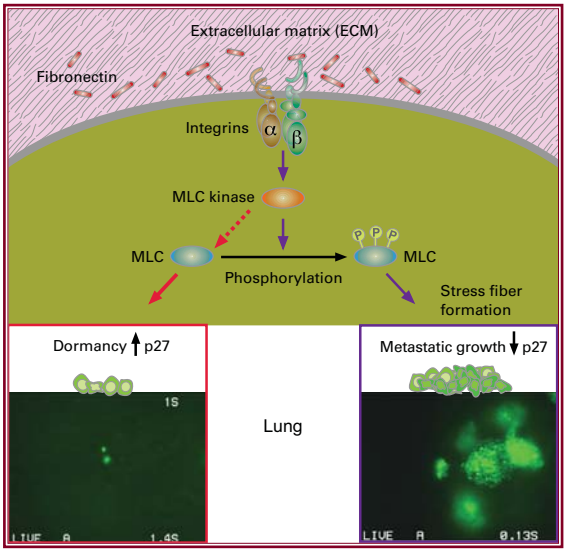
(*Photo: B. Branson*)

Robert H. Wiltrout, Ph.D.

(*Photo: R. Baer*)

Lee Helman, M.D.

Letting Sleeping Micrometastases Lie



(*Image: J. Kelly*)

A network of interactions within the extracellular matrix (ECM) activates dormant tumor cells, resulting in post-treatment metastases. When the intracellular enzyme MLC kinase is blocked, tumor cells remain dormant. This suggests that treatments that inhibit MLC kinase may effectively perpetuate dormancy in micrometastatic cells.

One of the most insidious aspects of the spread, or metastasis, of cancer cells is its stealth. Metastatic tumors can appear months or even years after treatment for primary cancers. Recent evidence suggests that during this time, tumor cells lie dormant in their new host tissues. Because most chemotherapy agents target actively dividing cells, the dormant tumor cells are able to survive this time untouched, waiting for a signal to awaken and grow.

The nature of the signal(s) is unclear, though this knowledge would give researchers and clinicians an opportunity to develop ways of keeping cancer cells permanently in a dormant state. A team of researchers led by Research Fellow Dalit Barkan, Ph.D., and Jeffrey Green, M.D., Head of the Transgenic Oncogenesis and Genomics Section in CCR's Laboratory of Cancer Biology and Genetics, applied a three-dimensional (3D) culture technique to model dormancy, allowing them to identify at least one of the external cues for waking dormant breast cancer cells. Their results were published in the August 1, 2008, issue of the journal *Cancer Research*.

The majority of *in vitro* cell culture experiments are conducted using two dimensional (2D) cultures. However, these cultures do not reflect the true nature of the tumor microenvironment. With their 3D cultures, Barkan, Green, and their collaborators attempted a more realistic assessment of the signals exchanged between dormant breast cancer cells and the extracellular matrix (ECM), the structural framework that provides cells with environmental stimuli for growth, survival, angiogenesis, and other activities.

What they discovered was a complex set of interactions within micrometastatic cells and between micrometastatic cells and the ECM that regulate dormancy. The researchers found that their cell lines exhibited remarkably different growth characteristics when grown in 2D versus 3D cultures, more accurately reflecting their metastatic behavior *in vivo*. According to their results, tumor cells remain dormant by applying a molecular brake on their life cycle. Expression of a protein called fibronectin, often found to be increased in the ECM of carcinomas, triggered rearrangements in their cytoskeletons that effectively released the brake. Blocking an intracellular enzyme called MLC kinase, which mediates fibronectin's cytoskeletal effect, prolonged cell dormancy in both 3D cultures and in an *in vivo* metastasis model.

The researchers noted that while it is unlikely that fibronectin is the only ECM factor involved in switching on dormant micrometastases, their results suggest that pathways that regulate the cytoskeleton might serve as good targets for treatments that keep dormant micrometastatic cells inactive permanently.

Read more about the research conducted by Drs. Green and Barkan at http://ccr.cancer.gov/staff/staff.asp?profileid=13662.

IL-7: Bringing New Youth to the T Cell Pool

When we are young, our immune system's pool of T cells is generated and kept fresh through the action of the thymus, a small organ located at the base of the neck. As we age, the thymus shrinks and becomes less active, and the job of maintaining our T cell repertoire gradually shifts away from the thymus to other lymphoid organs.

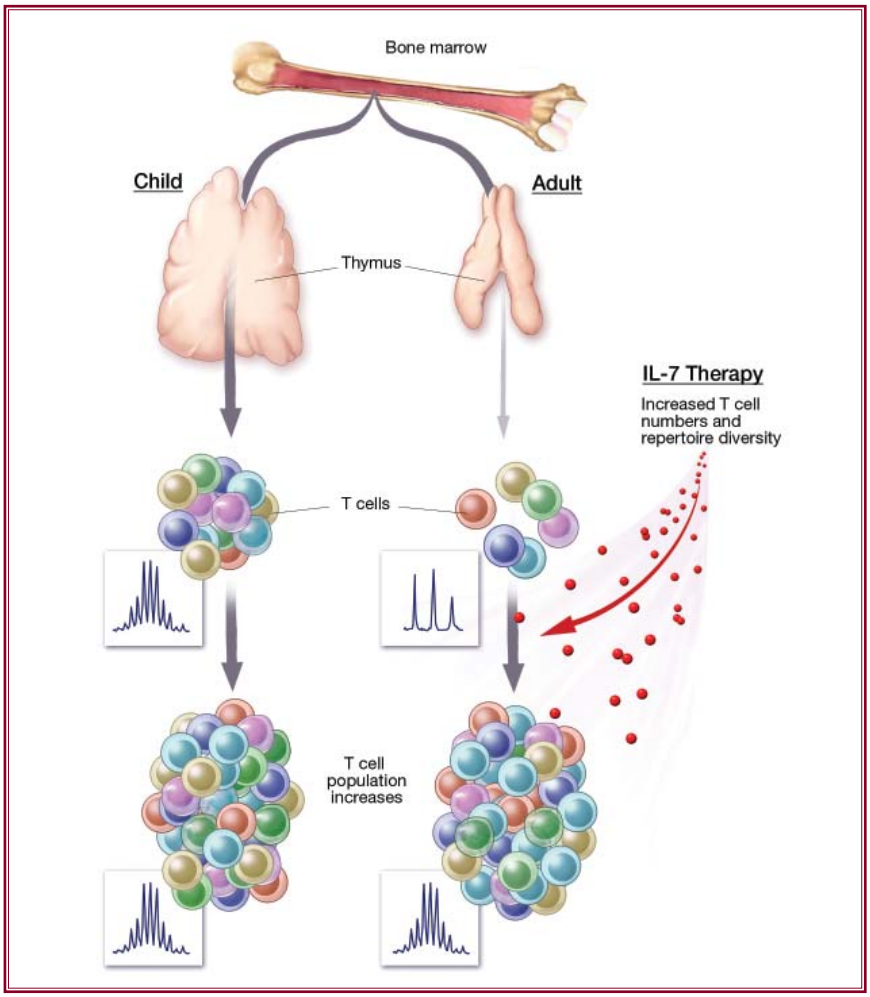
This shift in maintenance responsibility can be a cause for concern for cancer patients, particularly ones over the age of 45 or 50. Chemotherapy can deplete patients' T cells, leaving them vulnerable to infection for some time after completing treatment. The T cell pool of younger patients often comes back within months, mostly through increased activity of the thymus. However, the immune system of older patients, in whom the thymus is relatively inactive, struggles to restock its store of naïve T cells, reducing these patients' ability to adapt to new pathogens or to rely on their T cells to continue fighting their cancer.

In the June 23, 2008, online issue of the *Journal of Experimental Medicine*, a team of researchers led by Claude Sportès, M.D., Staff Clinician; Ron Gress, M.D., Chief of CCR's Experimental Transplantation and Immunology Branch; and Crystal Mackall, M.D., Chief of CCR's Pediatric Oncology Branch, reported on a study of the cytokine interleukin-7 (IL-7) as a means of reconstituting patients' T cell repertoires. Past studies have shown that IL-7 is required for maintaining an adequate T cell pool and that in animal models the cytokine can help restore a depleted repertoire of these essential immune cells.

Given these past data, Sportès, Gress, Mackall, and their collaborators reasoned that IL-7 might be able to rejuvenate immune function in cancer patients. After two weeks of treatment with the cytokine, the researchers found that the patients' numbers of helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells rose dramatically (for $CD8^+$ cells, the numbers increased over 400 percent) and stayed high for up to six weeks after the IL-7 treatment stopped. In addition, the new cells were overwhelmingly of a naïve phenotype, even in older patients. Thus, the treatment seemed to return some of the patients' immune system components to a younger state.

These findings could have significant clinical relevance in immune reconstitution and rejuvenation following a variety of insults on the immune system. Apart from helping rejuvenate cancer patients' immunity, IL-7 treatment could help improve the health of other immunocompromised patients such as those with HIV/AIDS or those in the normal aging population, or it could be used to boost the effectiveness of vaccines or forms of immunotherapy both inside and outside the field of cancer treatment.

Read more about the efforts of Drs. Sportès, Gress, and Mackall on their respective CCR Web sites at http://ccr.cancer.gov/staff/staff.asp?profileid=5907, http://ccr.cancer.gov/staff/staff.asp?profileid=5821, and http://ccr.cancer.gov/staff/staff.asp?profileid=5595.



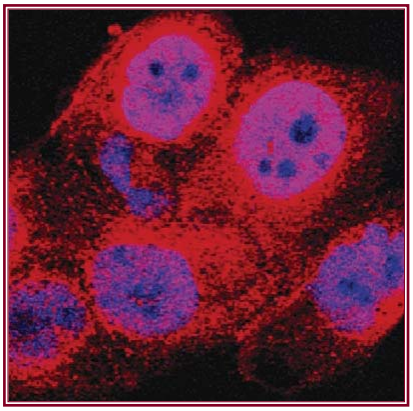
(*Image: NIH Medical Arts*)

As the activity of the thymus decreases with age, the number and the diversity of immune system T cells decrease; chemotherapy can further deplete the T cell repertoire. Treatment with the cytokine IL-7 has been shown to reconstitute the T cell pool, rejuvenating immune function in cancer patients.

Resistance Is Futile:

Examining the Causes of Cisplatin Resistance in Cancer Cells

The platinum-based anti-cancer drug cisplatin is a basic component of today's portfolio of chemotherapy drugs, being widely used to treat bladder, ovarian, testicular, lung, and other common cancers. Unfortunately, cisplatin-treated tumors frequently become resistant, for reasons that remain unclear. CCR researchers Michael M. Gottesman, M.D., Chief of the Laboratory of Cell Biology; Xing-Jie Liang, Ph.D., former CCR Fellow and current Director, Laboratory of Nanomedicine and Nanosafety, National Center for Nanoscience and Technology, Beijing, China; and colleagues set out to examine the root causes of cisplatin resistance. Their results, published in the September 15, 2008, issue of Molecular Cancer Research, isolate one of the many factors contributing to cellular resistance to cisplatin.



(*Image: X-J Liang, CCR*)

Increased levels of SIRT1 *(red)*, a histone deacetylase linked to cell metabolism, contribute to cisplatin resistance (CP-r) in tumor cells. By understanding SIRT1 and structural and functional changes to the mitochondria in CP-r cells, researchers hope to develop strategies to prevent cisplatin resistance and maintain the drug's efficacy.

Previous research by the team showed that cisplatin-resistant (CP-r) cells grow more slowly and consume fewer nutrients than those that are cisplatin-sensitive (CP-s). Because tumors can change their metabolism as a survival strategy when nutrients are scarce, the researchers decided to focus on examining the metabolic changes that occur in CP-r tumor cells.

The researchers generated CP-r cells by exposing liver and epidermoid carcinoma cell lines to cisplatin *in vitro*. Comparing these to CP-s cancer cell lines, the researchers found significant reductions in the CP-r cells' use of glucose (4–5 fold) and oxygen (30–60 percent). Mitochondria, the cell's metabolic engines, rely on oxygen and glucose to produce energy; therefore, the team also compared the mitochondria in CP-r and CP-s cells. They found that the mitochondria of CP-r cells are smaller and both functionally and structurally altered compared to CP-s cells.

Based on these basic differences, the team looked next at SIRT1, a histone deacetylase known to play a pivotal role in regulating cellular metabolism and responses to nutrient restriction. Measuring the SIRT1 levels in both cisplatin-resistant and -sensitive cells, the researchers discovered that the CP-r cells overexpressed SIRT1. Introduction of SIRT1 into cells increased cisplatin resistance, while knocking down SIRT1 expression using RNAi decreased resistance.

This direct link between the cisplatin exposure, resistance, and SIRT1 levels suggests that SIRT1 plays a key role in cisplatin resistance, a valuable insight that can help researchers develop strategies that decrease the potential for resistance and increase the efficacy of this important anti-cancer drug.

Learn more about the work of the Gottesman laboratory at http://ccr.cancer.gov/staff/staff.asp?profileid=5713.

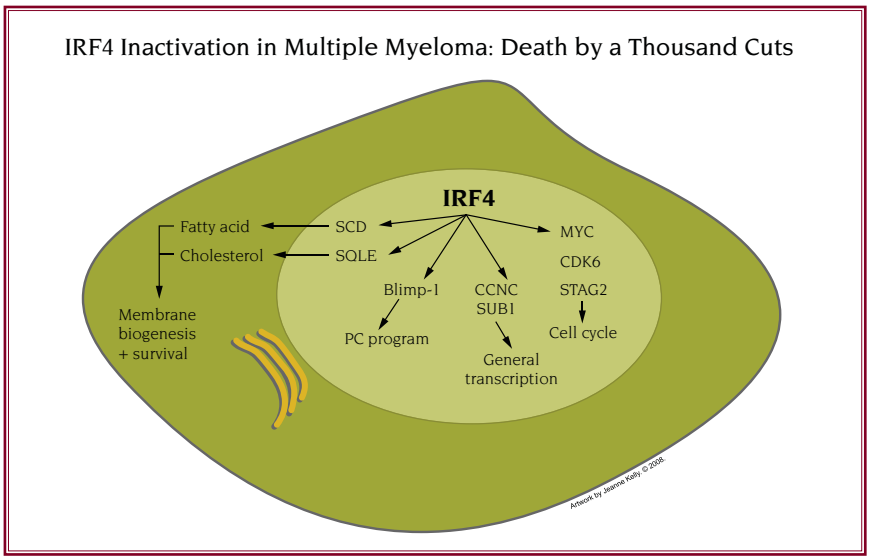
Building a Real-Time Resistance Fighter

More than a dozen multidrug-resistant cell lines, like the CP-r cell line studied by Gottesman and Liang, have been developed by the Gottesman laboratory and licensed to over 40 pharmaceutical or biotech companies over the past 20 years. The laboratory's research on changes in cancer cells' handling of drugs as a way to survive therapy, as well as the work of many other labs, has implicated 380 resistance-related genes, which the team is now assembling onto a "gene chip." Their goal is to develop a clinically relevant diagnostic tool that can be used to rapidly detect drug resistance in real time as it arises.

Achilles' (Other) Heel:

Non-Oncogene Addiction in Multiple Myeloma

Oncogene addiction has been regarded as the Achilles' heel of cancer, based on the idea that silencing an oncogene's expression will prove lethal in certain cancers.[1] *However, new research suggests that multiple myeloma—a cancer of antibody-producing plasma cells—may have a fatal vulnerability that is better described as a "non-oncogene addiction."*



(*Image: J. Kelly*)

Multiple myeloma cells' survival depends on the ability of the transcription factor IRF4 to activate genes that are quiescent in healthy plasma cells. This dependency suggests that, just as some cancers are said to have an "oncogene addiction," myeloma cells have a "non-oncogene addiction."

There is no curative treatment for the many subtypes of multiple myeloma, each of which utilizes distinct oncogenic pathways. Thus, developing therapeutic alternatives not based on type-specific oncogenes is very attractive to clinicians and researchers. Recent research highlighting the role of the protein IRF4 in the survival of myeloma cells suggests that this protein may provide a therapeutic target for all myeloma subtypes.

In the July 2008 issue of *Nature*, a team of NCI and NIH researchers, led by Staff Scientist Arthur Shaffer III, Ph.D., and Deputy Chief Louis Staudt, M.D., Ph.D., of CCR's Metabolism Department, reported results of a study utilizing small hairpin RNAs (shRNAs) to identify potential drug targets for multiple myeloma. The team observed that silencing the gene *IRF4* killed 10 different cell line models representing many subtypes of myeloma. Importantly, most of these myeloma models lacked any genetic abnormality in *IRF4* but were nevertheless completely dependent upon *IRF4* for survival, a phenomenon that the investigators characterized as "non-oncogene addiction."

In normal lymphocytes, IRF4 is a transcription factor, helping to initiate responses to foreign antigens and to generate plasma cells. To understand the molecular basis for *IRF4* addiction in multiple myeloma, the investigators characterized the repertoire of genes that are activated by IRF4 in myeloma cells. They found that IRF4 turns on genes in myeloma cells that are also induced during normal lymphocyte activation but are silenced in healthy plasma cells, from which myeloma is derived. Thus, *IRF4* controls an aberrant regulatory network in multiple myeloma.

Staudt, Shaffer, and their collaborators found a peculiar relationship between *IRF4* and the oncogene *MYC*, which has a prominent role in myeloma pathogenesis. In their experiments, silencing *IRF4* suppressed *MYC* expression and, conversely, silencing *MYC* suppressed *IRF4* expression. Their observations suggest a model in which *IRF4* and *MYC* reinforce the expression of each other in a cycle that perpetuates cancer cell proliferation and survival.

The findings suggest that blocking *IRF4* expression may be an attractive and broadly applicable therapeutic option for the many subtypes of multiple myeloma. More generally, the phenomenon of non-oncogene addiction promises to provide a new range of therapeutic targets in cancer.

To learn more about Dr. Staudt's research on hematologic malignancies, please see "Making Sense of Lymphoma: The Definition Makes a Difference" in CCR Connections, Vol. 1, No. 2, or visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5780.

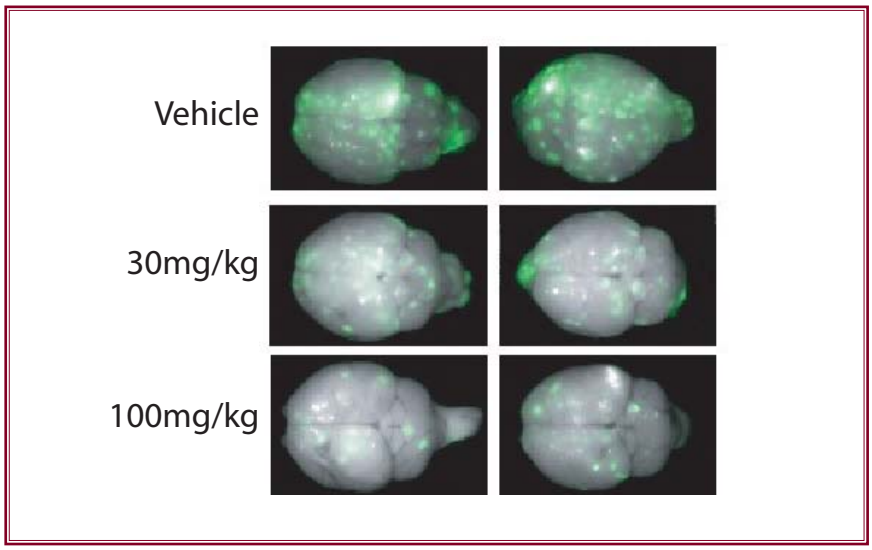
[1] *Weinstein, B. Addiction to oncogenes—the Achilles heal of cancer. Science.* 2002;297:63-64.

Small Molecule, Big Impact:

The Efficacy of Lapatinib in Breast Cancer Metastases in the Brain

The treatment of brain metastases linked to breast tumors overexpressing the protein HER2 represents a growing unmet medical need. HER2-positive tumors account for approximately 20 to 25 percent of all breast cancers, and 35 percent of HER2 metastatic patients will experience outgrowth to the brain. Treatment options for brain metastases are currently limited to radiotherapy, neurosurgery, and steroids, largely due to a lack of drug therapies capable of crossing the blood-brain barrier. For example, trastuzumab (Herceptin®), a monoclonal antibody against HER2, is too large to reach tumors that have spread to the brain.

A team of researchers led by Visiting Postdoctoral Fellow Brunilde Gril, Ph.D.; Staff Scientist Diane Palmieri, Ph.D.; and Principal Investigator Patricia Steeg, Ph.D., of the Women's Cancers Section in CCR's Laboratory of Molecular Pharmacology, recently completed a study demonstrating that the kinase inhibitor lapatinib (Tykerb®) effectively inhibited the growth of HER2-positive breast cancer cells that metastasize to the brain. The study results appear in the August 6, 2008, issue of the *Journal of the National Cancer Institute*.



(*Image:* B. Gril, CCR)

A lack of drug therapies capable of crossing the blood-brain barrier leaves HER2-positive breast cancer patients who develop brain metastases with limited treatment options. As a small-molecule, lipophilic drug, lapatinib successfully crosses the blood-brain barrier to reduce HER2-positive brain metastases *(green)* in preclinical models.

Lapatinib is a small-molecule drug that blocks both HER2 and another protein, epidermal growth factor receptor (EGFR). Breast cancers that overexpress HER2 and/or EGFR are more likely to metastasize to the brain. Because only small, lipophilic drugs can cross the blood-brain barrier, lapatinib may be an ideal candidate for the treatment of such cancers.

To test this hypothesis, Steeg, Palmieri, Gril, and their colleagues treated brain-seeking breast cancer cells with lapatinib *in vitro*. The results demonstrated that the drug inhibited the activation of both EGFR and HER2 pathways, thus restricting cell proliferation and spread. Furthermore, cell lines expressing high levels of both EGFR and HER2 were 30 percent more vulnerable to the drug than lines that expressed high levels of only one of the receptors.

The researchers then turned to *in vivo* models to confirm their results. Mice injected with HER2-positive breast cancer cells developed twice as many large metastases as those injected with cells expressing normal levels of the protein. While lapatinib was again significantly more effective against tumor cells that overexpressed both HER2 and EGFR, it could not completely prevent metastatic development.

Nevertheless, utilizing lapatinib as an adjuvant or preventive therapy for HER2-overexpressing breast cancer warrants further clinical investigation. Neurosurgery and radiotherapy may continue to offer the best treatment options for large metastases in the brain, but the study results demonstrate that lapatinib could be the first small-molecule therapy to successfully prevent the progression of micrometastases in a preclinical model.

To learn more about Dr. Steeg's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5851.

The Natural Products Repository:
A National Drug Development Resource

NCI's Natural Products Repository, a rich resource for potential anticancer agents, is experiencing a renaissance, according to two NCI-Frederick program leaders, **David Newman, Ph.D.**, *and* **Kirk Gustafson, Ph.D.**

"In the 1990s a great deal of attention was focused on new combinatorial approaches for generating large libraries of synthetic compounds," said Gustafson, Natural Products Chemistry Group Leader in CCR's Molecular Targets Development Program (MTDP) at NCI-Frederick. "The reality is, after 10 years, people were finding very few useful compounds in these synthetic libraries." Of about 155 small-molecule anti-tumor agents, 47 percent are natural products, derivatives of natural products, or mimics of natural products, including Gleevec and the other kinase inhibitors.

"Pharmaceutical companies dropped off of natural products, and we didn't," said Newman, Chief of the Natural Products Branch in the Developmental Therapeutics Program (DTP) at NCI-Frederick. NCI has the largest program to collect materials worldwide from marine, plant, and microbial sources. More than 200,000 natural products, both organic solvent and aqueous extracts, are available to internal NCI scientists and the extramural community. Any group that wants to develop a compound must sign a non-negotiable materials transfer agreement that protects the rights of the original source country.

The DTP works closely with CCR. "We collect materials and following purification (often by the MTDP) develop them up to Phase II clinical trials," Newman said. The MTDP also collaborates with other CCR investigators to develop molecularly targeted screens to test these extracts. MTDP scientists can go on to isolate and identify active materials and, if they look interesting, DTP can coordinate early animal studies, recollection, and resupply—which is not a trivial step—and then feed them into a complete development pipeline.

"If Kirk needs more material, we find it," Newman said. He has collection contracts with a marine collection group in Palau in the Pacific and also has contracted botanists who collect in rainforests.

The scientists have most recently been exploring compounds found in Australian marine sponges and the microorganisms that live symbiotically with these marine animals. Often the microbes carry the biosynthetic genes for producing the active compound found in the animal. Researchers have been using genomic techniques to fish out the relevant gene clusters from the microbes. Since culturing these microorganisms is often difficult, tremendous possibilities lay in capturing the biosynthetic machinery and putting it into an organism that researchers *can* culture and control—for example, yeast, or other single-celled organisms. "We're on the cusp of being able to do that," Gustafson said. "Our knowledge base is expanding rapidly and tools are getting better."

To learn more, see "The DNA of Drug Discovery," page 22.

(*Photo: P. Colin, Coral Reef Research Foundation, Palau*)

Some 200,000 extracts from terrestrial and marine species around the globe, such as the sponge *Phallusia julinea*, have been collected by the Natural Products Repository and archived in its facility at NCI-Frederick.

CCR Research

Fuels International IGFR Inhibitor Trial for Ewing's Sarcoma

The basic and translational research conducted at CCR can impact the lives of patients around the globe. Nowhere is this more apparent than in an ongoing international clinical trial for patients with Ewing's sarcoma, a soft tissue cancer primarily seen in children, teens, and young adults. This Phase II trial, which opened in December 2007, was devised as a collaboration between CCR, a consortium of U.S. centers, and sites throughout Europe.

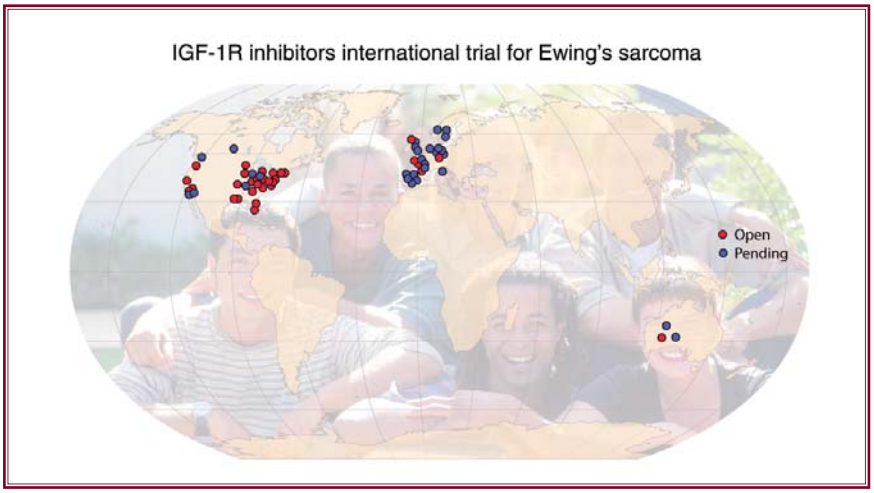
The new antibody, produced by Hoffman-LaRoche under the name R1507, inhibits the insulin-like growth factor 1 receptor (IGF-1R). "Tumor cells, in particular Ewing's tumor cells, appear to be dependent on signaling via an IGFR-mediated pathway," said Herbert Juergens, M.D., Professor of Pediatric Hematology and Oncology at the University of Muenster, a study site in Germany. Other sites outside the U.S. are in France, Italy, the Netherlands, and the United Kingdom.

"There is a strong sense that, if this pathway drives the growth of Ewing's sarcoma, there are other cancers that also may be driven by the same abnormality in the same pathway," explained Denise Reinke, N.P., President and Chief Executive Officer of the Sarcoma Alliance for Research through Collaboration (SARC), whose sites are accruing U.S. patients.

"This trial comes out of work by [CCR Scientific Director for Clinical Research] Lee Helman, M.D.," Reinke continued. "He extensively studied this pathway for more than 15 years, and now we have a drug that will go after that target."

Thirty to forty percent of patients with Ewing's sarcoma have a recurrence or have metastatic disease after frontline therapies. This study is recruiting patients whose disease is refractory to existing treatments. Patients include those with Ewing's sarcoma and several other types of sarcoma.

As of September 5, 2008, 169 patients had been accrued to the study. "We didn't expect to be here at this point," said Reinke. The accrual goal is 305 patients. "There's clearly interest in this as a potential treatment."



(*Image: J. Weddle*)

CCR, a consortium of U.S. Cancer Centers, and sites throughout Europe and Australia are collaborating in a Phase II clinical trial to test R1507, an IGFR inhibitor produced by Hoffman La-Roche, for the treatment of Ewing's sarcoma in children. If the treatment proves effective in this trial, researchers may begin investigating the role of the IGFR pathway in other cancers.

CCR's Elaine S. Jaffe, M.D., Receives High Honor from University of Barcelona

In May 2008, Elaine S. Jaffe, M.D., Chief of the Hematopathology Section in CCR's Laboratory of Pathology, was awarded an honorary doctorate from the University of Barcelona (UB), becoming only the third woman to receive the degree of Doctor Honoris Causa in the university's 555-year history. Nominated for the award by Elias Campo, M.D., a Professor of Pathology at UB and a former research fellow in the Jaffe laboratory, she was credited for her contributions to understanding the genetic and molecular mechanisms of lymphoid neoplasms and for her generosity in sharing knowledge through her leadership in international forums, including World Health Organization committees.

(*Photo: Courtesy of E. Jaffe*)

Staff News at CCR

announcements

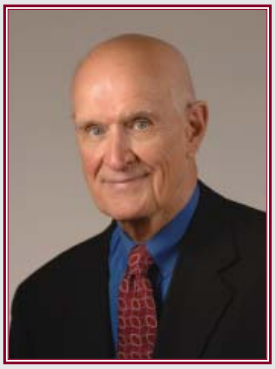
(*Photo: R. Baer*)

Crystal Mackall, M.D.

Mackall has been appointed Chief of the Pediatric Oncology Branch, having served as Acting Chief since 2005. She came to NCI in 1989 as a Clinical Fellow in pediatric hematology/oncology under the auspices of Ronald Gress, M.D., Chief of the Experimental Transplantation and Immunology Branch at CCR.

She and her team are currently developing immunotherapies that can be integrated into current treatment modalities. The vast majority of children with pediatric cancer are treated with multiagent chemotherapy, often resulting in lymphocyte depletion. To exploit these immune physiology changes, Mackall has developed immune-based therapies that are given during lymphocyte depletion, thus enhancing immune reactivity toward the tumor when a child's tumor burden is low. She has also led the clinical development of recombinant IL-7 (rhIL7), a cytokine with potent effects on T cell homeostasis and immune reconstitution.

Mackall completed a fast track B.S./M.D. program at the Northeastern Ohio Universities College of Medicine and a combination residency in pediatrics and internal medicine.



(*Photo: B. Branson*)

Samuel Wells, Jr., M.D.

Wells is a board-certified surgeon and expert in thyroid cancer. Currently a Professor of Surgery at Washington University and Executive Director of the International Thyroid Oncology Group, Wells divides his time between St. Louis and Bethesda.

With CCR colleagues, he has opened a new pediatric clinical study of hereditary medullary thyroid carcinoma (MTC). This inaugural trial of the new thyroid program will assess the safety, tolerance, and activity of vandetanib in children and adolescents with hereditary MTC.

He is a longstanding member of the Institute of Medicine and has received 13 major scientific awards, including the American Surgical Association Medallion for Scientific Achievement (2004). An honorary fellow in six international surgical colleges, he has also been president of seven major surgical societies, editor-in-chief of three major journals, and author or coauthor of over 250 peer-reviewed articles and 70 book chapters. From 1998–2005, he chaired the Oncology Group of the American College of Surgeons.

new tenure-track scientists

(*Photo: B. Branson*)

Mitchell Ho, Ph.D.

Ho joins CCR's Laboratory of Molecular Biology as a Tenure-Track Investigator. A recipient of the Mesothelioma Applied Research Foundation Award and Ovarian Cancer Research Fund Investigator Award, Ho will conduct mechanistically based, translational research leading to the development of more effective anti-cancer antibody therapies. His efforts focus on building new therapeutic antibodies directed at mesothelioma, ovarian cancer, and liver cancer.

Mitchell received his Ph.D. in immunology from the University of Illinois at Urbana-Champaign, and he received postdoctoral training here at NCI.

CCR Staff Elected to the Institute of Medicine in 2008

Elaine Sarkin Jaffe, M.D., Chief, Hematopathology Section, Laboratory of Pathology

William Marston Linehan, M.D., Chief, Urologic Oncology Branch

Medical Oncology Redefined:

A Conversation with the New Chief of the Medical Oncology Branch at CCR

Defining the discipline of medical oncology can be more difficult than one would initially think because it touches on so many aspects of cancer research and care. Most broadly, it can be defined as the care and study of adult cancers. Some categorize it as a subspecialty of internal medicine that provides chemotherapy and other non-radiation and non-surgical treatments, a definition that comes close to that of ***Giuseppe Giaccone, M.D., Ph.D.****, Chief of the Medical Oncology Branch (MOB) at CCR. "Medical oncology is the medical treatment of cancer," he noted, "which covers all that is not surgery or radiotherapy."*

(Image: Fenstein Kean Healthcare)

The Medical Oncology Branch brings resources, technologies, and people—researchers, clinicians, and patients—from across NCI and beyond together in ways that leverage the insights and capabilities of multiple clinical and scientific disciplines for the benefit of cancer patients worldwide.

That definition is changing, becoming more translational, more research oriented, and more reflective of the heterogeneity found among patients with the same or similar diagnoses. "Medical oncology also means understanding the range of treatments and identifying the best treatment for each unique patient," Giaccone said. "It no longer means treating everyone the same, a shift in practice that really is only happening now."

The nature of the research and organization of medical oncology is also changing to meet this new definition, a shift that is reflected in the efforts underway at CCR to revitalize the Medical Oncology Branch. Giaccone, a lung cancer specialist trained in the former NCI Medicine Branch, rejoined NCI last year as its Chief of Medical Oncology after 16 years at the Free University Medical Center in the Netherlands. His task: to revitalize the Branch as a translational research powerhouse by forging closer ties among the labs and sections comprising CCR's medical oncology community; to recruit new leaders in key disease areas such as gastrointestinal (GI) and head and neck; to foster relationships between the MOB and extramural investigators; and to build effective partnerships with industry.

CCR Connections had a conversation with Giaccone about CCR's vision for the new MOB and the Center's goals for collaboratively leveraging the unique resources of CCR and the MOB to accelerate the national cancer research effort.

Connections: How was medical oncology research conducted within NCI in the past?

Giaccone: Until recently, cancer biologists were not involved in patient treatment; the provision of systemic therapy largely fell to trained medical oncologists. With the growing translation of biological insights into targeted therapies, many CCR labs and branches have become more interested and involved in bringing treatments to our patients. While this is a very positive development, it requires increased coordination within the MOB and between medical oncologists and our colleagues in the laboratory. In fact, I believe the MOB should be at the forefront of delivering new treatments to our patients.

There are attributes of the old NCI Medicine Branch, particularly its collaborative, multidisciplinary nature, that can serve us well in translating the scientific and medical advances—such as advanced technologies for genomic screening, tools for linking patients' clinical histories and outcomes with the molecular characteristics of their tumors, and enhanced techniques, including imaging, for investigating the efficacy and activity of new therapies, particularly targeted therapies—in clinical practice.

All of these advances, if they are to impact cancer prevention and care, require the contributions of multiple fields of expertise in their development and application. Based on this reasoning, we have thus far focused on facilitating greater integration and coordination across the different components of CCR that engage in medical oncology research.

Connections: Please define CCR's vision for the new MOB.

Giaccone: Medical oncology can only work when the strengths and expertise of numerous fields—immunology, molecular biology, translational medicine, etc.—are leveraged in an integrated, coordinated fashion. The MOB is the largest CCR branch practicing the discipline of medical oncology. As such, it will provide a framework for conducting all medical oncology research at CCR, bringing all of the sections, branches, and laboratories together to make joint strategic decisions about how to move forward in advancing cancer research.

Connections: Where do you see the new MOB fitting within the larger medical oncology community?

(*Image: R. Baer*)

Giuseppe Giaccone, M.D., Ph.D. (*right*), a thoracic oncologist by training, examines a patient at CCR.

Giaccone: We were once highly regarded for our cutting-edge capabilities in translational and clinical research, and for the resources we brought to the table. However, as our internal research efforts became fragmented, we lost this reputation. With the revitalization of the MOB, we want the cancer community to understand that we have an important role to play in translational cancer research: that of a high intensity clinical research center that is ideally suited to bring advanced treatments to patients in a highly integrated research setting.

Over the last few years, both NCI and CCR have recognized the need to remove barriers to both internal and external collaboration and have undertaken significant efforts to do so. We need to partner with other institutions in order to share additional and unique areas of expertise. The new MOB will be an interactive part of the oncology community, with active involvement in a broad portfolio of multicenter studies, where we can leverage our unique resources to carry out experiments that are not feasible outside of CCR.

Medical oncology can only work when the strengths and expertise of numerous fields–immunology, molecular biology, translational medicine, etc.–are leveraged in an integrated, coordinated fashion.

Connections: One of the main factors in the fragmentation of the old Medicine Branch was the concern that this intramural program was too similar in scope and activity to external programs. How are you addressing this concern now?

Giaccone: The new MOB needs to be able to differentiate itself significantly from what can be done in academia or industry and position itself as a complementary resource for such groups.

We have unique abilities in translational medical oncology research due in large part to our unique patient populations. From day one, CCR has focused on rare tumor types. It is relatively easy for CCR to gather patients with rare cancers from around the country and the globe and run complicated studies—such as molecular imaging studies over a regular timeframe or mechanism-of-action studies—that would be difficult and expensive to coordinate on the outside.

Because we are a clinical research center and not an academic or community hospital, we are able to take that relatively small number of patients and study them very intensively, allowing us to gain a deep understanding of these tumors from multiple points of view, including genomics and imaging, two critical areas of targeted treatment research that can be very resource-, infrastructure-, and time-intensive.

Connections: Do you see greater collaboration with industry as part of the branch's reformation?

Giaccone: Absolutely. This collaborative openness must extend to industry as much as to academia. Most drug development in oncology is conducted by industry these days. The MOB is working closely with the pharmaceutical industry to design and conduct studies that would not be feasible elsewhere or that require particular kinds of expertise. And we need to go further, to work with our industrial partners to identify important questions that must be answered but which cannot be studied in the context of industry-sponsored studies.

We are in a very good place to run very early Phase 0 clinical studies—extremely small trials where you give a new drug to a limited number of patients under an exploratory investigational new drug (IND) protocol and develop reliable, reproducible assays that help determine whether the drug's behavior in people mirrors that in preclinical models. Comprehensive molecular studies on biopsies or molecular imaging studies on patients are very hard to conduct and are resource-intensive. But if they can be conducted in near real-time in an exploratory context, the data that they generate can help quickly and accurately determine next steps and properly define patient populations before moving into larger, later phase trials.

Connections: Is there a role for the so-called "big four" tumors (lung, breast, prostate, and GI) in the new MOB?

Giaccone: While rare tumors form a core focus of CCR, the four major tumor families will be well represented in our efforts, for two reasons. First, from a population standpoint, these tumors are the most important, affecting larger numbers of patients and causing the greatest mortality and morbidity. Second, a critical part of our mission is to train the next generation of medical oncologists and physician-scientists. We have one of the largest fellowship programs in the nation here at CCR. For the fellows to have the best training and gain the most from their experience, they need to be able to understand the common tumors before they can be expected to understand the uncommon ones.

We also have to consider how the major and rare tumors relate to each other and to CCR's mission. The major tumors each have many rare subtypes. Generally speaking, rare tumor types are biologically less complex than the major tumors. They tend to have fewer genetic alterations, making them easier to study biologically and facilitating their use as models for understanding the biology underlying the major tumors. The work of [Urologic Oncology Branch Chief] W. Marston Linehan, M.D., on kidney cancer and the *VHL* gene is a prime example of how one can leverage discoveries from a rare condition—namely, von Hippel-Lindau syndrome—to advance the understanding and care of more common conditions.[1]

Connections: Can you give any other examples of the kinds of collaborative research you have been discussing?

Giaccone: CCR is now working with a researcher from Washington University in St. Louis, Samuel Wells, Jr., M.D., to conduct a trial here at the NIH Clinical Center focused on a rare hereditary form of thyroid cancer called hereditary medullary thyroid carcinoma (MTC).[2] MTC accounts for 2 to 3 percent of all thyroid cancers, and only 25 to 40 percent of MTCs are hereditary. Thus, it is very difficult to collect a cohort large enough to do a study with meaningful power. Dr. Wells has teamed up with Frank Balis, M.D. [NCI Clinical Director and Head of the Pharmacology and Experimental Therapeutics Section in CCR's Pediatric Oncology Branch], to study a new targeted treatment option for patients with unresectable hereditary MTC, a study that likely would be impossible without CCR's research, resources, and reach.

Connections: What are the key elements for achieving this new strategic vision for the MOB?

Giaccone: Of all of the possible elements on the list, the most important is collaboration. You need a team approach and expertise from very different angles, from biology to patient care to symptom management, all combining to reach the best result. People in the different branches and sections recognize that we need to work together, not in isolation. But to bring us all back together, there needs to be a feeling that we all—all of the branches, all of the sections—are part of a larger enterprise.

Connections: What steps have been taken to make this vision of the MOB a reality?

Giaccone: Thus far, our efforts have primarily centered on bringing more integration and strategic planning into the clinical protocol development process. After consulting with the different MOB sections, my colleagues and I have developed a new planning step, called a concept review, designed to bring strategic consensus to protocol design. Before a protocol is written, we decide whether the question to be investigated is one that should be explored, and then we identify the resources needed, including those from other sections or branches. From there, we write the protocol collaboratively, ensuring that all details are addressed from the outset.

We also are actively involved in CCR's effort to reduce the time needed for protocol approval, with the goal of reducing that time to two months. This would make us extremely competitive with outside centers in terms of the speed with which we can translate discoveries into the clinic and also make us an attractive partner for collaborative efforts in clinical and translational research.

We are also actively reconstituting our lung, breast, prostate, and GI cancer programs. [MOB Investigator] William Dahut, M.D., has done well with the prostate cancer program for many years and will continue in his efforts to maintain its high standards. As head of the lung cancer program, I will be organizing

The MOB is a cancer research resource that exists to complement the excellent and mission-critical research that is being conducted nationwide.

the efforts of CCR's excellent team of lung cancer investigators. Leadership recruitment for the breast and GI programs, as well as our head and neck cancer program, is ongoing.

Lastly, CCR has initiated a Medical Oncology Center of Excellence (CoE). The CoE, which I am leading, is bringing collaborators inside and outside of NCI—people doing important work in areas related to translational medical oncology research like molecular diagnostics, molecular target development, early detection, tumor imaging, and early therapeutics development—together in a multidisciplinary way.

Connections: If there is any one message you would want to convey to our readership about CCR, the MOB, and how its reconstitution will affect translational cancer research nationally, what would it be?

Giaccone: The MOB is a cancer research resource that exists to complement the excellent and mission-critical research that is being conducted nationwide. The MOB is not here to compete with centers that participate in NCI's extramural program, but rather we exist to enrich their work by offering capabilities and expertise that are not available at the extramural centers, and we can leverage these capabilities in unique ways. As our transformation continues, we look forward to building closer ties to our colleagues in academia and industry, so that together we can make the best use of what the MOB and CCR as a whole have to offer.

[1] *To learn more about Dr. Linehan's work, see "A War on Kidney Cancer," CCR Connections, Vol. 1, No. 1.*

[2] *See "Staff News at CCR," p. 11.*

(Image: R. Baer)

Giuseppe Giaccone, M.D., Ph.D., Chief of the Medical Oncology Branch (MOB) at CCR

The career path of Giuseppe Giaccone, M.D., Ph.D., has spanned a revolution in cancer research and care on two continents. He first came to the United States and to NCI from Italy in 1988 to work in the Medicine Branch laboratory of John Minna, M.D. (now Principal Investigator of the NCI-sponsored Specialized Program of Research Excellence [SPORE] in lung cancer at the University of Texas Southwestern Medical Center), in the early days of research efforts to understand the genomic component of lung cancer. After two years, he left to conduct doctoral work at the Free University Medical Center in Amsterdam, the Netherlands, where he eventually became a Professor of Medical Oncology and Head of the university's Department of Medical Oncology.

A year ago, Giaccone returned to NCI as Chief of the newly reconstituted Medical Oncology Branch. "I learned of the opportunity to come back to NCI and lead the branch, and I was quite interested, in part because of the challenge, and in part because this is a unique place to work, with resources that you do not find anywhere else in the world."

Giaccone also returned to lead NCI's Lung Cancer Program. A thoracic oncologist by training, Giaccone will continue his research on targeted therapies for non-small cell lung cancer and how cancer cells regulate, or misregulate, apoptosis.

"I've now been back a little over a year, and while I feel that I'm still learning about the place, I have found very good people here. I am glad to be back and to contribute to CCR's efforts to advance translational cancer research."

To learn more about Dr. Giaccone and his work, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=12505.

Seeing the Multiple Dimensions of Cancer:

How Targeted Imaging Technologies Are Bringing New Clarity to Cancer Care

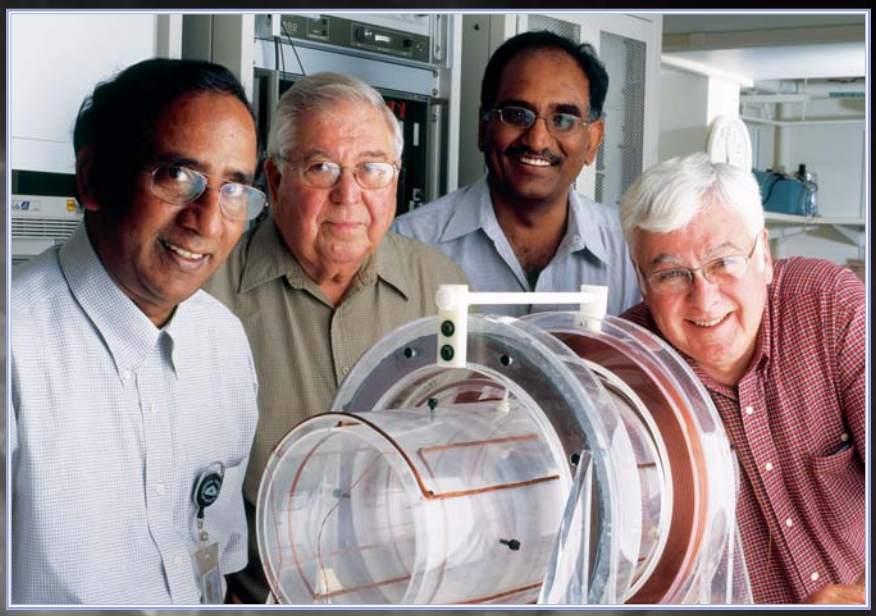
A surgeon, a radiologist, and an oncologist sit in a dimly lit room, banks of monitors in front of them. Their attention is focused on a collection of pictures: black-and-white, color, human outlines, brightly lit spots in some places, dark in others. At the press of a button, the radiologist sends a command to a group of computers. Data are exchanged, and the images merge together effortlessly into a single picture of a human form, superimposing physiology on anatomy. The bright spots fuse, revealing the location, viability, and vulnerabilities of a tumor.

This seamless scenario does not yet represent standard clinical practice. But it represents the ideal treatment planning or drug assessment scenario, one in which clinicians from different fields of oncology are able to share and integrate the data generated by a host of molecularly targeted imaging technologies—such as targeted optical fluorescent tagging, magnetic resonance imaging (MRI), and an emerging technology, electron paramagnetic resonance imaging (EPRI)—into single, holistic images that provide researchers and clinicians with a complete representation of the patient's tumor, including its location, its size, and its physiology.

Together, these technologies are fueling a new understanding of how tumor physiology and structure affect drug action while also bringing new precision to clinical treatment planning. The physician-scientists of CCR's Molecular Imaging Program (MIP) and Radiation Biology Branch (RBB) are leading the charge to refine these technologies and translate them into clinical practice, making the above scenario a reality.

The New Way: Seeing Is Believing

The traditional way of drug development, while effective and straightforward, is time-consuming and cumbersome. Researchers give the trial cohort a drug



(Photo: R. Baer)

Left to right: **Sankaran Subramanian, Ph.D., Staff Scientist; Mr. Frank Harrington, NIH Machinist; Murali Krishna, Ph.D.; and Jim Mitchell, Ph.D., show their original self-built magnet and field gradient assembly, which they used for electron paramagnetic resonance imaging (EPRI). This magnet was used to first demonstrate the feasibility of *in vivo* oxygen imaging.**

or treatment of interest, follow them for months or years by MRI or computed tomography (CT) scanning, and look for changes in tumor size.

> "Each technology has its strengths and weaknesses, and if we think broadly about how to leverage those strengths to answer specific problems, we can diagnose, track, and by extension treat cancers with greater specificity than is currently possible."

Traditional methods of treatment planning, particularly for radiation therapy or surgery, have similar limitations. Radiologists image the tumor using the same or similar techniques, with the goal of creating detailed three-dimensional representations of tumor size and location.

At the same time, functional imaging—technologies like positron emission technology (PET)—have rapidly advanced the ability of doctors and scientists to see the activity within a tumor, as represented in the case of PET by the relatively insatiable appetite of cancer cells for glucose.

But the current imaging modalities have limitations. PET can tell radiologists how much glucose a tumor is using but cannot shed light on other aspects of tumor physiology or anatomy. MRI and CT can help provide unsurpassed anatomical detail but have difficulty defining metabolic dimensions.

CCR's MIP is stepping in to bridge the functional and the structural. "The MIP was established four years ago to try to find new points of view and new solutions to challenges in cancer imaging," said MIP Head and Senior Clinician Peter Choyke, M.D. "Each technology has its strengths and weaknesses, and if we think broadly about how to leverage those strengths to answer specific problems, we can diagnose, track, and by extension treat cancers with greater specificity than is currently possible."

Imaging has always been a component of the translational research conducted at CCR, but resources dedicated to non-clinical work were often limited. The MIP is changing that, but it is doing so in a way that complements the long-standing efforts of the RBB. "We now have a strong, integrated, cancer-focused, *in vivo* imaging program made up of people with a broad but critical range of expertise," said Choyke. "With this disciplinary breadth, we can investigate the whole spectrum of imaging technologies and probes to create new families of clinically relevant image-based biomarkers."

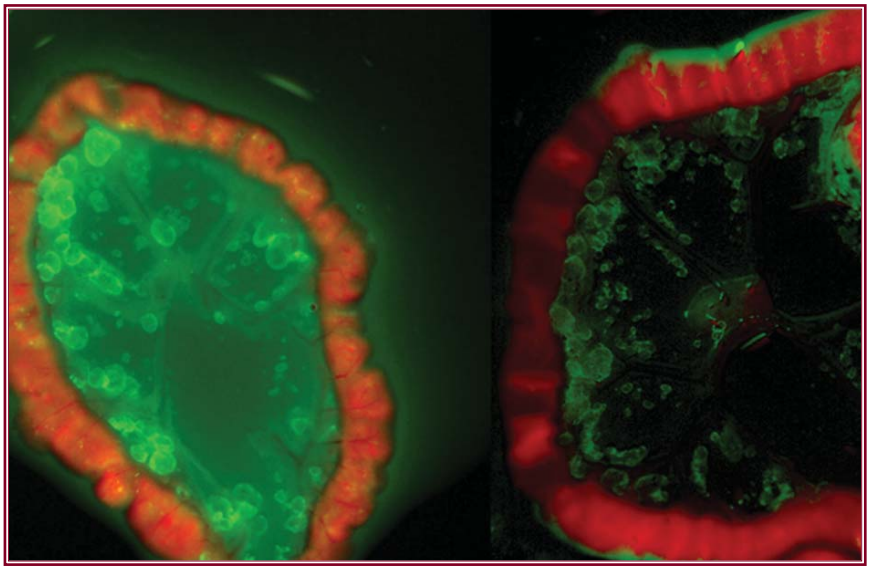
The availability of unique resources like the MIP's new dedicated clinical drug development imaging facility allows the program to serve as a focal point for research that is both high-risk and high-reward, like exploratory studies of new therapeutic agents and technology development (see "To Systematically Look Within").

Bringing Micrometastases into the Light

While radiology-based treatment planning methods provide anatomic information of unprecedented detail, once in the operating room, the most effective surgeries are those in which the surgeon can remove as much tumor as possible, including any metastatic colonies that may be present near the original malignancy. Currently, surgeons remove a margin, a buffer zone of apparently healthy tissue around the tumor, in the hopes of removing any micrometastases that may have spread, unseen, from the original cancer.

Hisataka Kobayashi, M.D., Ph.D., a Staff Scientist in the MIP, understands the challenges and importance of eliminating micrometastases early and efficiently, particularly for ovarian cancer patients. "Ovarian cancer is not a very aggressive cancer, but it is dangerous because it spreads silently. For this reason, gynecologic surgeons try to pick up as many metastatic nodules as they can." Also, because surgery, even when done endoscopically, is invasive, surgeons want to do as much as they can during a single operation.

But how can a surgeon know where the micrometastases are? Visual inspections by endoscope cannot reliably detect tiny tumors without some kind of guide or aid that makes the tumor stand out from the surrounding tissues. To address these visual limitations, Kobayashi and his colleagues—including MIP Visiting Postdoctoral Fellows Mikako Ogawa, Ph.D., and Nobuyuki Kosaka, M.D., Ph.D., as well as former MIP Clinical Fellow Yukihiro Hama, M.D., Ph.D., now an Assistant Professor at Japan's National



(*Image: H. Kobayashi, CCR*)

Figure 1: By using a cancer-specific ligand, like an antibody, conjugated to a fluorescent probe that glows only at low pH *(green)*, researchers can see metastatic ovarian cancer cells *(right, in a mouse model)* and determine whether the cells respond to therapy.

Defense Medical College—have developed a system that literally makes ovarian micrometastases light up.

The system makes use of a natural response to antibody or receptor-ligand binding, namely that once an antibody is bound to a cell, it will be taken up by the cell and then sent to the lysosome, a cellular compartment or organelle that uses low pH to digest internalized proteins. In Kobayashi's system, exposure to the acidity of the lysosome triggers the fluorescent tag attached to the antibody, making the cell glow (Figure 1). "Because we use only a cancer-specific antibody, we only highlight cancer cells, not normal cells," Kobayashi said. The system also takes advantage of a second aspect of cellular physiology. Only viable, healthy cells are able to maintain a low lysosomal pH; if a cell is damaged, its lysosomes become alkaline. Thus, if a cancer cell that has internalized Kobayashi's tagged antibodies is damaged—by chemotherapeutics, for instance—the lysosomal pH rises, and the tag's signal fades.

"If we give this tagged antibody to an ovarian cancer patient before surgery," according to Kobayashi, "the surgeon can look for glowing areas and know that they represent micrometastases. At that point, the surgeon can remove them or paint them with a chemotherapeutic agent and observe, in real time, whether the drug has any effect."

Though the system is only in the preclinical stage, it already shows promise. In the December 2008 issue of *Nature Medicine*, Kobayashi and his team reported on the system's specificity at highlighting lung metastases as peritoneal metastases of ovarian cancer in mouse models.

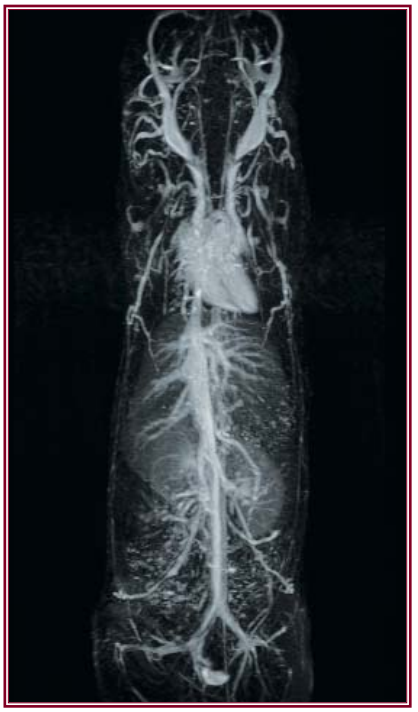
Kobayashi believes the fluorescent system could have widespread applications. "Endoscopic surgery lends itself well to image guidance, which is effectively what we are developing with this technology. It can be applied to any cancer for which there is an appropriate antibody or ligand. We could adapt this method as a way for surgeons to better determine the edges of a tumor while conducting resections. It could be used as a way of guiding robotic surgery, an area NCI is interested in pursuing. We could even use multiple fluorescent tags responsive to different aspects of physiology to increase the scope of visual information we can gain in real time."

Revealing Vascularity

Before the surgery can even take place, though, a surgeon needs to gather as much information as possible about the tumor's shape, location, and activity. Similarly, while deciding whether to employ chemotherapy, targeted therapy, or other treatment strategies, a medical oncologist should have as much information on tumor structure and physiology as possible.

To add physiological sensitivity to the anatomic detail provided by MRI, Choyke and his colleagues have turned to an imaging technique called dynamic contrast enhanced-MRI (DCE-MRI). "DCE-MRI falls somewhere between molecular imaging and anatomic imaging," said Choyke. The true difference between the two is reflected in time. Standard MRI takes a snapshot of a tumor's anatomy and location. By comparison, DCE-MRI is, as the name implies, dynamic, producing a representation of a tumor's blood flow over time.

At the heart of DCE-MRI is a running series of MRI snapshots taken at very short intervals using a contrast agent called gadolinium. This rare earth element interacts with the protons in water molecules, making them stand out more clearly on an MRI scan than they normally would. "Gadolinium actually changes the properties of the water in the body," Choyke explained. "When we run a DCE-MRI scan, the movie we produce actually captures the effects of gadolinium on the surrounding water, giving us a dynamic view of the agent's uptake into and clearance from the tumor."



(*Image: P. Choyke, CCR*)

Figure 2: Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) allows researchers to visualize entire circulatory systems, as with the mouse above, or the vasculature of tumors, making this technology an excellent tool for assessing anti-angiogenic therapies.

Because water is the main component of blood, the contrast agent makes anything containing significant amounts of blood, like blood vessels, shine brightly on the scans (Figure 2). "The angiogenic vessels in a tumor tend to be leaky, so they accumulate contrast agents rapidly and wash them out rapidly," said Choyke. "Measuring this ebb and flow of agent,

Standard MRI takes a snapshot of a tumor's anatomy and location. By comparison, DCE-MRI is... dynamic, producing a representation of a tumor's blood flow over time.

DCE-MRI becomes a way of identifying and monitoring highly angiogenic tumors." This capability to directly image angiogenesis positions DCE-MRI well as a tool for assessing anti-angiogenic therapies. "To tell if non-antibody-based tyrosine kinase inhibitors or antibody-based anti-angiogenics like bevacizumab are working," Choyke noted, "you need to be able to see the tumor and the drug's effect on the tumor over time. You need to know the tumor's angiogenic state before, during, and after treatment, and the closer you can get to gathering that information in real time, the better. With DCE-MRI, you can rapidly make those assessments."

The technique also provides greater flexibility for tumor diagnosis, staging, and screening. Choyke sees particular utility for the method in prostate cancer. "Prostate tumors are often hypervascular in comparison to the rest of the gland," said Choyke. "As an organ, the prostate is very challenging to image. It is located deep in the pelvis; it is an anatomically heterogeneous gland, and it is prone to hyperplastic changes that become more pronounced with age, the same age group that is at risk for prostate cancer. So, we are effectively trying to take a picture of an abnormality in a heterogeneous background in a small, remote organ."

There are additional reasons to have the tools available to image the prostate in detail. Prostate cancer tends to be localized, yet the majority of therapies are applied to the whole gland. "Ideally, we'd like to reduce the number of men undergoing whole gland therapies or radical prostatectomies, or we'd like to eliminate such therapies altogether and replace them with minimally invasive ablation techniques that could take care of the cancer without the side effects associated with more radical techniques."

Hypoxia in View

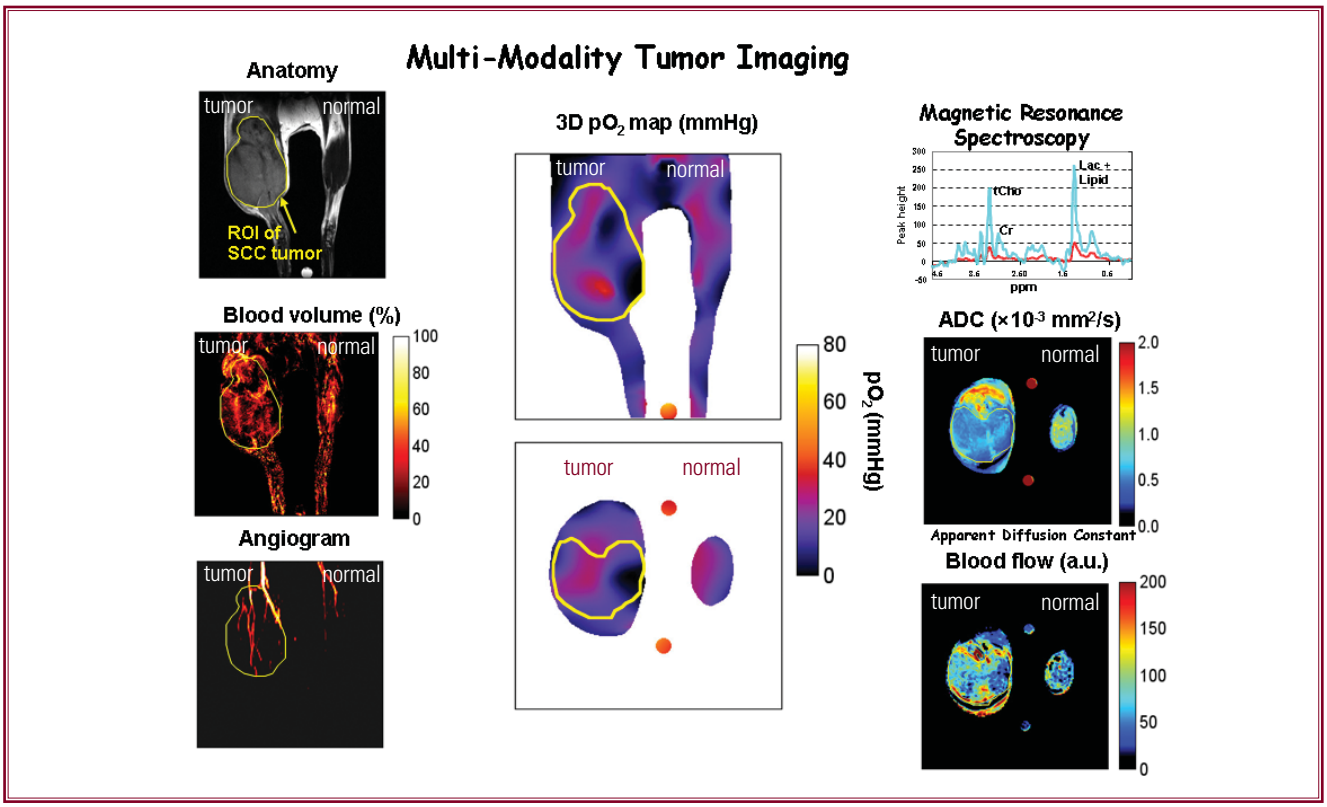
The translation of angiogenesis to oxygen concentration is not a one-to-one conversion. But knowledge of a tumor's oxygen level, or pO_2, can be crucial when planning treatment or assessing the effectiveness of an investigational therapy. "Tumors with significant hypoxia, or low pO_2, are very resistant to radiation therapy and maybe to chemotherapeutic agents as well," according to RBB Chief James Mitchell, Ph.D. "And for twenty years we have known that tumor hypoxia is directly tied to poor clinical outcomes, even in patients who undergo surgery."

"We have not had a readily available, noninvasive, and direct way to measure pO_2," said Murali Cherukuri Krishna, Ph.D., Head of the RBB's Biophysical Spectroscopy Section. "Indirect radiological measurements only provide qualitative information. Direct measurements with oxygen-sensing electrodes are accurate but are invasive, inappropriate for many tumors, and only give a localized snapshot of tumor pO_2."

"What we needed," Mitchell concluded, "was a quantitative method to map tumor hypoxia in deep sites in real time and in a way that can be coregistered with PET, MRI, or CT."

Krishna and Mitchell's answer to this need is a new imaging modality called electron paramagnetic resonance imaging (EPRI), an offshoot of nuclear magnetic resonance (a technology widely used for chemical analysis) that allows direct, quantitative assessment of oxygenation across a whole tumor (Figure 3).

Several features of EPRI work in its favor as a viable technology. The method employs the same equipment used for MRI. "The radio frequency we use for EPRI can be generated with an MRI scanner," according to Krishna. "In one sitting, we can generate three-dimensional EPRI oxygen maps and MRI anatomic maps of the same tumor."



(*Image: M. Krishna, CCR*)

Figure 3: Integrating images generated using multiple techniques can provide very comprehensive information about a tumor. For instance, overlaying MRI and EPRI data lets researchers assess structure, blood flow, blood volume, metabolite levels, and oxygenation in mouse model of squamous cell carcinoma. *(left leg, tumor; right leg, normal)*

But its development did not come without challenges. "EPRI looks for free radicals," said Krishna. "The body, especially the immune system, makes a number of endogenous free radicals. But none of them are stable or spectrally simple enough to be used for imaging. To make this technology work, we needed an artificial free radical that could be used as a tracer, something that would interact directly with the oxygen in a tumor and produce a simple, detectable signal."

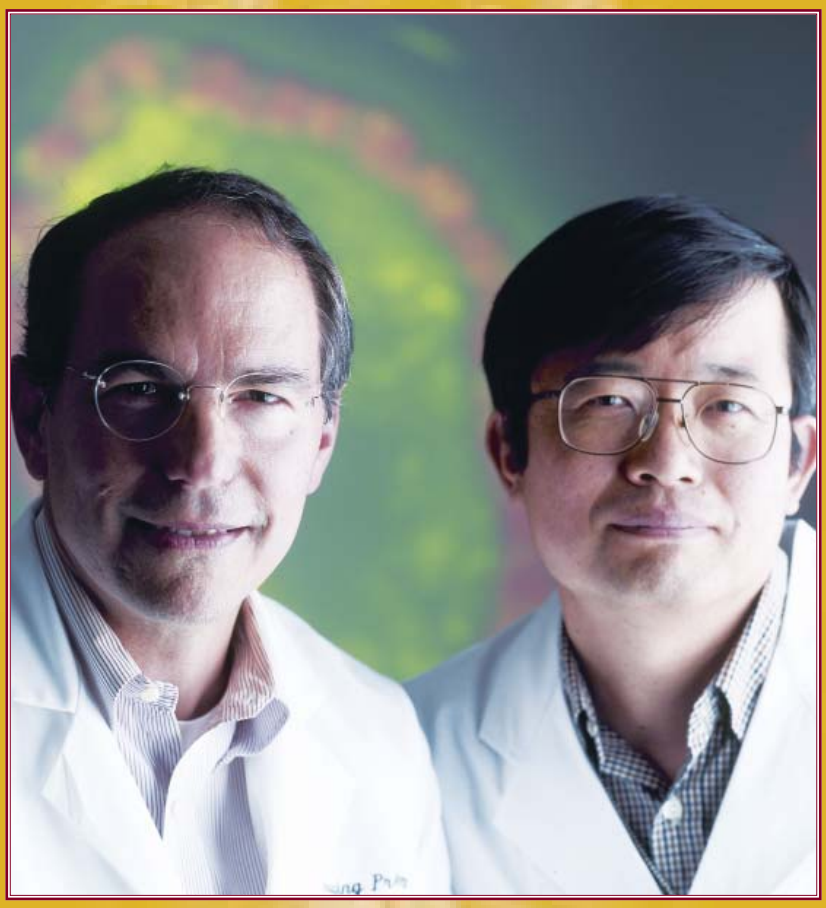
"GE has developed a family of tracers called TAM probes specifically for *in vivo* paramagnetic imaging," Mitchell noted. "Because the TAM signal on an EPRI scan increases linearly with oxygen concentration, imaging the tracer distribution within a tumor gives us a direct, quantitative, real-time image of its oxygen distribution."

Computational resources also proved to be a roadblock. "Paramagnetic signals last only one to two microseconds," Mitchell said. "The magnetic signals detected with MRI, by contrast, last about a second. The processing power needed to capture paramagnetic data simply hasn't been available until now."

Krishna and Mitchell—along with Postdoctoral Fellow Shingo Matsumoto, Ph.D., and Fuminori Hyodo, Ph.D., formerly a Postdoctoral Fellow in the Krishna laboratory and now at Kyushu University in Japan—published the results of a successful proof-of-concept mouse study in the April 2008 issue of the *Journal of Clinical Investigation*; the team is already pursuing translation to humans.

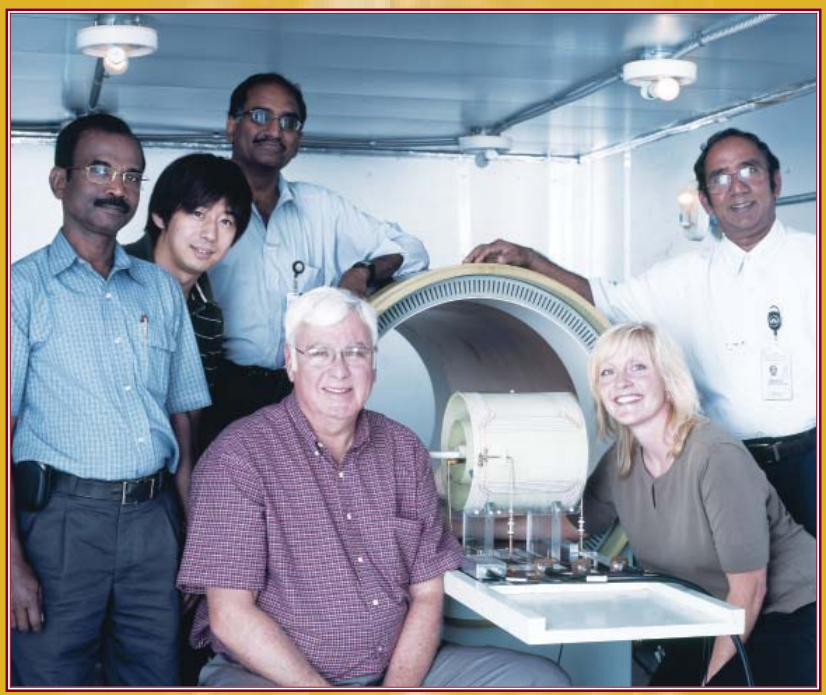
"With the ongoing development of technologies like DCE-MRI and EPRI, all here within the collaborative environment of CCR," Mitchell continued, "we now have the first real opportunity to look at tumor pO_2, metabolism, blood flow, vascularity, and anatomy and make them all correspond. We can't yet tell what the full impact will be on drug development and clinical care, but as these imaging modalities mature, we can tell that they will change the playing field."

To learn more about CCR's Molecular Imaging Program or the Radiation Biology Branch, visit their Web sites at http://mip.nci.nih.gov/ and http://ccr.cancer.gov/labs/lab.asp?labid=52.



(*Photo: R. Baer*)

Peter Choyke, M.D. *(left)*, and Hisataka Kobayashi, M.D., Ph.D. *(right)*



(*Photo: R. Baer*)

The EPR Imaging Lab
Sitting: Jim Mitchell, Ph.D., Anastasia Sowers, A.S.
Standing *(left to right)*: Nallathamby Devasahayam, M.S.; Shingo Matsumoto, Ph.D.; Murali Krishna Cherukuri, Ph.D.; Sankaran Subramanian, Ph.D.

To Systematically Look Within

(Photo: R. Baer)

Angela Stuber *(left),* Certified Nuclear Medicine Technologist, and Karen Kurdziel, M.D. *(right),* position their patient for a combined, single photon emission tomography/computed axial tomography scan (SPECT/CT scan).

Targeted imaging technologies such as dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and electron paramagnetic resonance imaging (EPRI) are giving precise insight into how cancer works at a molecular level. While these developments have the potential to revolutionize standard clinical care, they are also fueling a paradigm shift in drug development.

To have this kind of R&D impact, though, dedicated equipment and resources need to be available. Time on scanning equipment used in clinical trials is limited, which constricts CCR's ability to conduct early-phase translational drug development studies like exploratory clinical trials (small trials in which patients receive a trace dose of a developmental drug as a way of assessing whether the drug behaves in people as it does in preclinical models).

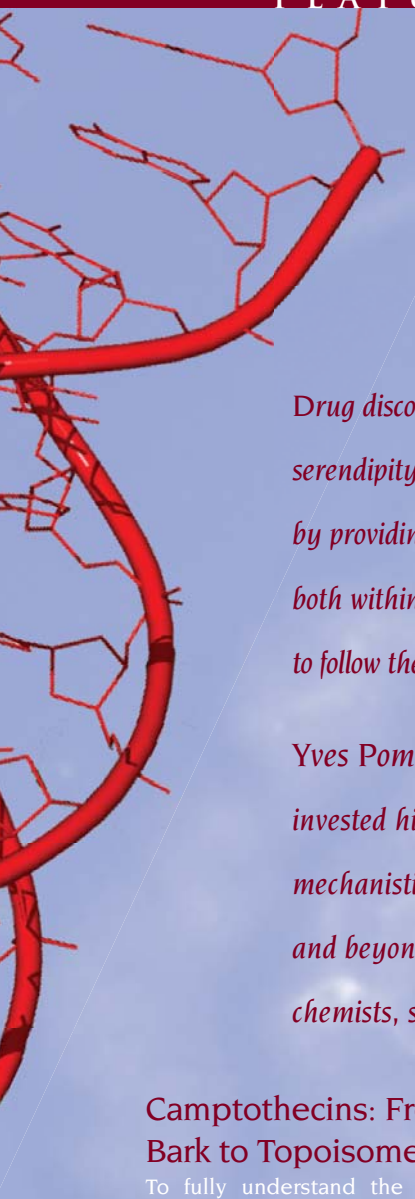
To bolster CCR's capacity for these kinds of research studies, the Molecular Imaging Program (MIP), with NCI's Developmental Therapeutics Program (DTP), opened the Molecular Imaging Clinic this past summer. "This facility is separate from the clinical facility," said Karen Kurdziel, M.D., a Staff Clinician with CCR and the Director of this new facility. "It is dedicated to drug discovery research protocols and lets us use imaging as a marker to make early go/no-go decisions in an exploratory context."

The new facility will house a comprehensive set of scanners, including PET, PET/CT, and 3T MRI, as well as full equipment for capturing vital signs and blood chemistry, all in close proximity. Together, this equipment will let MIP researchers and their colleagues from across NCI learn relatively quickly what a drug actually does. "We can take a drug, label it with an appropriate tag, and track it as it travels through the body," Kurdziel explained. "With this kind of information, we can see how much of a drug actually reaches the tumor and also where else it goes."

These studies can provide valuable insight into a drug's mechanism of action and the biology underlying side effects. "We have already started a study of paclitaxel (Taxol®), which no one has ever studied using imaging," said Kurdziel. "Its pharmacokinetics have been studied using plasma, blood, and urine, but with PET imaging, we can visualize the real-time whole body drug distribution. We have already found that it migrates to the gut and stays there, attacking the rapidly dividing cells in the gut lining, which may help explain the gastrointestinal side effects associated with paclitaxel treatment.

"This technology," Kurdziel continued, "can be used to determine how much of a dose actually penetrates the tumor. With the emergence of molecularly targeted drug therapies, PET imaging can be used to determine the dose needed, which may be much lower than the maximum tolerable dose that we use currently."

Kurdziel notes that data like this can also be used to create standard imaging-based markers to guide all cancer drug development. "If we can get the FDA to approve certain imaging markers as biomarkers, such as FDG for metabolism and FLT for proliferation, we can establish standard imaging endpoints that would allow drug developers to look for valid responses after weeks of treatment instead of years."



The DNA of Drug Discovery

Drug discovery, like research in general, relies on a fine balance between directed exploration and serendipity. With the goal of translating basic scientific insights into cures, CCR fosters this balance by providing the infrastructure to make new connections among seemingly disparate research efforts—both within the NCI and extramurally—and by providing new tools and opportunities for investigators to follow the therapeutic directions generated by their science.

Yves Pommier, M.D., Ph.D., Chief of CCR's Laboratory of Molecular Pharmacology, has invested his career in studying DNA processing mechanisms, with an eye towards turning his mechanistic insights into new generations of drugs. And thanks to innovative collaborations within and beyond NCI that have bridged his knowledge of molecular biology with the expertise of chemists, such drugs may be closer to hand.

Camptothecins: From Tree Bark to Topoisomerase

To fully understand the story behind Pommier's quest, one must look back 40 years. In the 1960s, while working on a contract with NCI, Monroe Wall, Ph.D., whose credits already included the purification of the anti-cancer wonder drug paclitaxel (Taxol®) from the bark of the Pacific yew tree, identified a second cancer-fighting compound—camptothecin—from the bark of a tupelo tree found only in China and Tibet. Wall studied camptothecin and synthesized derivatives, but without a known mechanism of action, the compounds languished at NCI's Natural Products Branch. Some 20 years later, in 1985, an NCI-supported academic/commercial collaboration of researchers at Johns Hopkins University, University of Florida, and SmithKline (now GlaxoSmithKline, or GSK) provided the first evidence that a DNA processing enzyme called topoisomerase I (topo I)—which makes cuts in DNA double helices, permitting them to relax for transcription or replication—was the camptothecins' molecular target.

At the time the camptothecins-topo I link was announced, Pommier's group was studying topoisomerase II (topo II), a related enzyme and known target of chemotherapeutic agents like doxorubicin. Thus, he was well positioned to study the cellular mechanisms of action of this new class of compounds. He and others confirmed that topo I was indeed the camptothecins' anti-cancer target and that the drugs turned normal topo I into a deadly enzyme by jamming it irreversibly onto the cell's DNA. Pommier's group also showed that human cancer cell lines could evolve resistance to camptothecins, invariably due to a mutation in the topo I gene. Within ten years of the confirmation of topo I's role, two camptothecin drugs had been FDA approved—topotecan (Hycamtin®) and irinotecan (Camptosar®).

> ...If there is one family of topo I inhibitors, might not there be another that would prove more powerful still?

Limited by their chemical stability and toxicity, camptothecins were not suitable for widespread drug development efforts. However, if there is one family of topo I inhibitors, might not there be another that would prove more powerful still? This was the question that Pommier and his colleagues decided to attack. But to do so, they needed help.

Panning for New Topo I Inhibitors

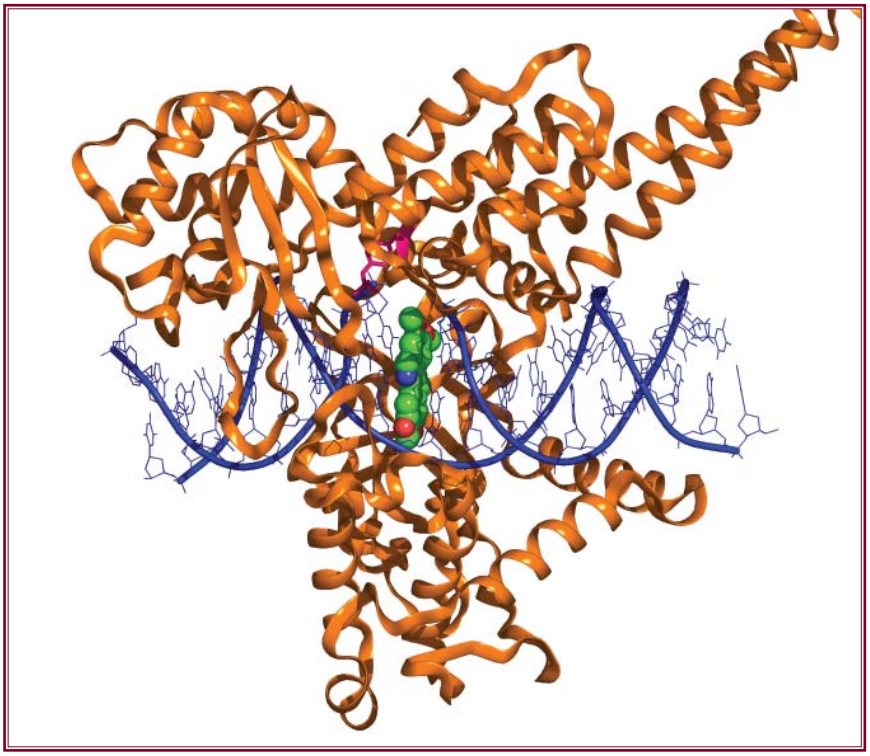
Prior to his death a few years ago, Ken Paull, Ph.D., worked with NCI's Developmental Therapeutics Program (DTP) to screen compounds for anti-cancer activities. NCI has 60 distinct standardized cancer cell lines, the so-called NCI-60, that its scientists use to screen compounds at five different concentrations for their ability to inhibit growth. Since no two cell lines are identical, compounds with different mechanisms of action affect the proliferation of individual cancer cell lines differently. Paull and his colleagues realized that by comparing dose-response profiles across all 60 cell lines, they could classify compounds with related mechanisms of action; drugs that affect all of the cell lines in a similar way are likely to operate via a similar mechanism. Paull formalized this logic in a computer algorithm called COMPARE.

Familiar with Paull's work, Pommier decided to see if COMPARE could pick out compounds that work like camptothecin. When they struck gold, the compound they identified, an indenoisoquinoline, turned out to be the byproduct of another serendipitous event captured by the NCI. The compound, synthesized by chemist Mark Cushman, Ph.D., at Purdue University, was the result of an "unexpected, undesired reaction," as he put it, that occurred as he attempted to synthesize the anti-leukemia agent nitidine chloride. Instead of discarding it, Cushman placed the indenoisoquinoline compound in the NCI-60 database, where it sat untouched for 18 years, until he received a phone call from Paull.

Cushman immediately set to work making indenoisoquinoline analogs—400–500 of them—which he sent to NCI for Pommier's group to test against purified topo I and in cell culture for structure-activity relationships. The data led Pommier and Cushman to focus on the two most promising candidates, which are now on the verge of entering the clinic for the first time. "At this point, we've done preclinical and toxicology work, and the clinical protocols have been written," said Pommier.

But advancing these compounds from the chemistry lab to the clinic would not have been possible without the DTP, which as a mission takes lead compounds that show promise in cell culture and puts them through the many hurdles of animal experiments and formulations that must be cleared before first-in-human trials. For example, the academic synthesis protocols that Cushman develops may bear scant relation to the synthesis processes necessary for the high-volume commercial manufacturing steps that a pharmaceutical company must employ. Similarly, the drugs that Pommier tests *in vitro* are all dissolved in DMSO, which is toxic to human beings, and so must be assessed for solubility in non-toxic solvents as well. The DTP has even enabled the development of a biomarker for topo I inhibition that research clinicians will use in their clinical trials, the phosphorylation of histone γ-H2AX. This biomarker was first associated with DNA damage by another CCR investigator, William Bonner, Ph.D. Another NCI colleague, James Doroshow, M.D., Director of NCI's Division of Cancer Treatment and Diagnosis, collaborates with CCR's Laboratory of Molecular Pharmacology and has been a key player in the development team leading to the clinical evaluation of the indenoisoquinolines at the NIH clinical center.

Pommier and Postdoctoral Fellow Thomas Dexheimer, Ph.D., continue to collaborate with Cushman to test new potential topo I inhibitors. "When you have one target, you want to have more than one type of drug," said Pommier. "Even drugs in the same family, such as irinotecan and topotecan, have different clinical profiles. We're making the assumption, but I think it is likely to be the case, that the indenoisoquinolines are going to have a different clinical profile from any of the camptothecins. And we have many arguments to say why they have advantages, but the proof will become apparent when we give these compounds to patients."



(*Image: Y. Pommier, CCR*)

Indenoisoquinolines *(green)* glue complexes of topoisomerase I *(brown)* and DNA *(blue)* together.

Nature Plus Nurture: The Consortium Approach

Citing the examples of paclitaxel and camptothecin, Pommier is convinced that Nature has many more hidden treasures that could benefit mankind's health: "Nature has taken a long time to optimize for us," he said. "Although we now have powerful methods for visualizing and predicting compounds' structural features and binding activities, rational drug design is not the only way forward." Rather, screening and rational drug design are complementary parts of an overall drug discovery strategy that Pommier and his colleagues are using to go after another cancer target, the DNA repair enzyme Tyr-DNA-PDE, or TDP. TDP repairs the stalled DNA replication caused by topo I inhibitors, so cells that are missing TDP are hypersensitive to topo I inhibitors.

> Pommier is convinced that Nature has many more hidden treasures that could benefit mankind's health.

Because TDP had no known inhibitors, Christophe Marchand, Ph.D., a Staff Scientist in Pommier's group, spearheaded high-throughput screening against TDP in collaboration with the NIH Chemical Genomics Center (NCGC) up the road in Gaithersburg, Md. Although Marchand had already developed an assay for the Pommier laboratory's in-house screening system when they began their collaboration, he needed to reoptimize it for the NCGC, more or less on his own.

"We got lucky," he said of the success of his early optimization attempts. After less than a year, NCGC was convinced that it could screen its entire compound library of over 300,000 compounds against Marchand's TDP inhibitor assay, a screen that was completed in the first week of June 2008.

The TDP project now includes more than just Pommier's group and the NCGC. NCI's Chemical Biology Consortium has since taken an interest in the work and set up an entire team of investigators, supported by dedicated project managers, to promote the development of TDP inhibitors "from bench to bedside." Across NCI, more than 20 investigators meet regularly to share data and plan new experiments, including using synthetic chemistry to design better inhibitors based on the structural analysis of lead compounds (see "SCSORS Takes the Lead").

Marchand counts the success of this project to date among his proudest achievements and is excited about the collaboration and the opportunities afforded by a large consortium in overcoming practical obstacles. "For the first time, I have the feeling that we are surfing on big waves."

"The resources are amazing, although they aren't always connected up as well as we'd like," Pommier noted when describing the path he took to establish a collaboration with the NCGC. "The NCI is a powerful place for this kind of work."

Learn more about Yves Pommier's research at http://ccr.cancer.gov/staff/staff.asp?profileid=5812 and http://discover.nci.nih.gov/pommier.

To learn more about camptothecin and other natural products, see "The Natural Products Repository: A National Drug Development Resource," page 9.

SCSORS Takes the Lead

To tap into the vast reservoir of possible synthetic organic chemistry, the NIH has developed a new Semi-Custom Synthesis On-line Request System (SCSORS) in conjunction with the company ChemNavigator, Inc. SCSORS has been funded mostly by NCI with additional financial support from the NIH Chemical Genomics Center (NCGC).

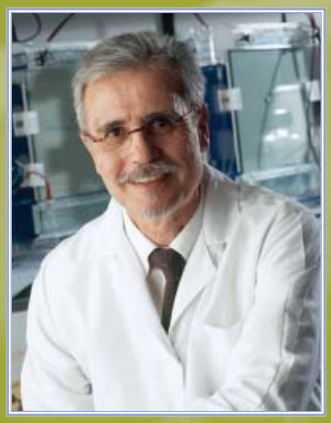
The new SCSORS project will provide the NIH (and the NIH Roadmap-associated screening centers) access to the world's supply of synthetic chemistry available for drug discovery. It will also help NIH scientists to access specific chemical samples, in amounts ranging from milligrams to kilograms, from thousands of synthetic chemists at suppliers registered in the system.

NIH researchers will be able to use SCSORS in three ways:

1) By proposing specific structures for which they request a SCSORS quotation
2) By submitting a structure—typically a lead generated from an assay—to ChemNavigator's affiliated chemistry procurement service, which will do a "medicinal chemistry expansion" of this structure and present a series of analogs for selection and approval before submitting them to suppliers
3) By requesting that a structure (or structures) be presented to suppliers as is, with the requests, "What can you do with this molecule? Which analogs do you think you can synthesize, and at what cost?"

The hope is that using the SCSORS strategy will allow the NIH to acquire chemical samples at less than 10 percent of the internal cost of synthesis while accessing global chemical expertise and diversity.

In the long-term, SCSORS will become an archive of commercially accessible custom chemistry products for pharmaceutical research. The project's leaders expect that its database will grow to over 250 million substances in the coming two years.



(Photo: R. Baer)

Yves Pommier, M.D., Ph.D.

Yves Pommier, M.D., Ph.D.

Pommier heads the Laboratory of Molecular Pharmacology at NCI where his research has centered on DNA processing mechanisms and on two enzyme classes in particular—cellular DNA topoisomerases and HIV integrase.

In addition to his focus on the role of DNA topoisomerases in cancer, Pommier began studying HIV integrase in 1993 in response to the widespread call to arms in the research community for the development of AIDS therapies. Pommier's group reported the first HIV integrase inhibitor and proceeded to develop several more.

Pommier joined the NIH in 1981 after receiving his degrees from the University of Paris, France. Although he does not do clinical work himself, he is glad that he was encouraged to receive both an M.D. and a Ph.D. He was quickly frustrated as a hematology/oncology resident by the paucity of treatments available for the cancers that ravaged his patients; he attributes the direction of his career in molecular pharmacology and translational research to these clinical experiences.

"You think differently," he says, explaining that he does not lose sight of the goal of turning research into cures. "I've had great fun [studying DNA processing]. But it would be so pleasing to discover one drug and make a difference."

(Photo: R. Baer)

Christophe Marchand, Ph.D.

Christophe Marchand, Ph.D.

Marchand's career epitomizes personal initiative. As an undergraduate in Reims, France, Marchand wrote to the organizer of an international meeting in Paris on DNA-drug targeting and convinced him to waive the attendance fees. At the meeting, he met his first mentor in the field, Cambridge University's Michael Waring, Ph.D., who took the fledgling scientist under his wing. For the next five years, Marchand spent every summer at Cambridge, returning in the fall with another publication under his belt.

Waring introduced Marchand to Claude Hélène, Ph.D., Head of an INSERM unit in the Laboratory of Biophysics at the French Natural History Museum, who supervised Marchand's doctoral work on DNA triple helices—molecules composed of three rather than two spiraling strands of nucleic acids. Marchand's passion for these intriguing molecules is still evident in his voice. "I had a revelation when I heard Hélène talking about DNA triple helices—there was a spark in my head—the applications seemed almost endless." The drug he developed for his thesis, which specifically identifies DNA triple helices, is now in the Sigma catalog.

Marchand, currently a Staff Scientist, has been in Pommier's group for ten years. His primary expertise is the study of HIV integrase, but he has broadened his focus to include the development of high-throughput screening assays. Although he does not rule out returning to France some day, he is pleased with his current position, which affords no shortage of opportunities for anyone with initiative.

(Photo: R. Baer)

Thomas Dexheimer, Ph.D.

Thomas Dexheimer, Ph.D.

Dexheimer came to the NIH two years ago, motivated to pursue postdoctoral work with Pommier after hearing him give a seminar at the University of Arizona (UA) where Dexheimer was completing his Ph.D. His doctoral work also focused on DNA with an eye towards drug discovery, so the transition was a natural one. In the lab of UA's Laurence Hurley, Ph.D., Dexheimer studied DNA secondary structures—G-quadruplexes, so called because of their four-stranded guanine-enriched composition. Most G-rich regions are in promoters, and Dexheimer had hoped to design drugs to stabilize G-quadruplexes in cells as a means of targeting proto-oncogene promoters.

Dexheimer is currently involved in both the TDP and topo I inhibitor projects. Although he arrived after the two lead indenoisoquinoline inhibitors of topo I were discovered, he continues to look for new compounds which may have different yet advantageous clinical profiles. He also hopes that since TDP's and topo I's mechanisms of action are linked, the two projects may intersect in combination therapies.

Dexheimer knows that the odds of turning a lead compound into a successful drug are very low. But that does not temper his excitement in the search for new classes of topo I inhibitors. "My father won the Wisconsin state lottery when I was in high school," he noted, a windfall that helped pay for Dexheimer's college undergraduate chemistry degree (and a few other things, like a hot tub). "I'm an optimist."

Laying the Groundwork for a Revolution

Over the last 100 years, lung cancer has grown from an obscure malignancy to the leading cause of cancer death globally. While public health efforts to reduce tobacco use can impact the rates of smoking-associated cancers, other methods must be brought to bear for the relatively small but significant number of lung cancer patients with no smoking history. The genomics revolution has brought about the promise of targeted therapy for these patients, as the work of past decades set the stage for the discoveries of today. ***Bruce Johnson, M.D.****, former Head of the Lung Cancer Biology Section in the NCI Medicine Branch (now the CCR Medical Oncology Branch) and current Director of the Lowe Center for Thoracic Oncology at Dana-Farber Cancer Institute and Principal Investigator of the NCI-sponsored Specialized Program of Research Excellence (SPORE) in lung cancer at the Dana-Farber/Harvard Cancer Center, offers his thoughts on how scientific foundations laid 20 years ago are now supporting a transformation in lung cancer care.*

It is hard to believe that in the early 1900s, lung cancer was rare enough to be considered a reportable disease. The renowned surgeon Alton Ochsner, one of the first to document the link between tobacco and lung cancer, once remarked that as a student in 1910, he was asked to view an autopsy of a lung cancer patient on the grounds that the disease was so rare he might never have the chance to see another case.[1] Contrast this view with the number of lung cancer cases that we see today: The American Cancer Society estimates that nearly 162,000 people will die of lung cancer this year just in the United States. Lung cancer now claims more people than colon, breast, and prostate cancer combined.[2]

However sobering these numbers may be, there is, of course, cause for hope. The number of people dying from lung cancer is going down. This trend is due to the nature of lung cancer as, primarily, a disease of tobacco use. The epidemic rise of lung cancer in the 20th century can, in large part, be tied to the rise in popularity of smoking in the years during and following World War I.[3] The continued development and deployment of effective tobacco control strategies, starting in the latter half of the century and carrying forward into the present day, promise to have a lasting dampening effect on lung cancer prevalence and mortality.

As the methods for lung cancer prevention have evolved, the methods for lung cancer therapy have similarly advanced. Surgery remains a mainstay of treatment, much as it has been for the last 50 or so years. Radiotherapy has improved, thanks to the development of techniques and technologies that allow the focused application of high doses of radiation directly to a tumor with minimal exposure to surrounding healthy tissues. Chemotherapy has also improved, but it has been applied in an overly broad way. The dominant paradigm has been to treat 100 percent of patients with the same approach to achieve a 20 to 30 percent response rate.

When I started working at the NCI's Medicine Branch as a Clinical Associate, my colleagues and I recognized that characterizing tumor samples genetically would be crucial for the ongoing development of lung cancer therapy. For instance, one of the first things that my mentor, John Minna, M.D. (now at the University of Texas Southwestern Medical Center), and I investigated was the link between *C-MYC* amplification and survival in small-cell lung cancer. But we also recognized that for such developments to come to fruition, we would need to have at our disposal a sizable sampling of tumors large enough to capture infrequent but clinically and biologically important mutations.

> The legacy of commitment to translational research and training at the heart of NCI's intramural program is a driving force behind the national lung cancer research agenda.

Based on this reasoning, Minna and Adi Gazdar, M.B.B.S. (also at UT Southwestern), set out to systematically generate cell lines from nearly every lung

cancer patient who came to the NIH Clinical Center. Because of our relatively low patient volume, we were fortunate to be able to study our patients very intensively. The patients we saw then numbered in the hundreds annually; in contrast, we see thousands per year just at Dana-Farber. With time and dedication, particularly on the part of the laboratory scientists who actually cultured the tumors that we collected, we were able to create 200 lung cancer cell lines (representing between 20 and 30 percent of patients who crossed the Clinical Center's threshold) while I helped annotate those lines with comprehensive clinical and outcome information for each patient.

At the time that we started these efforts, back in the 1980s, some thought that it was a lot of work for little benefit, that the resources we needed to do this systematic sampling could be better used in other ways. However, these efforts have proved to be more valuable than we suspected at the time. For instance, in 2004, my colleagues at Dana-Farber and I uncovered an association between specific mutations in the epidermal growth factor receptor (EGFR) and the responsiveness and outcomes of patients with non-small cell lung cancer (NSCLC) treated with the EGFR inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®). The first cell line that we found that matched the sensitivity to these two compounds that we saw in patients with this mutation, a cell line called NCI-3255, was one developed as part of this systematic sampling project. This cell line was collected from a woman with an adenocarcinoma who had no history of smoking, a clinical profile that matched the profiles of patients responding to these drugs and who also had the same mutation.

This same cell line also revealed to a trainee and now colleague of mine, Pasi Janne, M.D., Ph.D., one of the mechanisms by which initially sensitive lung tumors can become resistant to EGFR inhibitors, as generally happens within one to two years of treatment with gefitinib or erlotinib. Through the Lung Cancer SPORE program at the Dana-Farber/Harvard Cancer Center, we found that the tumors of some patients treated with these drugs developed a compensating EGFR mutation called T790M. To prove that this new mutation was responsible for this resistance, Janne exposed the NCI-3255 cell line to increasing concentrations of gefitinib for six months. Characterization of the now drug-resistant cell line revealed the same compensatory mutation.

In 2007, a Japanese group announced the discovery of a link between clinical outcomes in a small percentage of NSCLC patients and a genetic translocation called EML4-ALK. As with EGFR mutations, this translocation was discovered more frequently in women with adenocarcinomas who did not smoke; it appears to arise in only about two to three percent of NSCLC tumors. Having found that another of the cell lines we developed at NCI, called NCI-3122, contains this translocation, we have been able to characterize this translocation *in vitro*, develop an *in vivo* model, and begin to study ALK inhibitors as targeted lung cancer treatments.

The revelations we and others generate with these cell lines work both ways. By exposing an additional cell line started by Gazdar and Minna, HCC827, to gefitinib for one year, we discovered that a different genomic alteration, an amplification of the oncogene *MET*, can also give rise to EGFR inhibitor resistance. Going back to archived tumor samples, we have found the same amplification in 20 percent of gefitinib- or erlotinib-sensitive lung cancers that developed resistance.

The list of potentially druggable mutations discovered and characterized using these cell lines continues to grow. And it is doing so at a remarkable pace; the discoveries of the T790M mutation and *MET* amplification noted above happened in the span of two years. As the list grows, a new appreciation of lung cancer's molecular heterogeneity has emerged. Each of these mutations appears only infrequently, at rates ranging between 2 and 10 percent of NSCLC tumors. Because we created so many cell lines with CCR, it is possible to identify at least one cell line for each of these rare mutations, test the cells with different agents, select those agents to which the cells show the greatest sensitivity, and translate them into clinical application.

This heterogeneity in lung cancer tumors and cell lines provides an opportunity to generate an overall model of cancer genomics in translational research. An issue with which all physician-scientists struggle is how to gather enough of a population to study low-frequency events. Lung cancer is a very common malignancy; more than 200,000 people are diagnosed every year, the majority with advanced disease. Thus, by virtue of the sheer number of patients, even infrequent events like EML4-ALK will appear in enough patients to gather large relevant cohorts for clinical trials.

The legacy of commitment to translational research and training at the heart of NCI's intramural program is a driving force behind the national lung cancer research agenda. It should be noted that the leaders of five of the seven funded Lung Cancer SPORE programs in the U.S. are former members of the NCI Medicine Branch, including Minna and me.

And this legacy is fueling what could be a tectonic shift in lung cancer care. With a greater understanding of the frequencies and roles of such mutations in the general population of lung cancer patients, we may be on the verge of flipping the treatment paradigm: By grouping patients based on tumor genomics and treating them with the appropriate targeted therapies, instead of treating 100 percent of patients the same and achieving 20 percent success, we aim to treat 20 percent of patients the same and achieve 100 percent success.

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[1] *Spiro SG and Silvestri GA. One hundred years of lung cancer. Am J Respir Crit Care Med.* 2005;172:523-529.

[2] *American Cancer Society. "What are the key statistics about lung cancer?" http://www.cancer.org/docroot/CRI/content/CRI_2_4_1x_What_Are_the_Key_Statistics_About_Lung_Cancer_15.asp?sitearea=. Accessed* 07/11/08.

[3] *Spiro and Silvestri, ibid.*

(Photo: Courtesy of B. Johnson)

Bruce E. Johnson, M.D.
Professor of Medicine
Harvard Medical School
Principal Investigator
Dana-Farber/Harvard Cancer Center SPORE (Specialized Program of Research Excellence) in Lung Cancer

Ovarian Cancer:
A Silent Killer "Speaks" through Proteins

***Elise Kohn, M.D.**, is passionate about expanding our knowledge of ovarian cancer through teaching. When she is not mentoring the next generation of scientists and physicians, this 22-year veteran of NCI spends her days moving from bench to bedside—literally—as she leads both the Molecular Signaling Section ("bench") and the Medical Ovarian Cancer Team ("bedside") within the Medical Oncology Branch of CCR. And when she is not in the clinic or in the lab, Kohn is on the phone providing consultations for other patients across the country who seek her guidance after learning about her program through the Ovarian Cancer National Alliance, National Ovarian Cancer Coalition, and other ovarian cancer networks. CCR has provided Kohn with unparalleled opportunities to advance her science, which may not have received the same funding and support outside of the intramural program. This has allowed her to break ground in the clinical arena as she and her team find new ways to both diagnose and treat the no longer so-called "silent killer" known as ovarian cancer.*

(*Image: R. Baer*)

Elise Kohn, M.D.

The American Cancer Society estimates that 21,650 women in the United States will be diagnosed with ovarian cancer in 2008; 15,000 will die of their disease. The good news is that women who present with Stage I ovarian cancer have a greater than 90 percent chance of being cured. The bad news is that only 20 percent of patients are diagnosed at this early stage of disease. Less than 35 percent of patients with advanced-stage disease—80 percent of all women diagnosed—will survive beyond five years. This sobering statistic is the reason that ovarian cancer is the leading cause of gynecologic cancer death in the U.S. and why it ties with pancreatic cancer for fourth place in women's overall cancer mortality.

Barriers to Early Detection

Early detection, critical for surviving ovarian cancer, is one of the most imperative issues in ovarian cancer care, but it is most certainly not easy. Due to the elusive nature of the disease, there are a number of reasons why ovarian cancer used to be referred to as the "silent killer."

Ovarian cancer is difficult to detect. The ovaries lie deep within the abdominopelvic cavity, making them difficult to view or feel. It was initially believed that ovarian cancer lacked warning signs, although we now know that there are subtle symptoms that may suggest disease. In 2007, the American Cancer Society, the Gynecologic Cancer Foundation, and the Society of Gynecologic Oncologists released a consensus statement claiming that symptoms often do exist for ovarian cancer, even in the early stages. These symptoms include bloating, feeling full quickly, pelvic or abdominal pain, and frequent or urgent urination.[1] The problem with these symptoms is that they are common and occur with a number of ailments. But, if they occur almost daily and last for more than a few weeks, women should see a gynecologist. It remains to be seen whether this symptom checklist will help women detect ovarian cancer sooner rather than later.

Another hurdle to early detection is the lack of validated screening tools to identify disease. The Papanicolaou ("Pap") test is used to screen for cervical cancer and the mammogram to screen for breast cancer, but there is no validated

and robust test that can identify ovarian cancer. The biomarker CA-125, a protein in the blood that is sometimes elevated in women with ovarian cancer, is approved to monitor response to treatment as well as to detect recurrent ovarian cancer, but it is not sensitive and specific enough to identify early disease or to have an impact on survival. This lack of effective molecular diagnostics is why there is a great need to identify alternative biomarkers that can detect cancer at Stage I—when the disease is most amenable to cure.

CCR's Ovarian Cancer Medical Team is running a number of clinical trials to achieve two critical goals: to test the use of combinations of molecularly targeted therapies to treat recurrent and refractory disease; to identify diagnostic biomarkers for early detection and recurrent disease; and as a companion diagnostic with treatment.

We work from an understanding of the critical role that protein pathways, or networks, play in cancer. We postulated some time ago that future therapeutics will target entire protein networks, not just one protein. For this reason, we have invested our energies into the application of proteomics (the study of proteins and their networks) in both the laboratory and clinic. Blood and/or tissue samples are obtained from all patients for use in analyzing protein networks with the goal of developing life-saving diagnostic tests. This work has allowed the once-silent killer to be heard.

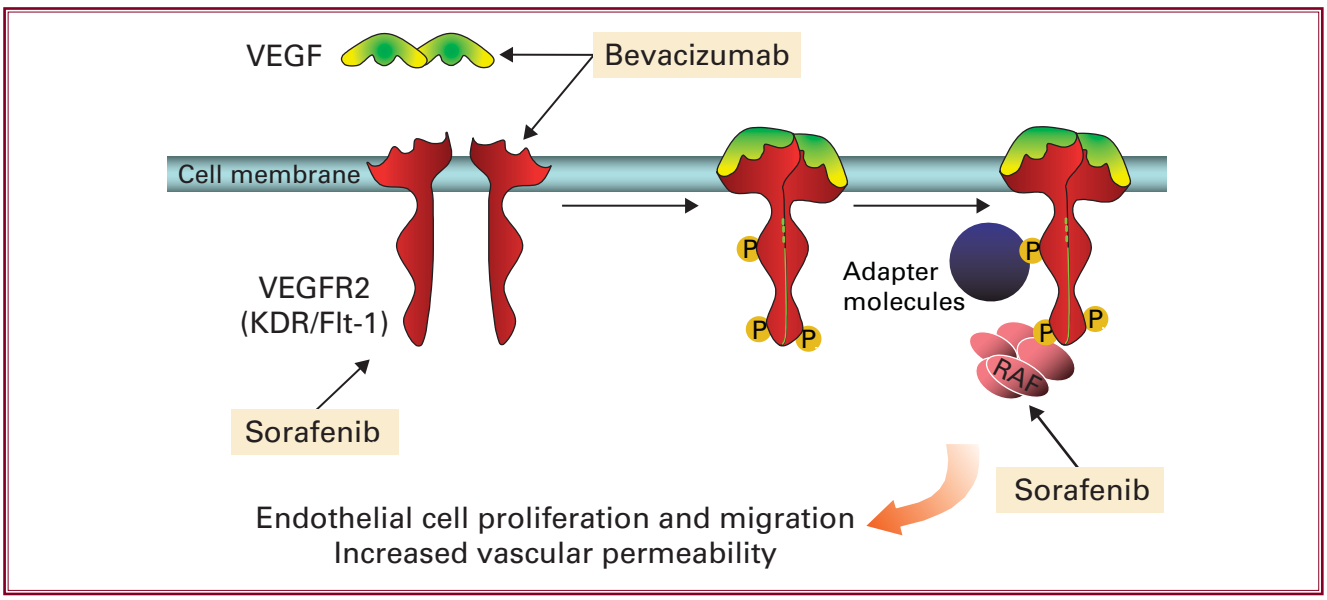
A Mix of Molecularly Targeted Therapies

Ovarian cancer will return in approximately 90 percent of patients who have advanced stage disease. Because recurrent ovarian cancer cannot be cured, it must be treated as a chronic disease, with the understanding that with chronicity comes a need for optimal benefit and minimal risk. We are running early stage clinical trials (Phase I and II) of targeted therapy combinations for recurrent and refractory tumors. These early trials will help determine how a drug might best be given, how often, at what dose and, most importantly, how safe it is in patients. The studies are also designed to address proof of concept, determining whether the drug (or combination) does what it is supposed to do.

Understanding the protein profile of a patient's tumor may help identify treatments that deliver the best outcome for the individual patient. My colleagues and I coined the concept of "personalized molecular medicine" in 2001.[2] If a signaling pathway is overactive in a patient's tumor, targeting that pathway at multiple points simultaneously may more effectively control the activity and at potentially lower doses of both agents.

Targeting the vascular endothelial growth factor (VEGF) pathway, well known to be a critical pathway for the process of angiogenesis is a hypothesis currently being explored in our clinic. Angiogenesis is a normal physiological process that occurs when new blood vessels grow from existing blood vessels. In 1971, the late Judah Folkman, M.D., first proposed that tumors relied on angiogenesis for survival; if they were denied this blood supply, the tumors would die. In 1974, Lance Liotta, M.D., Ph.D., demonstrated that angiogenesis was necessary for metastasis, the process of cancer dissemination. After decades of disregard, angiogenesis became widely accepted throughout the scientific and medical communities, and the field of anti-angiogenesis therapy was born. In the clinical trials being conducted here at CCR, such therapies are used in an attempt to "starve" the ovarian tumors.

My team and I have recently reported on the safety and efficacy of a combination of two agents that block angiogenesis: bevacizumab (Avastin®) and sorafenib (Nexavar®). Although both agents target the VEGF pathway, each does it through different mechanisms (Figure 1). Bevacizumab, FDA-approved for non-small cell lung cancer and metastatic colorectal and breast cancers, is an anti-VEGF monoclonal antibody that prevents VEGF from binding to its receptor (VEGFR). Sorafenib, FDA-approved for advanced renal cell carcinoma and hepatocellular carcinoma, is a small molecule drug that blocks VEGFR2 and downstream signals that are activated by VEGF.



(Image: J. Kelly)

Figure 1: Kohn and colleagues are the first to target the VEGF signaling pathway in series by combining the anti-angiogenesis treatments bevacizumab and sorafenib. Ongoing clinical trials indicate that this approach inhibits the pathway at two different points; as such the combination therapy holds promise for the treatment of refractory or recurrent ovarian cancer.

Our hypothesis is that targeting the VEGF pathway in series rather than in parallel will enhance the effects of both agents. We are also inhibiting the pathway at two different points—in endothelial (blood cells) and epithelial cells (ovarian tumor cells)—using this strategy. Our clinic is the first to target VEGF signaling in series with combination specific anti-angiogenesis therapy.

There are two clinical trials under investigation using this combined treatment. In a Phase I study, 62 patients with refractory, metastatic, or unresectable solid tumors of any type have been enrolled. This study is addressing identification of optimal doses, safety, and toxicity of this regimen in these patient populations. Tumor samples have been obtained from which to measure changes in the targeted protein networks and correlate them to a clinical outcome.

The second study using this combination therapy is a Phase II study specifically for patients with recurrent ovarian, fallopian tube, or primary peritoneal cancers. The objective of this trial is to confirm potential benefit of sorafenib and bevacizumab in these patients and to help guide further application of the regimen outside of NCI. Initial Phase I data in these patient populations showed promising activity in tumors known to have increased VEGF pathway signaling, but with synergistic anti-tumor activity at doses below the standard single agent treatment doses. Thirty-three percent of all treated patients had some reduction in tumor size—some quite rapidly—and many of the rest saw their tumors stabilize (Figure 2). Combination therapy reduced the blood supply to many patients' tumors. We observed a greater benefit than was expected in a Phase I clinical trial, and this has given us hope that these results will be reproduced in the ovarian cancer Phase II study.

We will analyze patient tumor samples, collected prior to treatment and while patients were on therapy, to investigate whether those who had a good response to treatment displayed an initially overactive VEGF pathway or one inhibited by treatment.

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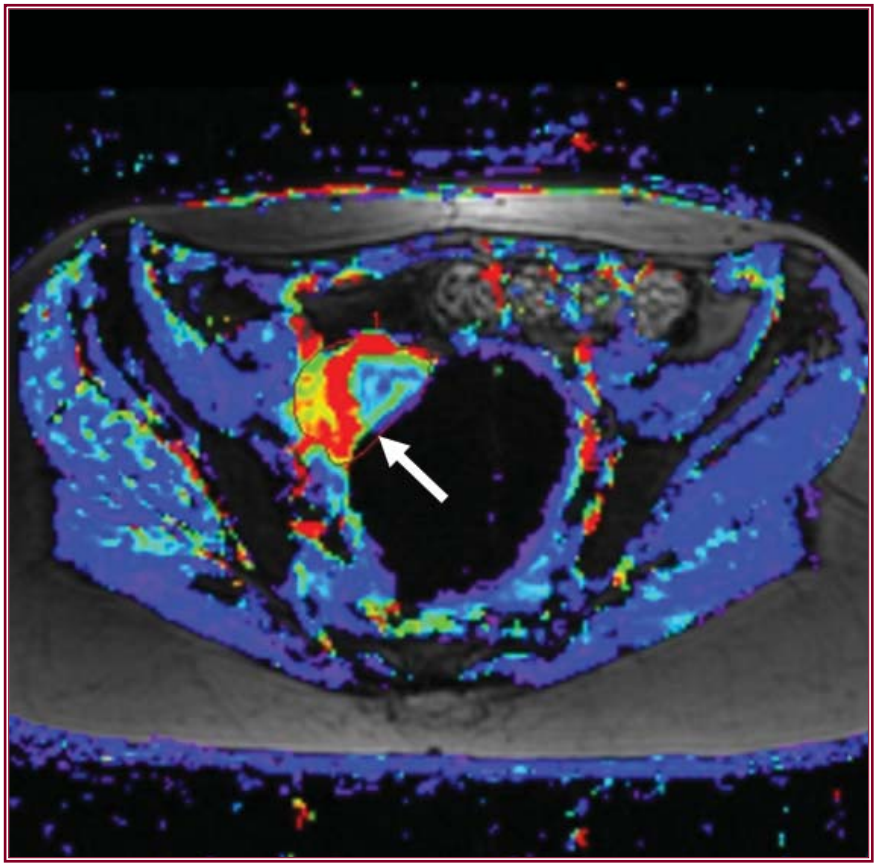

(*Image: P. Choyke, CCR*)

The resulting data could provide further justification for tailoring therapy to a tumor's protein profile and could result in a companion predictive test for this combination therapy, allowing doctors to monitor response during treatment.

Diagnostic Biomarkers

The lack of a validated screening test for ovarian cancer has prompted investigators to seek alternative diagnostic strategies. Tumors leak proteins into body fluids, including blood and urine, and some of these proteins may be able to alert doctors to the presence of disease. These cancer-related proteins are known as cancer biomarkers. By collecting these fluids, it may be possible to develop a biomarker that may diagnose cancer at an early stage.

Biomarker use is not a new concept. Elevated prostate specific antigen (PSA) is an example of a biomarker that can be detected in men who have organ-limited prostate cancer. Technologies for detecting proteins and our understanding of the underlying relationship between proteins and cancer have come a long way. These scientific advancements are being translated to clinical trials to benefit our patients.

My ovarian cancer team and I are in collaborations to analyze blood samples from ovarian cancer patients for protein "signatures," or patterns of proteins, that can predict early-stage ovarian cancer and cancer recurrence. In particular, candidate biomarkers will be compared against or tested alongside the CA-125 biomarker to determine whether they are more effective than this biomarker in predicting ovarian cancer's return.

In order to carry out this biomarker research, my colleagues and I are developing a repository, or bank, of blood samples from patients enrolled in one of the clinical trials. Because few, if any, cancers are characterized by a single reliable biomarker, such as PSA, this sample collection is critical. We will collect and analyze a large number of blood samples. Our trial is designed to accrue samples from 400 women with the goal of identifying signatures and biomarkers that may have true value in predicting ovarian cancer relapse.

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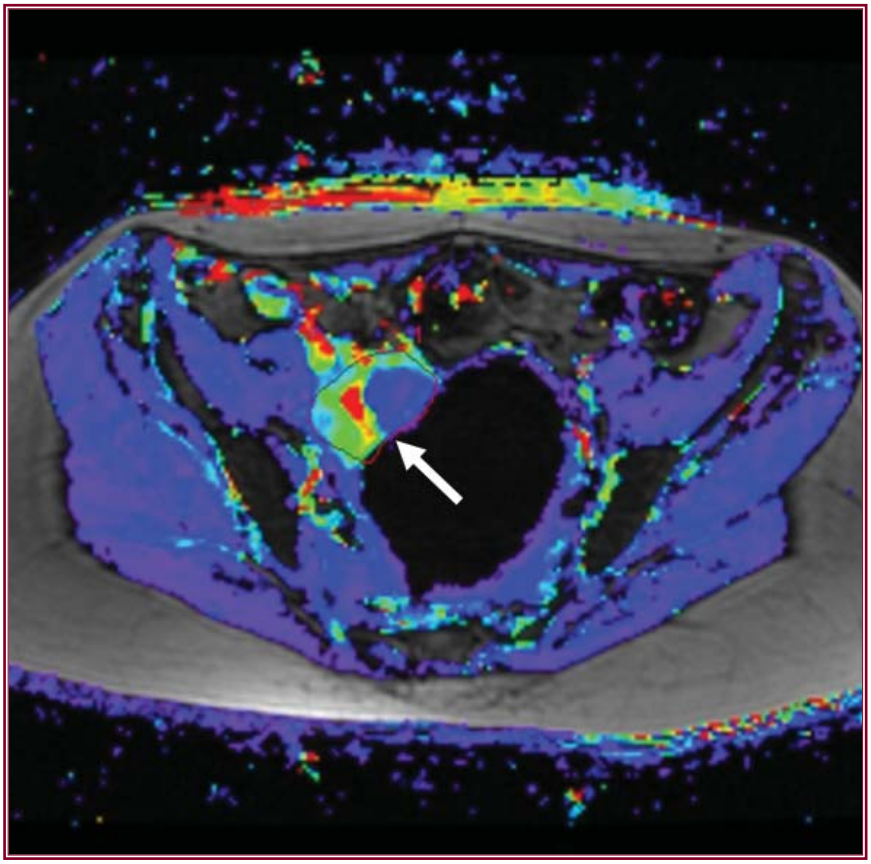
[1] *Goff BA et al. Development of an ovarian cancer symptom index: possibilities for earlier detection. Cancer.* 2007;109(2):221-227. *The full text of this publication is available at http://preview.tinyurl.com/5ox5zq.*

[2] *Liotta LA, Kohn EC, Petricoin EF. Clinical proteomics, personalized molecular medicine. JAMA.* 2001;286(18):2211-2214.

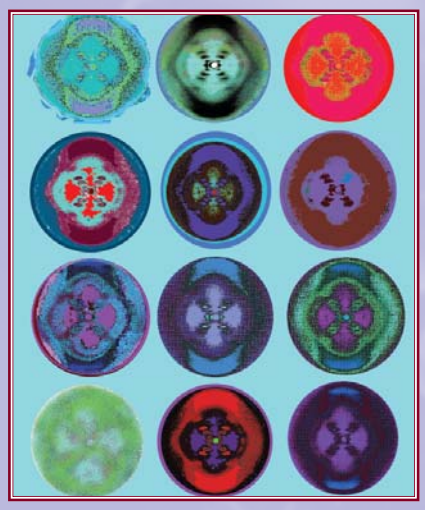
To learn more about Dr. Kohn's work, *visit http://ccr.cancer.gov/staff/staff.asp?profileid=5844*



(*Image: P. Choyke, CCR*)

Figure 2: The difference in a tumor's blood flow before *(left, red and yellow region next to arrow)* and after *(right)* treatment with a combination of anti-angiogenic therapies can be striking. In a Phase II clinical trial, thirty-three percent of patients treated with sorafenib and bevucizumab, which target different components of the angiogenic pathway, saw some reduction in tumor size.

Answering the Voices of Family History



(*Image:* A Vision of Rosalind Franklin *for* OCNA ©2006 Wyllie O Hagan)

"Vision of Rosalind" is an artistic rendition of Rosalind Franklin's pioneering first glimpse of the X-ray crystal structure of DNA, created by an artist and ovarian cancer survivor to honor Franklin's personal battle with the disease that ultimately took her life. The artist and Elise Kohn, M.D., met in 2006, the year Kohn received a Rosalind Franklin Excellence in Ovarian Cancer Research Award from the Ovarian Cancer National Alliance.

Katherine Horn, of Montgomery County, Md., came by her predisposition for cancer "honestly"; she carries a BRCA mutation, a type of genetic mutation that makes her more susceptible to breast and ovarian cancer. Cancer is prevalent throughout her family tree, including a male second cousin who had breast cancer, as well as two out of her three sisters who had previous bouts of breast cancer.

Early in 2005, Horn began noticing symptoms that included abdominal bloating and headed straight to her oncologist. "I knew with my family history that I was in big trouble," said Horn. In May of 2005, Horn's oncologist confirmed the diagnosis of ovarian cancer. Though devastating, the diagnosis did not come as a complete surprise, and though successful, the surgery revealed that the cancer had spread to the lymph nodes.

Horn responded well to treatment with paclitaxel (Taxol®) combined with intraperitoneal cisplatin, a recently recognized advance in the treatment of ovarian cancer, but in April 2006 a blood test revealed that her CA-125 (a protein biomarker associated with ovarian cancer recurrence and response to treatment) had gone up again. "I really wanted to go after it aggressively," Horn said, and she began another round of treatment.

Unfortunately, Horn's CA-125 levels started to climb again soon after her second treatment regimen ended. She went through this experience several times. Then her doctor, benefiting from a resident NIH nurse in his office who helps link patients with clinical trials at CCR, suggested that she join a clinical trial being run by Elise Kohn, M.D.

Though Horn met all of the trial criteria and began treatment in January 2008, an adverse reaction forced her to withdraw from the study. However, her positive experience at CCR and with the community of doctors and nurses there has led her to seek treatment in another clinical trial being run by Kohn, this one designed specifically for patients with the BRCA genetic mutation.

When speaking of Kohn, Horn emphasized the unique and supportive relationship that Kohn and the entire CCR staff strive to maintain with their patients. "It's that extra supportive layer," explained Horn. "My relationship with Dr. Kohn is not just a doctor-patient relationship. I know something about her life outside of CCR, and she knows something of mine.

"Not only are the doctors, nurses, and hospital staff wonderful," Horn continued, "but the physical facility itself is the most relaxing, comfortable hospital I've ever been in. As a former project manager for hospital renovations," she said, "I should know; I used to work in them!

I would definitely encourage people to try CCR," Horn said. "You're getting really avant garde medical treatment, and you're getting absolutely fantastic emotional and medical treatment from the staff."

Sharon Morris also understands the impact that a family history of cancer can have on both the past and the present. In her family, cancer is considered the "family curse." "I watched my father, my grandfather, my cousins, so many people in my family, die young," said Morris. Morris has the BRCA1 mutation. This mutation, a part of her family tree for generations, would come to affect her as well.

After taking time off to care for her mother, Morris, a former banker from New Jersey, was inspired to go back to school to become a certified surgical technician. Just after her graduation in December 2007, she began to notice unusual abdominal swelling, despite a good report following a gynecological exam the month before. Morris sought the advice of the obstetrician who helped deliver her two daughters. She was diagnosed with ovarian cancer by January 2008 and started treatment at the Robert Wood Johnson University Hospital in New Brunswick, N.J.

After two courses of standard treatment and surgery, Morris entered remission, but the cancer returned two months after she finished her second round of treatment. It was then that her oncologist suggested that conventional treatment might not be the answer for her, and she started looking into clinical trials. Unfortunately for Morris, she had an adverse reaction to the treatment in her first clinical trial and did not have positive results with the second. Morris started researching other possibilities for treatment. "From the day I was diagnosed," said Morris, "I would research, 24/7. If you could have a master's degree in ovarian cancer, I would have it."

Morris read about a new type of cancer treatment, the poly (ADP-ribose) polymerase inhibitor (PARP inhibitor). This type of drug has shown to be an exciting and promising advance for women with the BRCA1 and BRCA2 mutations, with the added benefit of fewer toxic side effects than standard chemotherapy treatments. Her interest in PARP inhibitors and a suggestion by her doctor led her to CCR, and she has enrolled in a PARP inhibitor-focused clinical trial being conducted by Kohn.

Though Morris only started her treatment with Kohn in June, her experience with CCR has been nothing but positive. "I have never gone to a place like CCR," explained Morris. "People at the NIH are in a class by themselves."

Given Morris's family history with cancer, she realizes that her participation in research at CCR is not just for her but for her entire family. "Everyone who has been afflicted with the BRCA1 mutation is gone," stated Morris. "But it ends here."

Morris is hopeful about her trial with Kohn and believes that the same feeling of hope can be found throughout the entire CCR community. "CCR doesn't talk about recurrence, progression, or survival statistics," explained Morris. "But when you do go to CCR, you *will* hear, 'Let's all hope together.'"

***CCR Connections* is now available online:**
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http://ccr.cancer.gov

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http://cancer.gov/about/default.asp

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Office of Training and Education
http://ccr.nci.nih.gov/careers/office_training_education.asp

Patient Information on Cancer and Clinical Trials

Open NCI Clinical Trials
http://www.cancer.gov/clinicaltrials

How to Refer a Patient
http://bethesdatrials.cancer.gov/professionals/refer.asp

NCI Cancer Information Service
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Understanding Cancer Series
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