National Cancer Institute

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CCR connections

CENTER FOR CANCER RESEARCH

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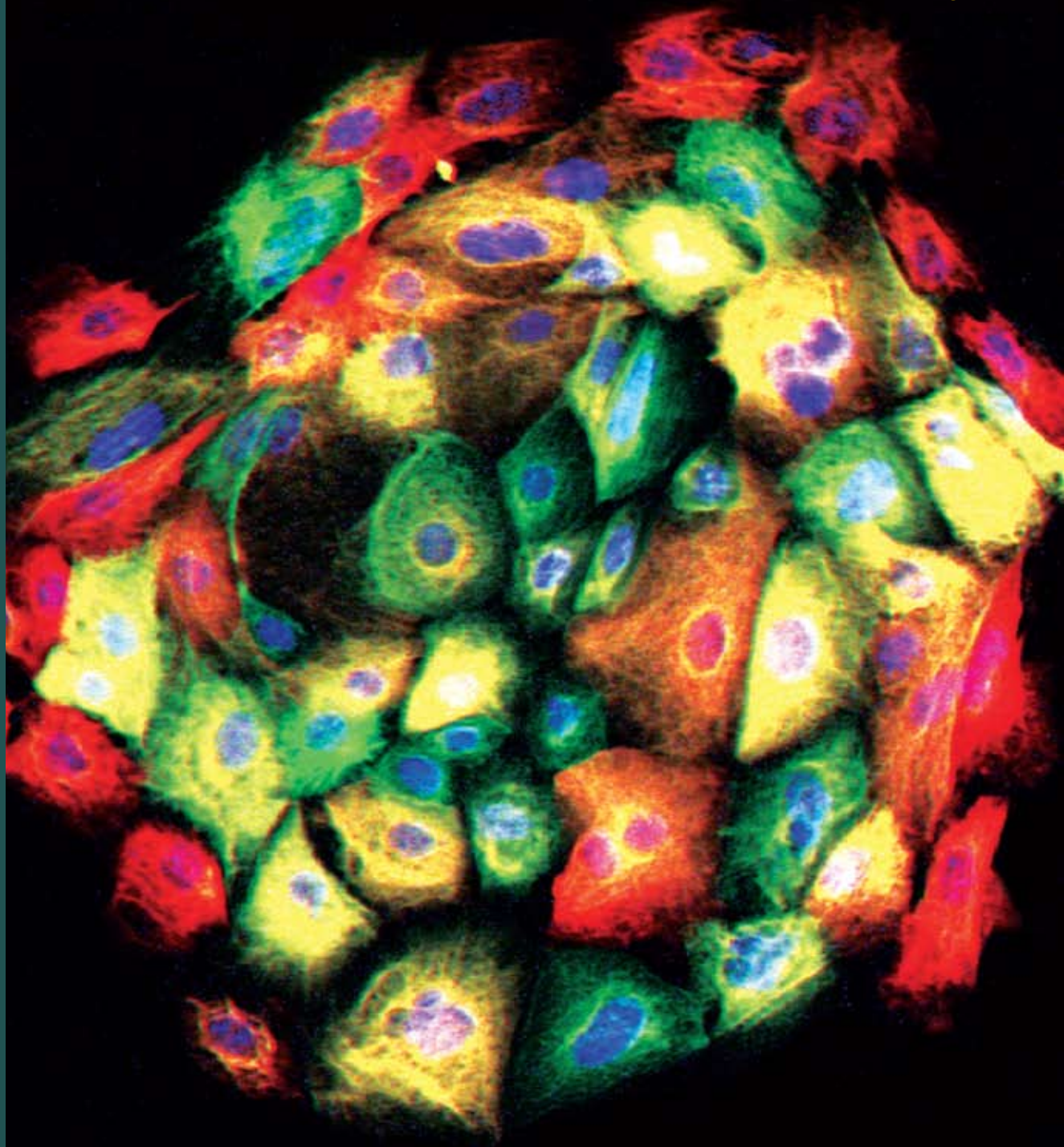

Of Mice and Men:

Tracking the Origins of Metastatic Prostate Cancer

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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CENTER FOR CANCER RESEARCH

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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

When Persistence Pays Off

As important as innovation, depth of expertise and determination are often key to great advances in biomedicine.

Some say that breakthroughs happen when people with a fresh, even naïve, perspective look at an old problem. But in science, particularly in the complex and detail-intense world of biomedicine, sustained work and investment in a particular set of problems, coupled with an open and creative mind, is often the most fruitful path to scientific advancement.

In this issue of *CCR connections*, we see several examples of researchers that have tackled the hard problems of a particular field for decades and consistently reaped great rewards. In "It's All About the Client," we learn how Len Neckers, Ph.D., identified the ubiquitous heat shock protein Hsp90 as an anti-cancer target 15 years ago. Defying conventional wisdom, he worked within NCI to develop the first clinical trial of an Hsp90 inhibitor and paved the way for the 13 Hsp90 inhibitors currently in clinical trials. Neckers and his team are continuing to establish the basic mechanisms of Hsp90 function and doing the preclinical work to optimize this therapeutic strategy.

Along with other NIH intramural colleagues, Gordon Hager, Ph.D., studied the role of chromatin structure in gene regulation long before most scientists believed it had any relevance and before the advent of research tools such as modern gene cloning technologies. Our feature, "Don't Throw Out the Packing Materials," explains how Hager's work has led to fundamental insights into the dynamic nature of gene regulation that have direct implications for understanding and overcoming the gene dysregulation associated with cancer.

One of our newest tenure-track Investigators, Christina Annunziata, M.D., Ph.D., has been studying the subtleties of a single molecular pathway—NF-κB—since she was a graduate student. As an inaugural participant in our Clinical Investigator Development Program, she had the opportunity to apply her insights and knowledge of this pathway from prior research in multiple myeloma and create a strong preclinical ovarian cancer program to evaluate NF-κB pathway inhibitors. "One Molecule, Multiple Cancers: The Devil is in the Details," describes her plans to translate this research into better treatment for patients.

We are also pleased to have an article from Joyce O'Shaughnessy, M.D., who developed a passion for clinical research in breast cancer during her years at NCI in the late 1980s. She brought that passion with her to Texas Oncology and Baylor College of Medicine, and in "Breast Cancer: The Triple-Negative Problem," O'Shaughnessy talks about the most recent fruits of her research—results of a very exciting phase 2 trial recently published in the *New England Journal of Medicine*, on the use of PARP inhibitors for the treatment of triple-negative breast cancer.

(*Photo:* B. Branson)

Robert Wiltrout, Ph.D.

At CCR, we are proud of the role we have played in selecting and supporting scientists who have the commitment and fortitude it takes to brave uncharted territories, and who stay the course and get answers from their scientific investigations. Without this kind of sustained effort and determination to grapple with the complex and sometimes very basic questions in biomedical research, clinical breakthroughs would simply not be possible.

Setting the Sun on Skin Cancer

Interferon-γ has been found to promote UV-induced melanoma in a mouse model.

A series of experiments designed to understand how solar ultraviolet (UV) radiation causes aggressive cutaneous melanoma has led to an unanticipated discovery that could upend assumptions about the relationship between interferon proteins and cancer. Interferon-γ (IFN-γ), which traditionally has been thought to contribute to an innate defense system against cancer, under some circumstances may promote melanoma and incite the development of tumors. This finding from Glenn Merlino, Ph.D., Co-Chief of CCR's Laboratory of Cancer Biology and Genetics, and Research Fellow M. Raza Zaidi, Ph.D., was published in the January 27, 2011, issue of *Nature*.

Over the past decade, these researchers used genetically engineered mice first to establish, and then to dissect, the connection between exposure to UV radiation and the initiation of melanoma. The current work—made possible through long-term collaborations with Edward De Fabo, Ph.D., and Frances Noonan, Ph.D., of George Washington University Medical Center—was their latest attempt to define the molecular mechanisms of this cause-and-effect relationship. The results of this study offer the possibility that the inhibition of IFN-γ immediately after sunburn might block the carcinogenic activation of melanocytes, the skin's pigment-producing cells, by UV radiation.

Crucial to the experiments was the development of a unique genetically engineered mouse in which the melanocytes were labeled with a green fluorescent protein. This fluorescent tag allowed melanocytes to be visually tracked and isolated and enabled researchers to evaluate, for the first time, their response to UV radiation while in their natural environment in a living animal.

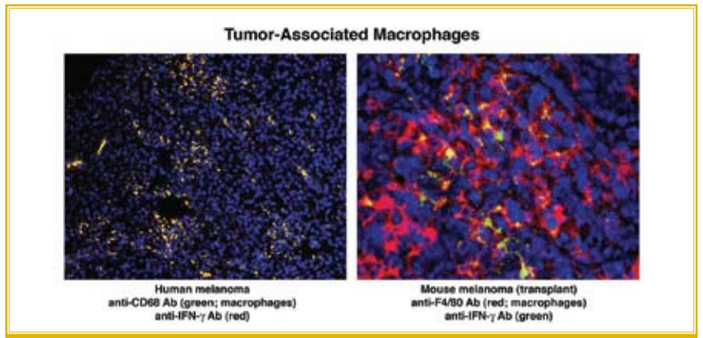
The researchers observed that UV radiation doses equivalent to those causing sunburn in human skin triggered aberrant growth and migration of melanocytes in mouse skin. UV radiation exposure also persistently activated genes known to respond to IFN-γ, including genes that may help tumor cells evade detection and attack by the immune system. When the activity of IFN-γ was inhibited, the growth and migration of melanocytes remained normal after exposure to UV radiation.

"Interferons have long been touted as anti-tumorigenic and cytostatic—that they have an inhibitory effect on cellular growth," said Dr. Zaidi. "The finding that IFN-γ can have a profoundly different effect—that it can exacerbate the growth of melanoma—is a paradigm-shifting discovery."

The team additionally showed that white blood cells known as macrophages were producing the IFN-γ. Macrophages significantly enhanced melanoma tumor growth when researchers injected them under the skin of healthy mice along with cultured mouse melanoma cells, and this effect was abolished by blocking IFN-γ activity. The researchers also identified IFN-γ-producing macrophages in 70 percent of 27 human melanomas they examined, supporting the possibility that IFN-γ plays a role in this type of cancer—not just in mice, but also in humans.

Moreover, Dr. Zaidi noted, inhibiting IFN-γ immediately after sunburn, an approach that he and his colleagues are pursuing, may prove to be an effective preventive strategy against UV radiation-induced melanoma. The discovery could one day lead to drug treatments that block this mechanism and thus the cancer's growth, potentially saving many from the lethal threat of skin cancer.

(*Image: R. Zaidi, CCR*)



A human melanoma tumor, *left*, and a transplanted mouse melanoma tumor, *right*, show infiltration of macrophages. While a subset of mouse tumor macrophages display expression on interferon-gamma (IFN-γ), virtually all the macrophages in human melanoma express this protein (identified by the yellow colored overlap of the two antibodies).

To learn more about Dr. Merlino's research, please visit his CCR Web site at http://ccr.nci.nih.gov/staff/staff.asp?Name=merlino.

Hitting the Target

Researchers have identified a possible target for treating the most aggressive form of lymphoma.

Single genetic mutations and, more commonly, combinations of mutations lead to the development of cancers such as lymphoma—a cancer of the blood that arises from infection-fighting white blood cells. Diffuse large B cell lymphoma (DLBCL), a type of non-Hodgkin's lymphoma, is the most common form of this disease and currently has a dismally low cure rate. There are three subtypes of DLBCL, of which the activated B cell-like (ABC) lymphoma has the worst outcome with a three-year survival rate of just 40 percent.

However, researchers have identified a recurring genetic mutation that could lead to targeted therapies for ABC lymphoma patients. Mutations of the *MYD88* gene (normally involved in the immune response to invading microorganisms) are found in 39 percent of patients with the ABC subtype of DLBCL and could drive the growth of some lymphoma tumors by activating multiple signaling pathways associated with cancer. A study published in the December 22, 2010, issue of *Nature* from the laboratory of Louis Staudt, M.D., Ph.D., Deputy Chief of CCR's Metabolism Branch, reveals a mechanism whereby a single alteration in the MYD88 protein sequence can cause uncontrolled cellular signaling, leading to survival of malignant cells.

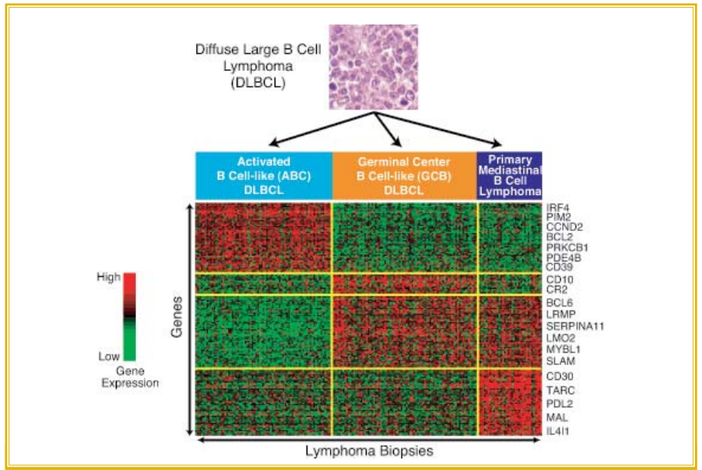
Dr. Staudt and colleagues have worked to identify proteins that play a role in the development of the ABC subtype as potential targets to improve the treatment of patients with this form of lymphoma. To identify these critical proteins, the researchers performed a screen in which thousands of genes were inactivated. They found that ABC lymphoma cells were killed when they inactivated the genes encoding MYD88 and IRAK1, another cell signaling protein that works with MYD88.

The scientists then looked for specific mutations in *MYD88* that might explain the survival-dependence they observed. Sequencing of the *MYD88* gene in 382 lymphoma biopsy samples revealed that 29 percent of ABC lymphoma samples had the same mutation, which altered a single amino acid in the MYD88 protein, but this mutation was rare or absent in other lymphoma subtypes. The mutant form of *MYD88* sustained the survival of the ABC lymphoma cells while the non-mutated version did not, suggesting that mutations in the *MYD88* gene could play an important role in the development of ABC DLBCL.

The researchers then examined proteins that interact with MYD88 in lymphoma cells. The mutant form of MYD88 spontaneously assembled a protein complex that included IRAK1, identified in the genetic screen, and a related protein, IRAK4. In this protein complex, IRAK4 functioned as an enzyme to modify IRAK1, which was required for the mutant MYD88 protein to promote lymphoma cell survival. This particular finding may hold promise for the treatment of lymphomas with MYD88 mutations since pharmaceutical companies are developing IRAK4 inhibitors for use in inflammatory and autoimmune diseases, noted Dr. Staudt.

"The results of this study may provide a method to identify patients with the ABC subtype of diffuse large B cell lymphoma whose tumors may depend upon MYD88 signaling," said Dr. Staudt. "And these patients may one day benefit from therapies targeting this and other regulatory pathways that sustain the survival of these lymphoma cells."

(*Image: L. Staudt, CCR*)



Biopsies of diffuse large B cell lymphoma (DLBCL) reveal varying gene expression levels in activated B cell-like DLBCL, germinal center B cell-like DLBCL, and primary mediastinal B cell lymphoma.

To learn more about Dr. Staudt's research, please visit his CCR Web site at http://ccr.nci.nih.gov/staff/staff.asp?Name=staudt.

Patent Pool Goes Global

CCR-developed HIV drug darunavir is the first patent licensed to the Medicines Patent Pool.

(*Photo: E. Branson*)

Hiroaki Mitsuya, M.D., Ph.D.

In recent years, there have been heated debates on how to ensure that patents do not stand in the way of access to medicines—especially for the world's poorest nations. According to UNITAID, an independent global health financing agency founded by the United Nations in 2006, treating a patient for one year with today's recommended first-line AIDS treatment costs between $151 and $1,033—in part because certain products are patented in some countries. This, according to the agency, is an almost two- to 13-fold increase from the price of the most affordable and widely used older regimen.

Enter the Medicines Patent Pool, a newly established UNITAID initiative that aims to find a route for expensive drugs to reach developing countries. By streamlining licensing processes for the production of generic versions of patented HIV/AIDS medicines, the Pool serves as a one-stop shop that will accelerate the pace at which newer medicines reach patients and will help drive down prices to low, sustainable levels by encouraging competition among multiple generic producers.

The Pool received its first, high-profile member when the NIH licensed a patent for the new AIDS drug darunavir in September 2010. The royalty-free license stipulates that this technology is to be available for the benefit of all low- and middle-income countries, as defined by the World Bank. Darunavir (Prezista™) is a novel protease inhibitor developed by Hiroaki Mitsuya, M.D., Ph.D., Head of CCR's Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, in a 13-year collaboration with Purdue University Professor Arun K. Ghosh, Ph.D.

"Darunavir embodies a breakthrough in the struggle against the notorious obstacle of current AIDS therapy: multidrug-resistant HIV variants," said Dr. Mitsuya. "The drug is active and has been proven clinically efficacious against multi-protease inhibitor-resistant HIV. More notably, darunavir effectively keeps HIV from becoming drug-resistant."

HIV rapidly mutates to resist new drugs, and many patients on first-line regimens will soon need to switch to second-line therapies that are more effective against drug-resistant HIV. And just as there is a need to continually develop new drugs, there is also a need to develop new approaches for improving drug access globally. With this first license in hand, the Pool is one critical step closer to achieving its goal of making life-saving medicines, like darunavir, more affordable and accessible to people in developing countries.

The Medicines Patent Pool provides a valuable model for attacking a longstanding problem: how to get patented products, developed through public and private investment, to developing countries today without having to wait many years for patents to expire in high-income markets. With darunavir, the NIH demonstrates its commitment to creative approaches to conducting research and development that meet global health needs. Dr. Mitsuya noted, "I am so pleased and honored that darunavir became the first drug to be licensed to the Pool." Although this is a significant step towards open access to patents for life-saving drugs, this alone will not transform the system unless the pharmaceutical industry follows suit. "The NIH's move is a good start," added Dr. Mitsuya, "but this should be only the beginning."

To learn more about Dr. Mitsuya's *research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=mitsuya.*

The Frontiers of Thymic Malignancy

The first International Conference on Thymic Malignancies at the NIH provided a forum to better manage this rare disease.

(*Photo: L. Eiben*)

The first International Conference on Thymic Malignancies speakers' dinner and reception were held at the Foundation for Advanced Education in the Sciences House.

As every oncologist knows, designing effective treatment and management strategies for individuals with relatively common cancers of the breast or lung can be challenging. For rare cancers, where the paucity of cases makes research opportunities scarce, that challenge is magnified enormously. Malignancies of the thymus—a gland that produces and "educates" critical cells of the immune system called T-lymphocytes—are such rare tumors whose biology is largely unknown. Surgery has been the mainstay treatment in early stages of thymic malignancy, but many patients with advanced stage tumors require aggressive multimodal treatment. Furthermore, the involvement of the thymus results in a broad array of symptoms associated with immune responses and autoimmune diseases.

To spearhead further basic and clinical research into thymic malignancies, Giuseppe Giaccone, M.D., Ph.D., Chief of CCR's Medical Oncology Branch, organized the International Conference on Thymic Malignancies—held on August 20–21, 2009, on the NIH campus in Bethesda, Md. and co-sponsored by the Medical Oncology Branch and the Foundation for Thymic Cancer Research—which brought together more than 100 scientists, pathologists, and clinicians from major institutions in the United States, Europe, and Japan with interest and expertise in the disease and its treatment.

"These are rare tumors with about 450 to 600 new cases in the U.S. every year," said Dr. Giaccone. "So it's an understudied disease, but the NCI has the ability to study rare tumors more easily than other institutions because we can bring patients from all over the country and all over the world here."

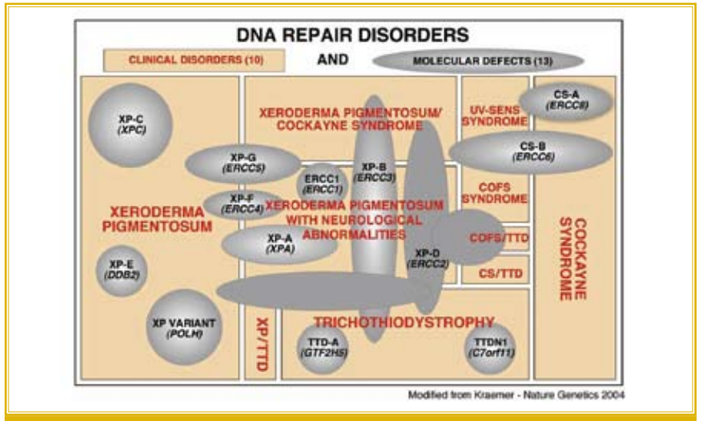
Dr. Giaccone published a monograph covering the major presentations given during the first International Conference on Thymic Malignancies in the October 2010 issue of *Journal of Thoracic Oncology*. The two-day meeting was organized as a workshop in which researchers presented lectures and posters featuring extensive reviews and novel findings on topics spanning the epidemiology of thymic malignancies to the basic biology of the disease, to the treatment of patients with the cancer. The group set out to establish research priorities, to develop potentially collaborative protocols, to standardize diagnosis and monitoring criteria, and to develop a patient database and tissue bank.

Several CCR investigators presented novel findings at the meeting. Ronald Gress, M.D., Chief of the Experimental Transplantation and Immunology Branch, discussed the layered levels of control between the thymus and the immune system. Dr. Giaccone and Arun Rajan, M.D., of the Medical Oncology Branch, presented case studies of the surgical results of two thymic malignancy patients. Drs. Giaccone and Rajan also presented data on studies of targeted therapies for thymic neoplasms. Ola Landgren, M.D., Ph.D., of the Medical Oncology Branch, also shared findings of a population-based study on mortality and morbidity patterns among 681 thymoma patients in Sweden.

As a result of this conference, the thymoma community recognized the need for an association to study thymic malignancies in a structured fashion. A meeting to ratify this structure, called the International Thymic Malignancy Interest Group, was held in New York City on May 5–6, 2010. Dr. Giaccone noted, "There are plans for similar meetings to be held regularly in the future in an effort to share current information and improved treatment options available to doctors and patients with thymic cancer."

A Look at Rare Diseases, from Molecules to Patients

Workshop on Xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy examines diseases of DNA repair, cancer, and premature aging.



(Image: K. Kraemer, CCR)

Clinical DNA repair disorders and their associated molecular defects.

Most people do not welcome the signs of aging, but for patients with Xeroderma pigmentosum (XP) and related diseases, the issue is not one of social status but of survival. XP causes patients to develop life-threatening skin cancers and a prematurely aged appearance of sun-exposed skin due to the inability to repair cell damage by ultraviolet (UV) radiation. There are other related disorders such as Cockayne syndrome (CS) and trichothiodystrophy (TTD) that also produce premature aging and are caused by inherited mutations that alter multifunctional protein complexes, which play essential roles in DNA repair and RNA transcription.

These rare diseases have a combined worldwide incidence estimated at less than one per 250,000 births. Yet, there are enough dedicated researchers and clinicians interested in XP, CS, and TTD, and enough cooperative patients and family members, that remarkable progress has been made recently in understanding the molecular basis of these complex disorders. This progress was evident at a workshop to address these rare diseases called Xeroderma Pigmentosum and Other Diseases of Human Premature Aging and DNA Repair: Molecules to Patients held in Chantilly, VA from September 21-24, 2010.

One hundred researchers, clinicians, affected patients, and representatives of patient support groups gathered at the workshop—co-organized by Kenneth Kraemer, M.D., Senior Investigator in CCR's Dermatology Branch; Vilhelm Bohr, M.D., Ph.D., Chief of the National Institute of Aging's Laboratory of Molecular Gerontology; and Laura Niedernhofer, M.D., Ph.D., Associate Professor at the University of Pittsburgh—to share, consider, and discuss the latest developments in understanding XP and other human diseases characterized by cancer, premature aging, and defects in DNA repair.

The third in a series, this workshop emphasized discussion, interaction, and open exchange of information and ideas among bench scientists, clinicians, patients, and patient advocates in order to establish new collaborations. The workshop sessions covered a variety of topics on XP, CS, and TTD including natural history and clinical features of disease, clinical and laboratory diagnosis, therapeutic approaches, molecular analysis of accelerated aging, neurodegeneration in Huntington's disease, as well as DNA repair and genome instability.

The meeting revealed areas in which great progress has been made, areas ripe for future study, and bottlenecks that are inhibiting progress in either basic understanding of the diseases or their clinical management. Several panel discussions and poster sessions provided the unique opportunity for extensive conversation and interaction between clinicians, researchers, patients, and family support group members in both formal and informal settings.

"The presence and participation of patients, their advocates, and family support groups was an important and enriching feature of this and the preceding two workshops in this series, in 2004 and 2006," noted Dr. Kraemer. "It is important to emphasize that studying relatively rare diseases such as XP, CS, and TTD may lead to insights that are relevant for more common diseases such as cancer and neurodegeneration. For example, XP patients have a 10,000-fold risk of developing new skin cancers and melanomas." The efforts of all the participants at this workshop may contribute to a greater understanding of rare diseases, as well as a better insight into the risk factors for common diseases in the general population.

Test Before You Treat

CCR researcher Frank Gonzalez, Ph.D., is recognized for the development of a life-saving diagnostic test to identify cancer patients that may experience 5-fluorouracil toxicity.

Frank Gonzalez, Ph.D., Chief of CCR's Laboratory of Metabolism, and his former fellow Pedro Fernandez-Salguero, Ph.D., now a professor in Spain, received the 2011 Federal Laboratory Consortium National Award for Excellence in Technology Transfer for developing and transferring a life-saving diagnostic test to the marketplace. The test has been nonexclusively licensed to several companies in Europe and the United States. Before administering the drug 5-fluorouracil (5-FU), it is now possible to screen patients for a mutation that puts them at risk for life-threatening toxicity.

Gonzalez and Fernandez-Salguero determined the molecular basis for 5-FU-linked toxicity. They discovered a splicing mutation in the dihydropyrimidine dehydrogenase (DPD) gene, which is normally involved in the degradation of the drug. Patients' sensitivity to 5-FU is directly correlated with a mutated DPD gene and low DPD activity levels, resulting in the accumulation of 5-FU in the body.

In the United States, approximately 275,000 cancer patients receive this drug annually. The transfer of this technology through nonexclusive licenses has enabled the wide dissemination of the diagnostic test. "As a result of these multiple licenses," noted Gonzalez, "many patients around the world can avoid being treated by a drug that may prove to do them more harm than good."

To learn more about Dr. Gonzalez's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=gonzalez.

Staff News at CCR

announcement

(Photo: G. Merlino)

Glenn Merlino, Ph.D.

Glenn Merlino has been named a Deputy Director of CCR. Merlino received his Ph.D. from the University of Michigan in 1980 and began his career at NCI as a Postdoctoral Fellow under Ira Pastan, M.D. He was named Chief of CCR's Laboratory of Cell Regulation and Carcinogenesis in 2004 and Co-Chief of CCR's Laboratory of Cancer Biology and Genetics in 2006. Merlino's research career has made contributions in the areas of receptor tyrosine kinase signaling, oncogenic transformation, transcriptional regulation, cell cycle regulation, multiple drug resistance, and genomic instability. He was the first to report the amplification/rearrangement of the EGFR gene in human cancer. Using transgenic mouse models, he was among the first to show that growth factors could function *in vivo* as oncogenes. Currently, Merlino and his colleagues in the Cancer Modeling Section—using genetically engineered mouse models of human cancer—are seeking to elucidate the complex molecular programs governing melanomagenesis and progression.

newly tenured CCR scientists

Stefan Ambs, Ph.D., M.P.H.
Laboratory of Human Carcinogenesis

Daniel Fowler, M.D.
Experimental Transplantation and Immunology Branch

Kevin Gardner, M.D., Ph.D.
Laboratory of Receptor Biology and Gene Expression

Dennis Hickstein, M.D.
Experimental Transplantation and Immunology Branch

Ola Landgren, M.D., Ph.D.
Medical Oncology Branch

Yun-Xing Wang, Ph.D.
Structural Biophysics Laboratory

new tenure-track scientists

(Photo: E. Branson)

Christina M. Annunziata, M.D., Ph.D.

Christina Annunziata is now a tenure-track investigator in CCR's Medical Oncology Branch. Her research investigates NF-κB signaling in an ovarian cancer model, and she maintains her clinical focus in the translational clinical studies of ovarian cancer.

(Photo: A. Sudarov)

Isaac Brownell, M.D., Ph.D.

Isaac Brownell joins CCR's Dermatology Branch. His research focuses on the regulation of stem cells in the skin and the use of mouse genetics to model carcinogenesis in the skin.

(Photo: E. Branson)

Udayan Guha, M.B.B.S, Ph.D.

Udayan Guha joins CCR's Medical Oncology Branch. His clinical interest is thoracic malignancies and his research interest is studying cancer-signaling networks using integrated proteomics, genomics, and mouse modeling approaches.

(Photo: E. Branson)

Rosandra N. Kaplan, M.D.

Rosie Kaplan joins CCR's Pediatric Oncology Branch. She is a clinician and physician-scientist with active translational and clinical research interests focused on the mechanism of cancer metastasis.

(Photo: D. Sone)

Teri N. Kreisl, M.D.

Teri Kreisl is now a tenure-track investigator in CCR's Neuro-Oncology Branch. Her research focuses on imaging biomarkers in primary brain tumors.

(Photo: A. Lal)

Ashish Lal, Ph.D.

Ashish Lal joins CCR's Genetics Branch. His laboratory focuses on elucidating the function of specific cancer-associated microRNAs using molecular and genetic approaches. His lab is also investigating the role of mutations in tumor suppressor proteins such as p53 on microRNA biogenesis in cancer cells.

(Photo: E. Branson)

Daniel R. Larson, Ph.D.

Dan Larson joins CCR's Laboratory of Receptor Biology and Gene Expression. His laboratory focuses on the regulation and function of RNA in a cell-biological context, including transcription, splicing, post-transcriptional processing, and decay.

(Photo: M. Spencer)

Jayne Stommel, Ph.D.

Jayne Stommel joins CCR's Laboratory of Molecular Pharmacology. Her research focuses on oncogenic kinase signaling in glioblastoma multiforme.

(Photo: P. Tofilon)

Philip Tofilon, Ph.D.

Philip Tofilon joins CCR's Radiation Oncology Branch. His research investigates radiation-induced translational control of gene expression, as well as the radiobiology of glioblastoma stem cells.

(Photo: M. Welch, SPGM, NCI-Frederick)

Christopher Westlake, Ph.D.

Chris Westlake joins CCR's Laboratory of Cell and Developmental Signaling. His research investigates membrane trafficking pathways important in ciliopathy, diseases linked to primary cilia dysfunction, and cancer.

Recent CCR Awards

2011 Albert B. Sabin Gold Medal Award

Sabin Vaccine Institute

For exemplary contributions to disease prevention through the development or use of vaccines

Douglas R. Lowy, M.D.
Office of the Director, National Cancer Institute

John T. Schiller, Ph.D.
Laboratory of Cellular Oncology

2011 ASCO Pediatric Oncology Award and Lecture

American Society of Clinical Oncology

For outstanding leadership or achievement in the field of pediatric oncology

Lee Helman, M.D.
Office of the Director, Center for Cancer Research

2011 ASCO Statesman Award

American Society of Clinical Oncology

For extraordinary volunteer service, dedication, and commitment to ASCO

Lee Helman, M.D.
Office of the Director, Center for Cancer Research

The Pioneer Award

Mesothelioma Applied Research Foundation

For work on targeting mesothelin, a protein highly expressed in mesothelioma, and for the treatment of patients with mesothelioma

Raffit Hassan, M.D.
Laboratory of Molecular Biology

2011 AACR-ACS Award for Research Excellence in Cancer Epidemiology and Prevention

American Association for Cancer Research-American Cancer Society

For outstanding research accomplishments in the fields of cancer epidemiology, biomarkers, and prevention

John T. Schiller, Ph.D.
Laboratory of Cellular Oncology

2011 AACR Future Leaders in Cancer Prevention and Epidemiology Research Special Symposium

American Association for Cancer Research

For outstanding early-career scientists in cancer research whose work reflects innovation, scientific independence, motivation, and creativity

Brid Ryan, Ph.D.
Laboratory of Human Carcinogenesis

Lila Gruber Memorial Cancer Research Award

American Academy of Dermatology

For lifetime achievements in the field of cancer research

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

2011 American Academy of Microbiology Fellow

Michael J. Lichten, Ph.D.
Laboratory of Biochemistry and Molecular Biology

2010 AAI Distinguished Service Award

American Association of Immunologists

For outstanding service to AAI through creative and successful initiatives that have benefitted the immunology community

Arthur Andrew Hurwitz, Ph.D.
Laboratory of Molecular Immunoregulation

Radiation Research Society's Failla Award

For a distinguished career in radiation research

James B. Mitchell, Ph.D.
Chief, Radiation Biology Branch

Intel Science Talent Search Semifinalist

For her project, Hydrogen Sulfide: A Novel Molecular Target for Breast Cancer Therapy

Kelley Ivins-O'Keefe
Laboratory of Pathology

Award for Excellence in Technology Transfer

Federal Laboratories Consortium Mid-Atlantic Region

For their outstanding work transferring their technologies to the marketplace

A Life-Saving Diagnostic Test for Cancer Patients

Frank J. Gonzalez, Ph.D.
Chief, Laboratory of Metabolism

Novel Protein-Like Therapeutics for the Treatment of Cancer

Nadya Tarasova, Ph.D.
Cancer and Inflammation Program

Michael Dean, Ph.D.
Laboratory of Experimental Immunology

Sergey Tarasov, Ph.D.
Structural Biophysics Laboratory

Hong Lou, Ph.D.
Cancer and Inflammation Program

Therapeutic Antibodies for the Treatment of Cancer

Ira Pastan, M.D.
Chief, Laboratory of Molecular Biology

The Presidential Volunteer Service Award

The President's Council on Service and Civic Participation

Pamela Webb
Pediatric Oncology Branch

The Scientist Magazine: Best Places to Work for PostDocs 2011

Ranked 14

National Cancer Institute, Bethesda/Frederick

In Conversation:
Research Fellow Ram Savan, Ph.D.

CCR: Congratulations, Ram, on receiving the Milstein Young Investigator Award from The International Society for Interferon and Cytokine Research last year. We understand that the award honors scientists who have made an impact on interferon or cytokine research early in their careers. Could you tell us about your research?

Ram: I work with Howard Young, Ph.D., Deputy Chief of the Laboratory of Experimental Immunology. The award was for work related to the regulation of INF-γ, which is a focus in our laboratory. We've long known that the mRNA for INF-γ is rapidly degraded, almost as fast as it is made. We discovered a microRNA—miR-29 that actually stabilizes the mRNA transcript. From our experiments, we believe that mir-29 binds to the mRNA recruiting a complex that restricts the access of RNA degrading machinery.

CCR: But, aren't most microRNAs thought to actually help destroy, rather than preserve, gene transcripts?

Ram: Exactly. MicroRNAs recruit RISC complexes. RISC stands for "RNA-induced *silencing* complex." When we first observed this microRNA region near the INF-γ gene, we thought it would enhance degradation of the transcript. Imagine our surprise when we added miR-29 and saw more INF-γ than before. We did a lot of different experiments to convince ourselves (and others!) that this was real.

CCR: So, you have been working on this project during your 4 years at CCR?

Ram: Yes, but also on two completely different projects. One, which has just been published in *Nature*, identifies a microRNA-binding regulatory element in the HLA-C gene that influences levels of HLA-C allotype cell surface expression, affecting the immune response to HIV. This project was in collaboration with Smita Kulkarni, Ph.D., and Mary Carrington, Ph.D. The other project looks at a new role for the IL-22 receptor in inflammation, which could be important for certain lymphomas.

(Photo: J. Sommers, SPGM, NCI-Frederick)

Ram Savan, Ph.D.

CCR: Those are some very diverse projects. How have you done it all?

Ram: Through collaborations, no question. We are part of the Cancer and Inflammation Program, headed by Giorgio Trinchieri, M.D., and it is the most collaborative scientific environment I have ever experienced. It combines two laboratories—ours and the Laboratory of Molecular Immunoregulation—including 15 Principal Investigators. Everyone is incredibly open about their data and keen to help.

CCR: What are your plans for the future?

Ram: I am looking for a faculty position and to run my own laboratory. I want to take what I've learned here about microRNAs and focus my own lab on post-transcriptional regulation. There are so many intricacies to decipher; it's immensely complex. I really want to study the role of post-transcriptional regulation under infectious conditions, like in chronic HIV patients.

CCR: We wish you great success in your work. Do you have any advice for aspiring Fellows?

Ram: My advice? Always have a back-up project. For the first couple of years that we were unraveling our paradoxical miR-29 results, I had a very hard time. I was learning a new technology and getting unexpected readouts; it was very confusing. So, I created back-up projects and then got very lucky. You don't want to end up with nothing when a risky project goes wrong.

Of Mice and Men:
Tracking the Origins of Metastatic Prostate Cancer

In most cases, prostate cancer is a treatable disease. Typically slow growing tumors that occur in men at a median age of 70 years are often treated effectively by interfering with androgen hormone signaling. But in 10 percent of cases, prostate cancers metastasize, become resistant to androgen deprivation therapy, and turn lethal. Kathleen Kelly, Ph.D., Chief of CCR's Cell and Cancer Biology Branch, has a long-standing interest in understanding the transformation from normal prostate cells into primary cancer and then into metastatic disease. Led by a desire to identify the earliest origins of prostate cancer, Kelly turned to a model system that allows her to study the cells that give rise to the disease as well as trace its metastatic spread.

Cancer's Original Sin

Prostate tumors initially require androgen hormone signaling to survive, so androgen-deprivation therapy (ADT), using drugs that inhibit androgen-receptor signaling, has been a highly effective therapeutic option for many patients. Over the years, the drugs that can inhibit androgen receptor signaling have improved such that the time between when the prostate cancer patient is treated and when he succumbs to the disease has increased. But, when the cancer progresses, it is almost always linked to the development of androgen-independence or "castrate-resistant" prostate cancer. Metastasis is invariably associated with a castrate-resistant form of the disease.

"When I first started thinking about the source of metastases," said Kelly, "one of the things I found very interesting about the healthy prostate is that when you take away androgen, the prostate shrinks and involutes. And when you add androgen back, it grows." There aren't very many dividing cells in the normal prostate, but manipulations of androgen provided a striking demonstration of the existence and importance of androgen-independent stem cells in the healthy prostate.

"One of the ideas in the field that hasn't been proven or disproven yet, is that in prostate cancer, an immature undifferentiated cancer cell ultimately gives rise to resistance and metastases," said Kelly. "The hypothesis is that this cancer stem cell doesn't require—or has unique mechanisms for obtaining—androgen receptor signaling, so it survives androgen-deprivation therapy."

To test the hypothesis, Kelly wanted to look at the cells that initiate prostate cancers and follow their progression.

Building a Better Model

Many human cancers can be effectively studied through xenografts, in which primary tumor cells are injected into mice with compromised immune systems so that they do not reject the foreign cancer cells. Prostate cancer is unusual in that it is extremely difficult to reproduce in such a model system. Furthermore, although they can be kept alive, primary prostate tumor cells do not thrive in culture conditions.

There are a handful of prostate cancer cell lines, which were mostly derived from metastases and not primary tumors, so they have multiple mutations and have been in culture for

(*Photo: R. Baer*)

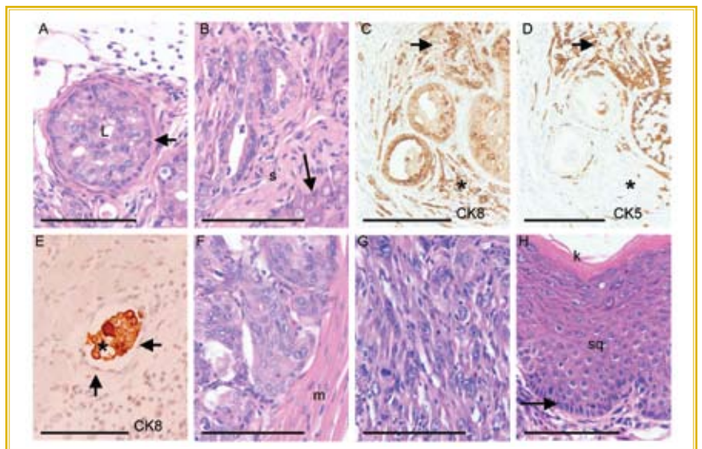
Kathleen Kelly, Ph.D., and members of her laboratory.

many years. Thus, they are problematic as a tool to study the properties of cancer stem cells, as they might not exist in a living organism.

"The approach I decided to take was a mouse model," said Kelly. "I chose an aggressive model, because I thought there was a higher chance we could study a metastatic process."

Kelly, with her graduate student, Philip Martin, D.V.M., made a mouse with deletions of two tumor suppressors—*PTEN* and *TP53*—in prostate epithelial cells. Mutations of *PTEN* and *TP53* occur at fairly high frequency in human populations and are often associated with aggressive, castrate-resistant, and metastatic disease.

(*Image: K. Kelly, CCR*)



Kelly and her colleagues have created a mouse model of prostate cancer with tumors that display a diversity of tumor types: (A) prostatic intraepithelial neoplasia; (B) adenocarcinoma; (C) and (D) prostate tumors positively stained for two epithelial filament markers (arrow: CK8 and CK5) or only one (asterisk: CK8 but not CK5); (E) vascular invasion; (F) prostatic urothelial carcinoma; (G) sarcomatoid carcinoma; (H) basal/squamous carcinoma.

In addition to the two genetic deletions, Kelly and Martin introduced a light-emitting reporter gene—luciferase—which allowed tracking of transplanted cells that carried the genetic deletions.

"The idea we had was that we would be able to sort through the tumor cells and find the tumor-initiating cells in these mice," said Kelly, "but first we had to fully characterize our model." So using his training in veterinary pathology, Martin led a full longitudinal study of their mouse model. (See "The Veterinary Perspective.")

"One of the most important things we were able to show is that, unlike other mouse models of prostate cancer, this one produced cells with metastatic potential," said Martin. The mice rapidly developed tumors composed of multiple cell types, which were lethal in approximately seven months.

Panning for Cells

In the Kelly lab, Research Fellow Wassim Abou-Kheir, Ph.D., has studied the progenitor cells undergoing transformation in the prostate of these genetically modified mice. He used selective culturing conditions to study the self-renewing capabilities of prostate cells extracted from these mice. He found that the number of progenitor cells in these mice was strongly amplified and that the cells had a greatly increased ability to self-renew compared to cells from normal mice. "They can be cultured indefinitely and will continue as progenitors. We believe that the tumor-initiating cells are within this self-renewing population," said Kelly.

Meanwhile, at a nearby bench, Research Fellow Paul Hynes, Ph.D., is searching for tumor-initiating cells by teasing apart identifiable cells from the primary prostate tumors in these mice. The process of fractionating the cells involves separating them out on the basis of protein markers on their cell surface. "He's finding that there is an undifferentiated tumor-initiating cell that can give rise to both basal and luminal cells," said Kelly, referring to the two major cell types in the prostate.

The team has also found such bipotential progenitor cells in cell lines that they have created from single tumor cells, i.e., clonal cell lines, and analyzed. These cells are also immature, can give rise to both basal and luminal cells, and metastasize when grafted into the mouse prostate.

The Veterinary Perspective

(*Photo: R. Baer*)

Philip Martin, D.V.M., and Kathleen Kelly, Ph.D.

In 2003, Mark Simpson, D.V.M., Ph.D., Head of the Comparative Molecular Pathology Unit of CCR's Laboratory of Cancer Biology and Genetics, launched a new training initiative under the NIH Graduate Partnership Program titled the Comparative Molecular Pathology Research Training Program. His aim was to provide opportunities for veterinarians to gain postdoctoral training in pathology and human biomedical research. The program operates with multiple NIH institute intramural programs and university partners, with training leading to a Ph.D. and eligibility to certify as a specialist in veterinary pathology.

"We recognized that an incredible set of advancements were forthcoming from the genomic revolution and the sequencing of the human genome," said Simpson. "But understanding the function of most genes requires their study in the context of the biology of a model organism, that is, an animal."

A veterinary pathologist, Simpson knew that this training would support a bridge between molecular discovery and whole animal pathophysiology. "What was needed was to bring more veterinarians to the NIH and provide them with the opportunity to get research training in human biomedical research," said Simpson. "Training alongside medical and basic scientists fosters a shared vocabulary and approaches to translational research."

Philip Martin, D.V.M., arrived at the NIH in 2005, part way through a residency in veterinary pathology. "The program was perfect for me because it allowed me to fulfill my residency requirements, including the national board certification exam, as well as pursue a Ph.D., which is important for pathologists who are interested in animal models of human disease."

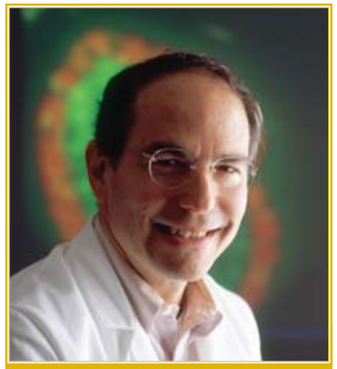
Martin is currently finishing up his dissertation in the laboratory of Kathleen Kelly, Ph.D., but, in the meantime has accepted a full-time position as a pathologist at CCR's Center for Advanced Preclinical Research (CAPR) in Frederick, Md. CAPR is dedicated to improving preclinical evaluation for effective cancer diagnosis and treatment.

"If we're going to study mice as models of human disease, we need to be sure that it is a relevant form of disease," explained Martin. "The need to analyze the pathology is extremely important. Many models, upon rigorous pathological examination, are not the type of cancer that the investigators were hoping to study."

"Having Philip's perspective in the lab has really helped me understand the strong and weak points of mouse models," said Kelly.

Simpson hopes that this initiative will contribute not only to prevention and treatment of disease in humans, but also in animals. "There's a special perspective we bring because of our orientation to disease in multiple species." By integrating the comparative perspective with human biomedical research, Simpson aims to train D.V.M./Ph.D. scientists who are capable of leading research collaborations at the forefront of scientific discovery.

For more information about the Comparative Molecular Pathology Research Training Program, please visit http://ccr.cancer.gov/resources/training/applications/programInformation.asp.



(*Photo: R. Baer*)

Peter Choyke, M.D.

"We are really interested in determining how tumor-initiating cells are related to the different cell lineages and then understanding what their response is to androgen and androgen deprivation," said Kelly.

Kelly and her team have found that some of these clonal cell lines are sensitive to androgen deprivation, but there is a component of cells that does survive androgen deprivation. Kelly noted that in a very recent paper from Memorial Sloan Kettering Cancer Center, researchers also found cells from human prostate cancer-derived cell lines that have tumor-initiating capacity and are insensitive to androgen.

"From our mouse work, and now this human cell line, it does appear that a relatively undifferentiated tumor-initiating cell will lead to luminal adenocarcinoma of the prostate," concluded Kelly.

Understanding more about the cells responsible for driving tumor formation will provide new insights into how to more effectively diagnose potentially progressive disease and target these specific populations therapeutically.

Tracking the Spread

Another challenge for prostate cancer research lies in the differences in androgen sensitivity that result when cells are removed from the environment of the organism. "It's complicated to take apart the response," said Kelly, noting that cells are just not as sensitive to androgen deprivation in culture.

Among the clonal cell lines that Kelly's team has generated from their mouse model, a few behave like human prostate cancer cells in that they are androgen-sensitive and give rise to adenocarcinoma. "So we are looking at metabolically labeling and tracking these cells in the mouse," said Kelly.

Peter Choyke, M.D., Program Director of the Molecular Imaging Program at CCR, runs both preclinical and clinical imaging facilities. "Our goal is to develop molecular imaging tools that are translatable into people with the hope that we can diagnose, stage, or monitor cancer patients in a noninvasive way over time," said Choyke. Among the tools in his research armamentarium is a micro-positron emission tomography (PET) scanner for use on small animals.

Choyke and Kelly are planning to use PET to look at metabolic changes in prostate cancer cells relative to normal tissue, both as a tool to better understand prostate cancer progression in the whole organism and as a means to improve the ability to image these cancer cells in men.

"There's a lot of interest in the metabolomics of cancer and, from an imaging perspective, one of the interesting aspects of prostate cancer is that, early on, it is not particularly well visualized by the standard PET scan," explained Choyke. Whereas most tumors differentially depend on glucose uptake, prostate cancers early in development do not, and PET scanning relies on cells taking up radiolabeled glucose (18F-fluorodeoxyglucose or FDG).

"As the prostate cancer advances and becomes more malignant, it starts to take up more FDG. So, there is some kind of glucose utilization switch that occurs later in its development," said Choyke.

From studies that Kelly has conducted on prostate cancer cells, she has additional reasons to believe that prostate cancer cell metabolism is altered during the progression of the disease. In addition to changes in glucose metabolism, she believes that changes in fatty acid metabolism might also be important, and that early prostate cancers differentially utilize fatty acids. Choyke and Kelly plan to study this by using PET to monitor uptake of the fatty acid precursor, 11C-acetate, which corresponds to the activity of an enzyme that synthesizes fatty acids.

> There's a lot of interest in the metabolomics of cancer.

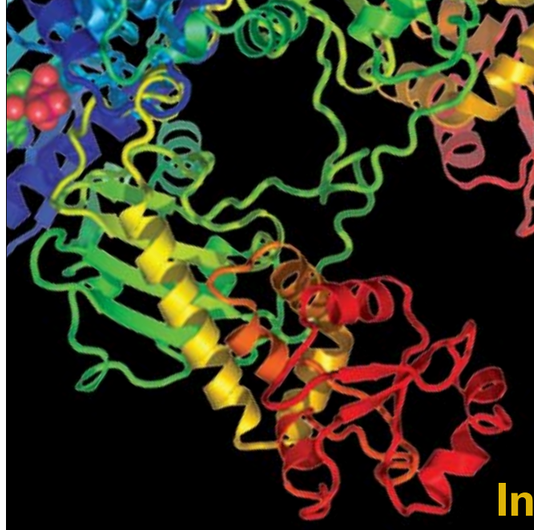
Specifically, Kelly will use a model in which their clonal cell line is introduced into the prostates of mice that have been castrated and implanted with testosterone pellets. Subsequent removal of the pellets will mimic androgen-deprivation therapy and result in the development of androgen-independent malignancies.

"We want to know whether a marker of fatty acid metabolism—11C-acetate—would be a sensitive way of finding prostate cancer cells either in a primary state or following androgen deprivation," explained Kelly.

One of the biggest challenges to studying and treating any metastatic disease is being able to find and track the cancer as it spreads. Ultimately, Kelly hopes that studying this progression in a carefully characterized and controlled mouse model will provide insights to address that challenge in man.

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

To learn more about the Molecular Imaging Program at CCR, please visit Dr. Choyke's CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=choyke.



It's All About the Client

The Development of Hsp90 Inhibitors as Anti-Cancer Agents

Approximately one to two percent of all proteins found in a cell are the evolutionarily conserved heat shock 90 (Hsp90) proteins. These proteins, so ubiquitous and functionally complex, are a challenge to study in mammalian cells. For a long time after the identification of Hsp90, the most anyone knew was its size (90 kilodaltons), that it was activated as part of the cellular response to stress (e.g., heat), and that it performed "housekeeping functions" in the cell. In the 1990s, Len Neckers, Ph.D., Senior Investigator in CCR's Urologic Oncology Branch, was among the first to recognize that Hsp90 inhibitors could be powerful drugs in the fight against cancer. Today, 19 inhibitors of Hsp90 have been approved for clinical trial as targeted anti-cancer agents, and the Neckers laboratory is combining yeast genetics with work in mammalian cell lines and mouse models to define the next generation of Hsp90 inhibitors.

The Trials of Hsp90

Hsp90 works hard, serving over 200 "client" proteins in the cell, helping them to fold correctly as they take up their rightful positions in the cell. For reasons that are still not well understood, Hsp90 has a special fondness for oncoproteins whose structures shift according to functional state. Among Hsp90's clients, a surprising number are well recognized targets in oncology, including HER2, a member of the epidermal growth factor receptor (EGFR) family, the fusion protein kinases BCR-ABL and EML4-ALK, the receptor tyrosine kinases KIT and MET, and the steroid hormone receptors for androgen and estrogen. As a result, Hsp90 is predicted to have activity against a variety of cancers.

> Among Hsp90's clients, a surprising number are well recognized targets in oncology.

"Theoretically, heat shock proteins are a very interesting class of drug targets because they are involved in oncogene protein folding in many different tumor types, so their inhibitors should cause downregulation of multiple oncogenes. So, they have the potential to treat different tumor types," said Giuseppe Giaccone, M.D., Ph.D., Chief of the Medical Oncology Branch at CCR. "And

> Hsp90 inhibitors may be particularly effective in combination with other therapies.

the preclinical data are really incredible—these drugs work at nanomolar concentrations *in vitro*."

Because Hsp90 affects multiple oncogenic pathways, Hsp90 inhibitors may be particularly effective in combination with other therapies to lessen development of resistance. "One of the major reasons for drug resistance is the cancer's resourcefulness: if you shut down one pathway, then a parallel pathway will take over. Hsp90 inhibition could prevent activation of the parallel pathway. So there is a clear role for Hsp90 inhibition in resistant tumors, either upfront to prevent the development of resistance, or later to reverse the resistance once it starts," said Giaccone. Several clinical trials are under way to test Hsp90 inhibitors in drug-resistant settings.

Neckers agrees that Hsp90 inhibitors could be highly effective in combination with agents targeted against particular oncoproteins. But his research also points to a way forward that utilizes alternative strategies to interfere with Hsp90 in cancer cells. This is important because the first generation of inhibitors have not yet achieved the expected success. "These should be the perfect cancer drugs," said Neckers, "but so far, the activity in patients is less than you would predict."

In the Beginning

The Neckers laboratory did not always study Hsp90. "My lab in the late 1980s was working on something completely different: antisense technologies," said Neckers. However, he was increasingly concerned about off-target effects, that is, interactions that could not be predicted by the sequence of the antisense probe. Neckers had just completed a successful review of his laboratory by the Board of Scientific Counselors, so he felt he had an opportunity to explore new research directions.

(*Photo: R. Baer*)

Len Neckers, Ph.D.

Luke Whitesell, M.D., Ph.D., now at the Whitehead Institute for Biomedical Research at M.I.T., was working as a Research Fellow in the Neckers laboratory and had decided to search for novel inhibitors of protein kinases to study their effects on tumor cell morphology. Learning of the antibiotic geldanamycin, isolated from *Streptomyces hygroscopicus*, that was reported to inhibit the viral oncoprotein v-SRC, Whitesell acquired it and a set of structurally similar compounds called benzoquinone ansamycins from the Natural Products Repository of NCI's Developmental Therapeutics Program (DTP).

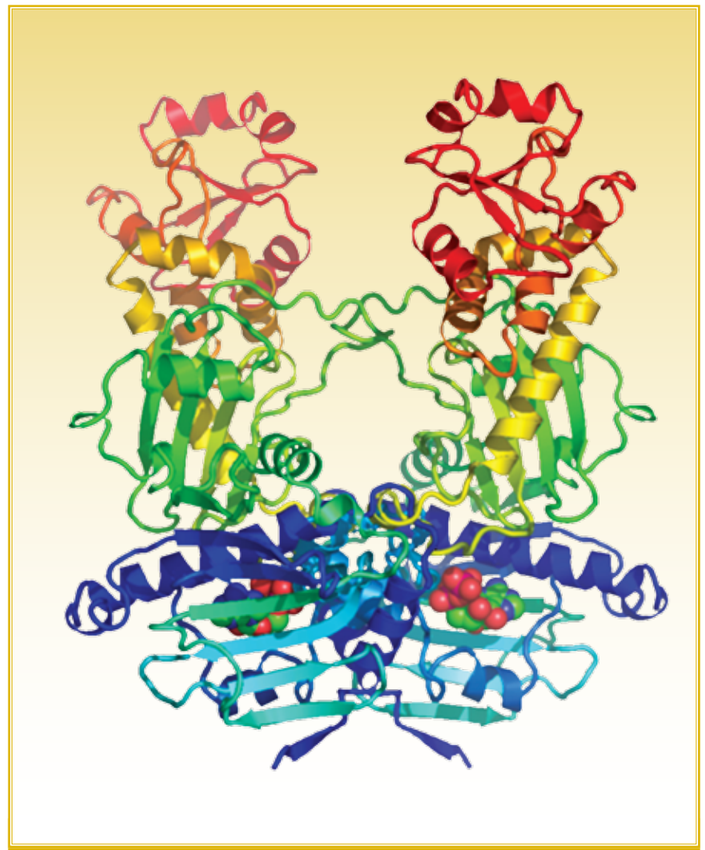
Although the compound did block the ability of *v-SRC* to transform cells in culture, Whitesell and Neckers soon found that this was not because the compound was directly interfering with the activity of the v-SRC protein. Instead, geldanamycin caused the v-SRC protein to degrade. When they did an experiment to pull out cellular proteins that bound directly to geldanamycin, only one came up—a protein 90 kilodaltons in size, which subsequent experiments confirmed was Hsp90.

"Initially, we thought this was so depressing," said Neckers. The team was disappointed to pull down a huge amount of what seemed to be a boring cellular housekeeping protein, and nothing else. Fortunately for cancer research, that disappointment didn't last for long.

"A light bulb went off, and we realized this may be more interesting than we thought. What if this heat shock protein was associating with v-SRC and what if the loss of v-SRC had something to do with the binding of geldanamycin to Hsp90?" asked Neckers.

A series of experiments revealed that geldanamycin bound to an ATP-binding site in the N-domain of Hsp90 and inhibited its ATPase function, that is, the ability of Hsp90 to use energy from ATP to operate as a protein chaperone. Once free of Hsp90, v-SRC became vulnerable to protein degradation machinery in the cell. "That showed us that Hsp90 binds to a client protein and protects its stability," said Neckers.

(*Image: L. Neckers, CCR*)



Molecular model of heat shock protein 90 (Hsp90).

Although geldanamycin is itself too toxic to be used in humans, Neckers worked with NCI clinical translation programs—DTP and the Cancer Therapy Evaluation Program (CTEP)—to develop a discontinued compound from Pfizer, 17-AAG, a close chemical relative of geldanamycin, which had been identified in a HER2 inhibitor screen. They tested 17-AAG in preclinical models, verified its Hsp90 inhibitory activity, and began a phase 1 clinical trial in the late 1990s. Neckers noted that his former NCI colleague, Edward Sausville, M.D., Ph.D., now at the Greenbaum Cancer Center at the University of Maryland, was instrumental in initiating these clinical trials.

"We found that the less frequently the drug was administered, the higher the doses were that could be tolerated before toxicity became a problem." At that point, drug companies began to get interested in Hsp90 as a target, considering the ATP-binding site as "druggable."

Turning to Yeast

Mehdi Mollapour, Ph.D., came to the Neckers laboratory as a Visiting Fellow with a very different background in Hsp90 research. Several years after the Neckers lab

> Neither dose was effective in preventing tumor growth in mice, but together, they were highly effective.

identified geldanamycin's target and mechanism of action, Mollapour was working on his doctoral degree at University College London where investigators first crystallized Hsp90 from the yeast, *Saccharomyces cerevisiae*, and visualized the ATP binding site in its N-domain.

"Up to that point, it was very controversial as to whether Hsp90 bound and hydrolyzed ATP. By crystallizing the protein in the presence of ATP, we could see the ATP binding site. That really changed the whole field," said Mollapour.

Mollapour was motivated to join the Neckers laboratory because of the finding that geldanamycin bound to the same site on Hsp90 as ATP. "I wanted to learn more about cancer, but also continue to build on my expertise," said Mollapour.

Both Mollapour and Neckers had become interested in how Hsp90 itself is regulated. Neckers and other investigators had observed that all the Hsp90 inhibitors that have been tested to date in animals uniformly concentrate in tumors, whereas they are cleared relatively quickly from the blood and from normal tissues. Neckers had also seen that Hsp90 in tumors seemed to be more sensitive to drugs like 17-AAG and felt that if there were differences in Hsp90 in different cell types, they were most likely a result of modifications made to the Hsp90 protein after it was translated from mRNA.

"Before I joined Len's lab, I had a series of questions about Hsp90, and I felt that the next big question was how this protein gets modified post-translationally," said Mollapour. "When I emailed him about coming to the lab, I asked Len about post-translational modifications and remarkably, he was interested in addressing the same question."

Up until Mollapour joined the laboratory, Neckers and his colleagues had focused on Hsp90 in mammalian cells. But because there is so much Hsp90 in these cells, it is difficult to study the protein by genetic manipulation. Yeast, on the other hand, provides a perfect model system for using genetic tools to study how modifications to Hsp90 affect its function.

Who Is Regulating Whom?

"We published a paper in *Molecular Cell* last year that is a perfect example of integrating insights from yeast into translational research," said Neckers.

Mollapour and Neckers demonstrated in yeast that Hsp90 was modified—phosphorylated—by a protein kinase that is itself a client of Hsp90. They identified the site of phosphorylation in Hsp90 in yeast and confirmed this by mutational analysis. They further demonstrated that if they prevented Hsp90 phosphorylation by silencing or inhibiting the protein kinase, Hsp90 inhibitors were more effective.

"We went on to show in prostate cancer cells that preventing Hsp90 phosphorylation made these cells dramatically more sensitive to our Hsp90 inhibitors," said Neckers. Most recently, they have shown a synergistic effect *in vivo* between very low doses of both the kinase inhibitor and the Hsp90 inhibitor. Neither dose alone was effective in preventing tumor growth in mice, but together, they were highly effective.

"We started something in simple eukaryotic cells—in other words, yeast—and made that huge jump to mammalian systems; first to cancer cell lines and then to mouse cancer models," said Mollapour. Along the way, we benefited enormously from the diverse expertise of our collaborators, which helped us to bring all the pieces together expeditiously. "My next goal is to see whether this principle of a client protein modifying Hsp90 is true for other protein kinases," said Mollapour.

"The kinase inhibitor we used is in phase 1 and 2 clinical trials for different cancer types," said Neckers. "So we hope that soon it will be feasible to combine this kinase inhibitor with an Hsp90 inhibitor in a combinatorial clinical trial."

Improving Efficacy

"We are continuing to collaborate with companies that have Hsp90 inhibitors in the clinic," said Neckers. He is particularly interested in the efficacy of Hsp90 inhibitors in cancers that are driven by known client proteins like HER2 and mutated EGFR. "HER2 remains among the most sensitive client proteins of Hsp90 that have so far been identified in tumors."

(*Photo: R. Baer*)

Len Neckers, Ph.D., and Mehdi Mollapour, Ph.D.

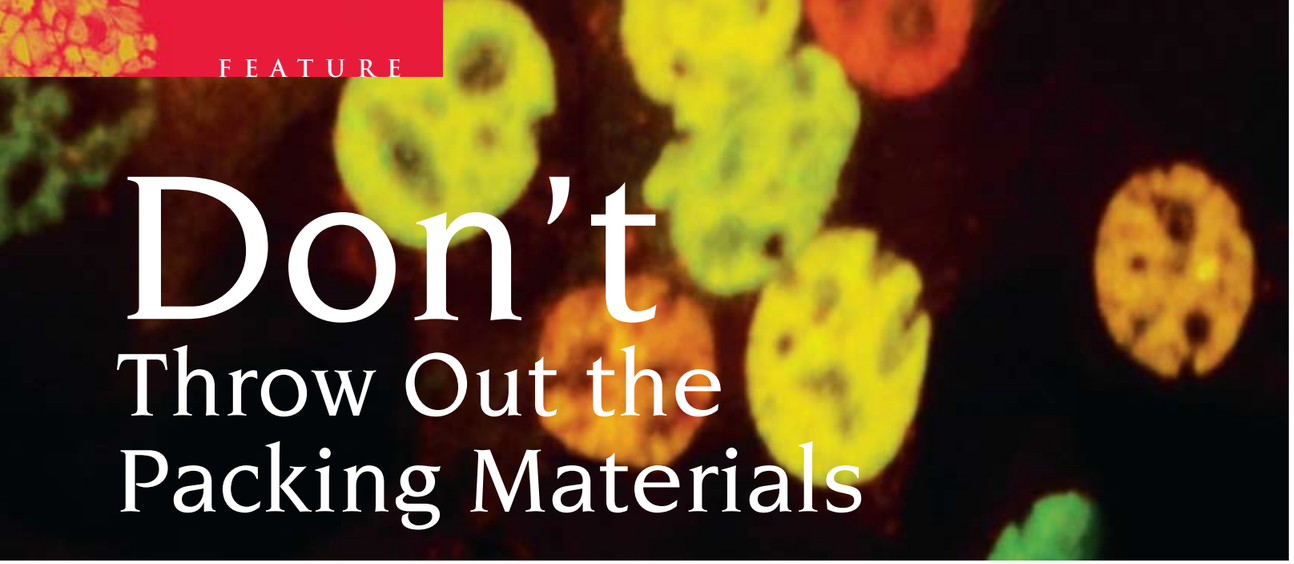
The most famous example of HER2-positive tumors is in a subset of breast cancers, for which the targeted drug Herceptin is prescribed. "Herceptin frequently stops working after some time, but in initial trials with the Hsp90 inhibitor 17-AAG added in, there was additional clinical benefit," said Neckers. HER2 is also overexpressed in a certain percentage of patients with bladder cancer. "The program in the Urologic Oncology Branch for bladder cancer is expanding, so we are hoping to test Hsp90 inhibitors on these cancers as well."

Interestingly, normal EGFR is not highly dependent on Hsp90, but mutations in EGFR that confer drug resistance to the targeted agent, Tarceva, also confer dependence on Hsp90. Accordingly, clinical benefits of 17-AAG and other Hsp90 inhibitors in drug-resistant non-small cell lung cancer have also been observed.

"A lot of what we do now in our laboratory is to focus on identifying more modifications of Hsp90 and understanding how they regulate function and specificity," said Neckers.

Neckers and his team have identified several modifications —phosphorylation and acetylation sites—on Hsp90, not all of which have the same impact on function. They have found sites that inhibit the binding of client proteins, and increase or decrease Hsp90's sensitivity to inhibitors. Neckers is hopeful that within all this information, there is going to be a way to explain and exploit the difference between Hsp90 in tumors and healthy tissues.

To learn more about Dr. Neckers' research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=neckers.



Don't Throw Out the Packing Materials

Most illustrations of DNA depict a kind of ladder spiraling off into the distance, the ladder being the famous DNA double helix consisting of paired nucleotide bases. Although it has long been known that mammalian DNA is packed very tightly and systematically with specialized proteins into material called chromatin, researchers are only now beginning to appreciate the importance of chromatin structure in gene regulation. Gordon Hager, Ph.D., Chief of the Laboratory of Receptor Biology and Gene Expression, has built his considerable scientific achievements on the study of nuclear hormone receptors, using the glucocorticoid receptor (GR) as a prototype. Not without controversy, his research has brought him inexorably closer to the pivotal role and complex dynamics of chromatin structure in the control of gene regulation.

"In the 1980s," recalled Hager, "chromatin was a bit of a dirty word." A wave of experiments done by several different laboratories that were designed to transcribe genes from chromatin fractions had ended in the purgatory of experimental artifacts a few years earlier. Wary scientists shied away from studying chromatin as anything more than DNA packing material. "And there were some groups, including mine, Carl Wu's, Gary Felsenfeld's, and Bob Simpson's that did a lot of the early chromatin work because it simply couldn't get funded extramurally," explained Hager, referring to his current and former NIH Intramural Research Program colleagues.

Hager's laboratory was focused on nuclear hormone receptors—receptors that bind hormones like glucocorticoids, which allow them to interact with particular response elements in the DNA to regulate gene transcription. For reasons that are still only partially understood, the murine mammary tumor virus (MMTV) contains a regulatory element that binds GR when MMTV is integrated into cellular DNA. The team discovered that when MMTV integrated into the mammalian genome, chromatin structural elements called nucleosomes were invariably positioned over the GR binding sites.

This discovery was soon followed by studies showing that GR binds directly to a nucleosome and that, as a result, the nucleosome undergoes a structural transition. To measure the change in chromatin structure, the team used an assay known as DNAse hypersensitivity. DNAse or deoxyribonuclease is an enzyme that will chew up DNA entirely if incubated long enough. However, when incubated only very briefly, DNA is fragmented at easily accessible sites in the chromatin structure that are termed hypersensitive. GR binding sites corresponded to sites of DNA hypersensitivity.

"We proposed that the glucocorticoid receptor was binding to DNA and causing chromatin reorganization at a specifically positioned nucleosome," said Hager.

(Photo R. Baer)

Gordon Hager, Ph.D.

> The structure of chromatin was playing more than just a passive role in gene regulation.

A Few Years in the Doghouse

"In 1987, I proposed at a Keystone Meeting in Park City, Utah, that somehow this chromatin reorganization was part of the mechanism by which the glucocorticoid receptor regulated gene expression," said Hager. Hager was presented with a "Renegade Award" at the meeting, which was not meant as an accolade. "We were in the doghouse," said Hager.

Within a few years, however, the field had shifted its perspective. Evidence began to accumulate suggesting that the structure of chromatin was playing more than just a passive role in gene regulation. A Postdoctoral Fellow in Hager's lab, Trevor Archer, Ph.D., now Chief of the Laboratory of Molecular Carcinogenesis at the National Institute of Environmental Health Sciences, published an influential experiment in 1992 that presented the first direct evidence that the structure of chromatin could prevent a transcription factor from binding to its promoter element. A few years later, C. David Allis, Ph.D., now Tri-Institutional Professor at The Rockefeller University, and colleagues identified a known transcriptional regulator in yeast as an enzyme that modified histone proteins in chromatin.

"And bingo, chromatin was not such a dirty word anymore," said Hager.

It is now clear that remodeling of chromatin is a key event in transcriptional regulation. Many chromatin remodeling factors have been identified and an entire field of epigenetics has emerged to investigate the influence of chromatin modifications on functional gene expression.

There and Back Again

"Most of molecular biology until about the mid-1990s was dead-cell biochemistry," said Hager. "No matter what you do—a DNA footprint or a chip experiment—the first thing you do is kill the cell and then, often, you do 'terrible' things like crosslink the proteins everywhere."

Hager and his colleagues wanted to study gene regulation in living cells. With the advent of green fluorescent protein (GFP), which could be introduced genetically to label proteins of interest, a Postdoctoral Fellow in the lab created GRs tagged with GFP and showed that their fluorescent signature could be visualized under a microscope in living cells.

"And then we remembered cell line 3134," said Hager.

Back in the laboratory's early days, Hager and his team had used the MMTV promoter element as an experimental tool to study glucocorticoid receptor function. "Accidentally, we had a cell line where this structure had amplified itself into a 200-copy tandem array sitting in one place on chromosome 4. That's two million base pairs of DNA with about 1000 GR binding sites in this one place in the chromosome." A more perfectly optimized system for visualizing GR binding could not be readily imagined.

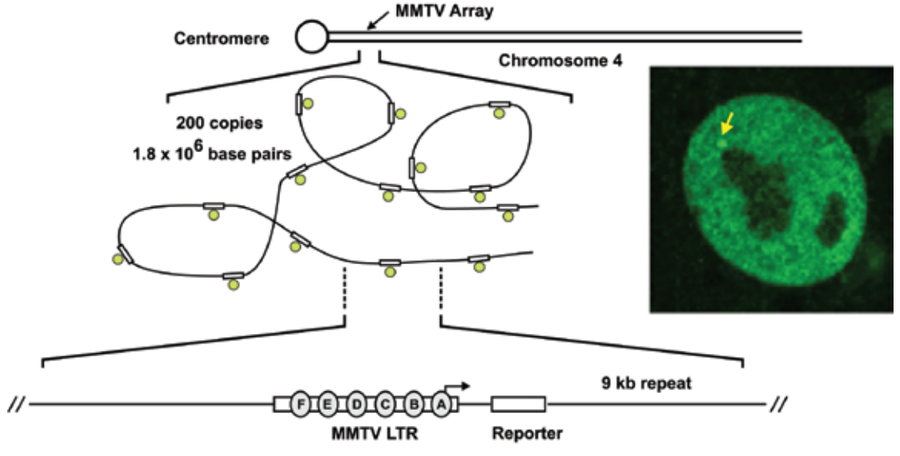
Under a fluorescence microscope, Hager and his colleagues were able to see GRs accumulating on this massive stretch of binding elements. They could also study its kinetics through a technique known as photobleaching. When fluorescent molecules are subjected to light of a particular wavelength, they lose their activity and are no longer visible. By shining a laser on the chromosomal segment, Hager's team could discover whether and when the GRs were replaced by new unbleached molecules.

"We found that they were almost instantaneously replaced," said Hager. "And we were back in the doghouse."

Not only was the result surprising, the idea that DNA binding proteins were operating on such a fleeting timescale contradicted many accepted

> The idea that DNA binding proteins were operating on such a fleeting timescale contradicted many accepted experimental paradigms.

(Image: G. Hager, CCR)



To study nuclear hormone receptor binding, Hager and his colleagues created a cancer cell line with approximately 1000 hormone response elements from the murine mammary tumor virus (MMTV) present in an array on chromosome 4. Activated steroid hormone receptors tagged with green fluorescent protein (GFP) translocate into the nucleus and bind to the array, visualized here as a sharp increase in intensity (yellow arrow) in the cell nucleus.

experimental paradigms for which protein-DNA binding was essentially considered fixed. However, other groups began to do the same kind of experiments with different proteins and the dynamic nature of these interactions gradually came to be accepted.

Hit and Run

To understand the relationship between GR binding and chromatin remodeling, Hager and his colleagues began to look at chromatin remodeling proteins, massive ATP-dependent enzyme complexes that literally grab hold of nucleosomes and alter their higher order structure. Biochemical experiments indicated that the chromatin remodeling protein complex, hSWI/SNF, could create chromatin transitions in the presence of GR. But, they also suggested, paradoxically, that activation of the hSWI/SNF complex disrupted GR binding.

Hager's team decided to use ultrafast UV laser-crosslinking to examine this phenomenon in more detail. They incubated GR and hSWI/SNF with chromatin containing the MMTV array of promoter elements and then studied the resulting interactions at different points in time by taking samples and rapidly crosslinking everything with the UV laser. Their results revealed strong evidence that initially, GR binds to the promoter and recruits the hSWI/SNF complex, but then is displaced during chromatin remodeling.

"The GR proteins are binding to a structure that is being destroyed by the very hSWI/SNF enzyme that it is recruiting," said Hager. "These hSWI/SNF enzymes are giant complexes running around the DNA, so it makes sense that sooner or later they're going to run into the DNA binding protein."

This model of transient, reversible interactions of transcriptional regulators with DNA is now well known as the "hit-and-run" model. It appears to be a central mechanism for all of transcription biology.

Recently, the Hager lab has turned to high-throughput imaging to study the mechanisms by which nuclear receptors migrate to their targets. Using small interfering RNAs (siRNAs) to knock down individual proteins, the team can search for molecules that will prevent migration or clustering of GRs along an MMTV array structure.

Ty Voss, Ph.D., who runs the NCI High-Throughput Imaging Facility, is collaborating with Hager's group and other investigators to optimize and run their assays using high-throughput microscopy. "These microscopes are capable of taking maybe 25-50,000 pictures a day at 300 nanometer resolution, giving very fine details of subcellular organization," said Voss. For siRNA screens, cells are placed on plastic plates with 384 wells, with different siRNAs introduced into each well. An automated liquid handler can process several thousands of such samples in an hour. Voss programs the microscope to automatically analyze features of interest, like the fluorescence generated when GRs bind the MMTV array.

"That's the latest stage in the evolution of this living cell technology. You don't have to know anything about pathways—you could, in principle, look at every gene in the genome," said Hager.

Scaling Up

"About four years ago," explained Sam John, Ph.D., a Staff Scientist in the Hager lab, "we decided to stick our toes into the genomics waters and see how cold they were." It turned out that the temperature was just right, and the lab shifted from focusing on one or two genes to studying transcriptional regulation and chromatin structure in the context of the entire genome.

> "We decided to stick our toes into the genomics waters and see how cold they were."

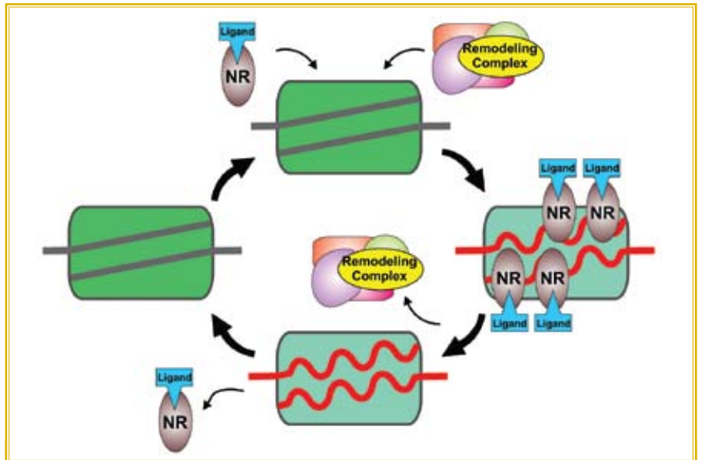
DNase hypersensitivity assays—the technique that Hager and his colleagues used when first suggesting a role for chromatin structure in GR binding and activation—got a boost into the 21st century with the creation of Digital DNase or DHS-Seq by John Stamatoyannopoulos, Ph.D., Hager's collaborator and friend at the University of Washington. Instead of studying a single hypersensitive site, DHS-Seq allows the genome-wide mapping of all the hypersensitive sites in a given cell.

"We found that, with a handful of exceptions, every place the GR protein hits the genome, corresponds with a hypersensitivity site," said Hager. The number of binding sites they were surveying was on the order of 100,000. The surprise came, however, in the order of events. For 85 percent of GR binding sites, the hypersensitivity, i.e. remodeling and opening of chromatin structure, was already present before GR binding.

Furthermore, Hager and his colleagues found that both DNase hypersensitivity and GR binding across the entire genome were dependent on cell type. "When we compared accessible chromatin regions, we found that the organizational overlap was very, very small. And when we looked at where GR bound in cell lines, very different patterns emerged."

"The convention has always been that GR interactions with chromatin result in hormone-dependent changes in chromatin structure," said John. "It turns out that the organization of chromatin at baseline is also an important determinant of how a transcriptional regulator finds and binds its target sites in chromatin." By extension, the structure of chromatin at baseline appears to be important for defining a cell.

(Image: G. Hager, CCR)



Hager and his colleagues have proposed that the recruitment of remodeling complexes by nuclear hormone receptors leads to a transient opening of nucleosome positioning in chromatin. This modified chromatin structure will be accessible to multiple transcription factors during the lifetime of the modified state. After completion of the remodeling cycle, the nucleosome returns to its previous configuation, and a new cycle is initiated.

DNase hypersensitivity sites—not genes—appear to account for 60 to 70 percent of all targets identified in genome-wide disease association studies. That is, many single nucleotide polymorphisms are not located in genes but rather are found in hypersensitivity spots. "This is going to be key in cancer biology—mutations in people that cause dysregulation of their regulatory elements," concluded Hager.

Far, Far Away

Chromatin structure is not just important within localized regions of DNA. Recently, chromosome conformation-capture technologies are being used to identify distant sequences of DNA that come together when DNA forms loops.

"The best data we have is in T cells; as they mature and differentiate, the whole nuclear material gets reorganized," said Hager. Genes that are activated come together in clusters, called hubs. "This is the next frontier in cell biology—how to understand the structure of the nucleus. That takes us back to this dynamic question: If the proteins that are binding to these sites are coming and going so fast, how can they possibly get hold of the DNA for long enough to form a long-range interaction?"

"For our group, the dynamics of chromatin remodeling is a key issue. And it all started back in 1987 with that first experiment that put us in the doghouse," said Hager. "I always wind up at the end of my seminars saying something like 'biology is chemistry.' These are chemical reactions, but we often view them as static macromolecular cartoons… The next breakthrough will come from observations of single molecules moving in living cells."

To learn more about Dr. Hager's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=hager.

Breast Cancer:
The Triple-Negative Problem

Joyce O'Shaughnessy, M.D., spent 10 years at NCI, initially as a Clinical Associate, then as a Senior Investigator and Special Assistant to NCI Director, Samuel Broder, M.D., and finally as a Senior Investigator in NCI's Intramural Breast Cancer Research Program. O'Shaughnessy is now a Medical Oncologist with Texas Oncology and the Baylor-Sammons Cancer Center in Dallas, Texas. She is also Co-Chair of the Breast Cancer Research Program at U.S. Oncology, a practice management company that operates clinical trials across its national network through a structure that resembles NCI Clinical Trials Cooperative Groups. A major focus of her clinical research is on triple-negative breast cancers.

Triple-negative breast cancers are defined by what they are lacking—they do not have the three molecular receptors known to fuel most breast cancers: estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2). Metastatic, triple-negative breast cancer has a very poor prognosis, with a median survival of only about one year, and there is no standard-of-care therapy.

Triple-negative cancers share similarities with hereditary *BRCA1*-related breast cancers, namely dysregulation of *BRCA1*, which leads to defects in repair of double-stranded breaks in DNA. Thus, we and others have wondered whether we could develop therapeutic strategies that exploit this defect in DNA repair. Poly-ADP ribose polymerase (PARP) inhibitors were identified as agents that could further disrupt DNA repair in breast cancer cells, thus rendering them particularly vulnerable to DNA-disrupting chemotherapeutic agents like gemcitabine and carboplatin.

We recently conducted a phase 3 clinical trial of these standard chemotherapeutic agents with and without the PARP inhibitor, iniparib. We saw a promising signal of iniparib benefit in second and third-line patients but, disappointingly, the overall population did not benefit. We believe that this inhibitor may provide even greater overall benefit if we can identify the right subset of patients. As with many cancers, a particular challenge these days is to better define the subtypes of triple-negative breast cancer. Not all of them have the same DNA repair problems; not all of them even have the same cell types of origin.

Working with Populations

I am involved in a wide variety of clinical trials for high-risk, potentially lethal breast cancers. The majority of my work involves patients with triple-negative breast cancer because it is such a large unmet medical need.

I have developed a particular interest in correlative tissue biomarker studies for triple-negative breast cancer. A few years ago, my colleague Lisa Carey, M.D., and I reported data at the San Antonio Breast Cancer Symposium that the epidermal growth factor receptor inhibitor, cetuximab, showed some activity in breast cancers that are triple negative. We are working on a follow-up study with an intensive biomarker discovery component, in which we hope to understand how to predict the really long benefit—the multiyear remissions—that we have seen in a subset of patients.

One of the reasons I came to Texas Oncology is because I felt they were very prescient in starting community-based clinical research back in the late 1980s. Although large clinical trials were once the domain of NCI and the academic medical centers, currently, accrual to larger phase 3 trials, even within the NCI cooperative groups, mostly comes from the community setting. And U.S. Oncology has at least 10 practices that do collaborative phase 1 work, making many of those investigational agents available outside the academic setting. I remember a time in the early 1990s when I looked around and realized that there was just a tremendous amount of research going on in the community. In part, I attribute that proliferation to the NCI's training of so many talented Oncology Fellows who are able to carry on the principles of clinical research outside of academia.

Joyce O'Shaughnessy, M.D.

Working with Individuals

In addition to conducting large-scale clinical trials, I am also engaged in a project with the Translational Genomics Research Institute (TGen) in which we harvest and analyze transcriptome data from patients' metastatic triple-negative breast cancer tissue. We are not just describing the mutational abnormalities; we are using this data to identify the most productive targetable mutations in an individual patient and then treating the patient with corresponding investigational drugs or off-label agents.

For example, we found clear indications of important mutations in the phosphatidylinositol 3 (PI3) kinase pathway in the first patient whose tumor we sequenced, and on the basis of those mutations, I made a referral for her to start on a phase 2 study of a promising PI3 kinase inhibitor. This is a patient I have been caring for, for years—she was diagnosed about four years ago, received preoperative chemotherapy followed by a mastectomy, and then found that the cancer recurred two years later in her lungs, lymph nodes, and chest wall. She has since been treated on two clinical trials, but her cancer has progressed each time. Between those therapies, we harvested tissue and it has taken a few months to get the sequencing results. But now, she is being treated with an agent that specifically targets a driving mutation in her cancer.

> I am definitely encouraged by our advances in the last two decades.

Where We Go from Here

The standard cytotoxic agents are, by themselves, only going to cure a small minority of newly diagnosed patients. To me, a cure is not necessarily a complete eradication of the disease at a microscopic cellular level; it is never seeing that life-threatening breast cancer again in a woman's lifetime. This may involve long-term therapies—already, breast cancer patients may take anti-estrogen therapies for a decade or more. I think about the current AIDS therapies, which basically suppress but do not eradicate HIV and which provide convergent combination therapies focused on one or two essential HIV enzymes.

I am encouraged by the fact that we are increasingly differentiating among the several different types of breast cancers, we are understanding some of the driving biological factors in these cancers, and we now have some solid leads for therapeutic interventions. Clinical research in breast cancer is dramatically different from what it was during my early years at the NCI. In those days, when we did clinical trials, it didn't matter what subtype of breast cancer you had. If you had breast cancer, you could enroll. So, I am definitely encouraged by our advances in the last two decades, but we still have miles to go.

One Molecule, Multiple Cancers:

The Devil is in the Details

"Inside the beltway," is a phrase normally reserved for discussions of careers in national politics, referring as it does to the highway that surrounds the Washington D.C. metropolitan area. However, Christina Annunziata, M.D., Ph.D., has also developed her career as a physician-scientist inside the beltway, first as a medical student and resident at Georgetown University and then rising through the ranks of CCR training opportunities to become a tenure-track Investigator. Over the years, Annunziata's responsibilities have involved her in many NCI protocols, but her own research has remained firmly rooted in the family of transcription factors, NF-κB. As a student with Jeffrey Cossman, M.D., at Georgetown, she studied nuclear factor kappa B (NF-κB) signaling in Hodgkin's disease. As a Medical Oncology Fellow with Louis Staudt, M.D., Ph.D., in CCR's Metabolism Branch, she studied the role of NF-κB in multiple myeloma. Now her laboratory investigates the effects of NF-κB signaling in ovarian cancer. Annunziata believes that understanding the nuances of NF-κB function in distinct cell types could lead to effective pharmacological interventions for cancer.

It was definitely the opportunity for strong and dedicated research in a clinical environment that drew me to NIH. I met Lou Staudt, when I was still a doctoral student, and it was a natural fit for me to continue my postdoctoral research in his laboratory. NF-κB is a very interesting family of molecules. It consists of five subunits that form various combinations of dimers capable of coordinating the expression of multiple genes. It is present as an inactive form in the cytoplasm of most cells, where a variety of external cellular signals can prompt its rapid separation from a molecular complex and migration to the nucleus to modulate gene expression. NF-κB signaling is important for many different cell types, however, the pathway functions differently according to cell type.

In multiple myeloma, we found that NF-κB signaling was turned on in most of the tumors we studied. In many cases, that may have been the result of influences from the surrounding tissue—the tumor microenvironment—but in some cases, the tumors had autonomous aberrant NF-κB activity. However, we didn't identify just one specific mutation. We found that there were multiple points throughout the pathway that were dysregulated in different myeloma subtypes. In one case, a protein might be amplified, in another case a negative regulator might be lost. We did these analyses by first looking at changes in gene expression, but in many cases, validated our findings by looking at protein levels. We believe that one therapeutic action of the protease inhibitor, bortezomib, now approved for the treatment of multiple myeloma, may be to inhibit NF-κB signaling.

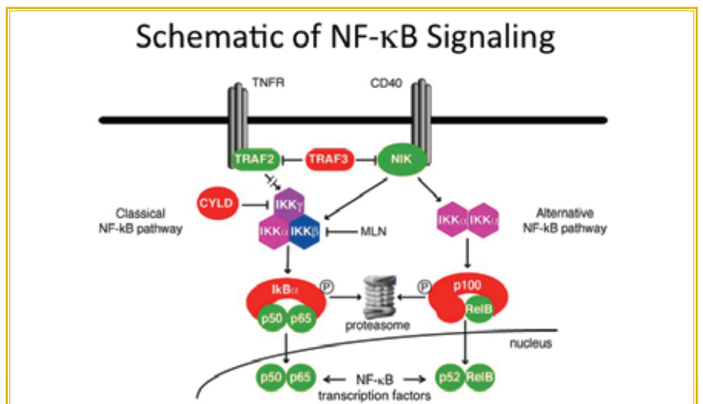
(*Photo: E. Branson*)

Christina Annunziata, M.D., Ph.D.

NF-κB Signaling in Ovarian Cancer

While working in the Staudt laboratory, I was performing clinical duties one day per week with Elise Kohn, M.D., in CCR's Medical Oncology Branch (See "Ovarian Cancer: A Silent Killer "Speaks" through Proteins," *CCR connections* Vol. 2, No. 2). I began seeing patients with ovarian cancer, and I began to wonder whether NF-κB signaling might also play a role in this disease. I could not find much in the scientific literature that addressed this question, and I wanted to move my research more towards helping the patients I was actually seeing. So, when I began my research program as an Assistant Clinical Investigator, I began to study NF-κB signaling in ovarian cancer (See "CCR's Clinical Investigator Development Program," Page 31).

(*Image: C. Annunziata*)



The NF-κB signaling pathway can be activated by a variety of receptors on the surface of cells to activate transcriptional networks in the nucleus. Several signaling components within the cell regulate NF-κB activity.

My laboratory used a small-molecule drug and small interfering-RNA molecules (siRNA) to inhibit NF-κB signaling in ovarian cancer cell lines. We found that we could define a genetic signature that reflected the upregulation of NF-κB signaling in ovarian cancer and that inhibiting NF-κB signaling affected both this genetic signature and measures of aggressiveness in disease. The signature included genes associated with proliferation, survival, inflammation, adhesion, invasion, and angiogenesis; i.e., all the hallmarks of cancer.

It turns out that NF-κB regulates a very different set of genes in ovarian cancer as compared to multiple myeloma cells. That is hardly surprising, given the very different developmental and functional profiles of the two kinds of cells, but it does mean that the NF-κB activation signature needs to be identified for each cancer type.

We collaborate with Michael Birrer, M.D., Ph.D., who moved from

Smac, IAP, and Cancer Therapy

Members of the inhibitor of apoptosis protein family (IAP) are exciting targets in cancer research these days. Originally studied for their effects on blocking cell death through inhibition of caspase proteins, IAPs are now known to directly affect multiple cellular processes. Cancer researchers are most interested in the ability of certain IAPs to regulate cell survival and tumorigenesis through activation of NF-κB signaling.

IAPs appear to protect cancer cells from signals related to inflammation in the tumor microenvironment, for example, tumor necrosis factor α (TNFα). Alterations in IAPs are found associated with many human cancers and are typically associated with poor prognosis, disease progression, and chemoresistance.

Five biotechnology and pharmaceutical companies currently have early stage clinical trials under way for drugs that interfere with IAPs. These drugs are called Smac mimetics, because they operate like cellular Smac molecules to tag IAPs for degradation. Smac mimetics cause the rapid depletion of certain IAPs and show potent anti-tumorigenesis activity in cancer models.

CCR to the Dana Farber/Harvard Cancer Center a few years ago. He has access to a lot of ovarian cancer patient samples, and we have been lucky to work with him to verify that the genetic signature we identified in ovarian cancer cell lines is also found in primary tumors. In fact, we have shown that the NF-κB signature in primary tumors is associated with poor prognosis.

Finding the Right Drug

There aren't many direct NF-κB inhibitors available, mainly because of issues related to toxicity. So, we're looking at other points in the pathway that might be more amenable to therapeutic intervention. I am currently working with an investigational drug from Tetralogic Pharmaceuticals, TL-32711, which mimics the cell signaling molecule, Smac. Under certain conditions, Smac shifts the balance of cell signaling from NF-κB-related proliferation to controlled cell death by apoptosis. (See "Smac, IAP, and Cancer Therapy"). TL-32711 seems to be more potent and specific than other drugs in its class because of reduced cross-reactivity.

We are now studying the effect of Smac mimetics on ovarian cancer cell lines. I also plan to test this drug in mouse models. NF-κB is an important part of normal cellular signaling, particularly in the immune system, which is one reason for the toxicities associated with direct inhibitors of NF-κB. Thus, it will be important to study our Smac mimetic in a mouse model with a normal immune system, so that we can observe the effect of this drug on normal and tumor-infiltrating immune cells. Many mouse models of cancer rely on human cancer cells grafted into a mouse with a deliberately dampened immune system, making such studies impossible.

If, as we predict, Smac mimetics alter expression of the genes we identified as a signature of NF-κB activity, we will want to move our work into human trials for recurrent ovarian cancer. Although such a trial would not be restricted in its enrollment, our hypothesis would be that patients with the highest levels of NF-κB activity would be most responsive to the drug. As the trial proceeds, we would look at the treatment response relative to the level of gene expression from an initial biopsy. In that way, we would hope to hone in on the most responsive patient population.

Finding the Right Cancer

I am currently an Investigator on about 10 clinical protocols at CCR, at least half of which are for ovarian cancer. Although my research focus is on NF-κB signaling, there are several other candidate targets for this disease including angiogenesis and poly ADP-ribose polymerases (PARPs). Because some ovarian cancers have dysfunction of *BRCA* 1 or 2, including germline mutations, these ovarian cancers have compromised DNA repair. Inhibiting PARP-associated mechanisms of DNA repair is thought to overwhelm the cell's ability to withstand standard chemotherapeutic agents that disrupt DNA. These were the hot topics at the annual meeting of the American Society of Clinical Oncologists (ASCO) last year, but there are many genetic mutations implicated in this disease, albeit at relatively low frequencies.

A major limitation with all our clinical trials is that we have no way to identify which patients are most likely to respond to a given therapy. Ovarian cancers come in four different histological varieties: serous, clear cell, endometrioid, and mucinous. Interestingly, clear cell ovarian cancer shares some similarity in molecular mutations with clear cell renal cancer and may have a higher response to the drugs sorafenib and sunitinib that are used for renal cancers. So, clear cell ovarian cancer may be a molecular subtype that also has a histologic definition. But, that is an exception rather than the rule.

Perhaps even more than in other cancers, it seems that ovarian cancer is extraordinarily heterogenous at a molecular level. For instance, looking at data on genomic instability from The Cancer Genome Atlas (TCGA), you can see distinct hotspots of genetic abnormalities for glioblastoma and lung cancer patients, but so far, you don't see that kind of clustering for cases of ovarian cancer. It might be, therefore, that only five percent of ovarian cancers will respond to a particular drug so, without the appropriate molecular testing, clinical trials will continue to see very low response rates.

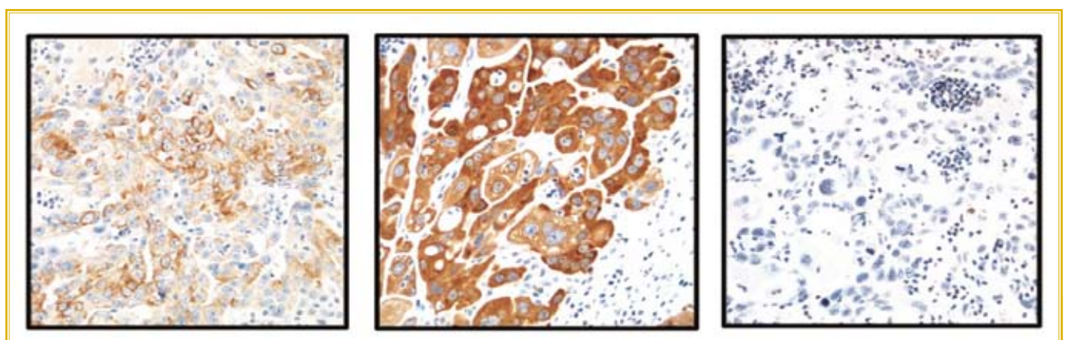
Our trials are designed so that patients can stay on the therapy as long as there is a response and side effects are manageable. Patients may, in fact, stay on a drug regimen indefinitely. For instance, in the case of the angiogenesis inhibitors bevacizumab and sorafenib, we've had several patients treated for two to three years (See "Warrior Drugs," page 32). Although there are side effects including high blood pressure and rashes, this drug combination seems to have tolerable levels of toxicity over time, which patients deem an acceptable impact on their quality of life.

There will likely come a point when the tumor evolves to evade a particular drug, and we will have to switch to another drug or another combination of drugs. So, options are important, which is one reason I continue to pursue my work in NF-κB. My personal goal, probably like many of my colleagues here at NCI, is to bring my research into the clinic.

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

> Ovarian cancer is extraordinarily heterogenous at a molecular level.

(*Image: C. Annunziata*)



Cells taken from primary ovarian tumors show selective expression of NF-κB family proteins. Cells stained in blue show expression of IKK-α (*left*) and IKK-β (*middle*) stained in brown, but not IKK-ε (*right*).

CCR's Clinical Investigator Development Program

Now a tenure-track Investigator, Christina Annunziata, M.D., Ph.D., came to CCR as a Clinical Fellow in 2002. Subsequently, she and Heidi Kong, M.D., (a recently recruited tenure-track Investigator in CCR's Dermatology Branch) became two of the early participants in the Clinical Investigator Development Program, a 3-year opportunity that serves as a transition between a Fellowship and a position as an independent Principal Investigator. "The program really enabled me to build momentum in my research," said Annunziata.

The goal of the program is to enable promising board-eligible or board-certified translational physician researchers at early stages of their careers to become competitive for tenure-track appointments in academia or comparable positions in government and industry. Program participants are selected via a competitive process and are designated as Assistant Clinical Investigators. Mark Udey, M.D., Ph.D., Chief of the Dermatology Branch and a Deputy Director at CCR, has been instrumental in the program and likens the position to that of an Instructor in traditional academic institutions.

Grant writing skills are emphasized, and participants must apply for an NIH Career Development Award by the end of the second year. The program also includes a component of formal coursework. "This requirement is intended to impart some structure to the program," said Udey, "and we wanted to ensure that our investigators have command of core knowledge central to clinical research."

Applications to the program are accepted annually; up to three positions are available every year. For more information about the Clinical Investigator Development Program, please visit http://ccr.cancer.gov/careers/clinical_programs_invest.asp.

Warrior Drugs

With the right combination of drugs, Patricia Beyea expects to see her ovarian cancer become a managed chronic condition.

Patricia Beyea's mother died at the age of 52 from breast cancer, so when Patricia felt a lump in her own breast at age 34, she knew it was serious. Ignoring the physician who advised her to wait six months after a negative needle biopsy, she sought another opinion. Shortly thereafter, in 1986, she had a modified radical mastectomy and reconstruction for a breast cancer that had spread to a lymph node.

"That was the first time I saved my own life," said Patricia.

Given her family history and later discovery that she had inherited a mutation in the *BRCA1* oncogene, Patricia has been on a vigilant cancer watch with her doctors ever since. Nine years went by before blood tests revealed the first new sign of cancer. This time, it was ovarian cancer.

"Back then, they were doing in-hospital chemotherapy following surgery," said Patricia. "A 36-hour treatment with carboplatin and taxol every three weeks—a horrible, tough treatment." But, the cancer went into remission for another decade.

Patricia retired from her job as a physical education teacher in New York and moved to a small town in Florida. Then, in September 2004, Hurricanes Frances and Jeanne came through, forcing her from her home and into a trailer provided by the Federal Emergency Management Agency (FEMA). "Everything on the outside looked fine, but inside my house was a watermark of 24 inches – sewer water, contaminated water. You had to throw everything out."

In the busy time that followed, a few things got neglected, including her normal yearly checkup. "All of a sudden I started having abdominal pains. I went from feeling fine to feeling

(*Photo: E. Branson*)

Christina Annunziata, M.D., Ph.D., and Patricia Beyea

I couldn't handle the pain unless I was in a hospital on morphine."

This time the chemotherapy that followed her surgery was done on an outpatient basis, but that didn't make it easier to bear. "It's like you're dying but you're still alive," said Patricia.

The cancer returned twice more after much shorter intervals of remission. After a third recurrence in four years, her surgeon was unable to remove any of the tumor because it was completely surrounded by blood vessels. Patricia could not bear the thought of more standard chemotherapy, which seemed increasingly ineffective.

"I told my oncologist I wanted to enroll in a clinical trial," said Patricia. "And he asked me where I wanted to go."

Patricia began her quest for an investigational therapy at M.D. Anderson Cancer Center, but was concerned about signing an agreement making her liable for any costs incurred that were not covered by her insurance. "That's when the head of the division told me I should self-refer myself to the NIH."

In September 2008, Patricia enrolled in an NIH protocol for treatment of ovarian cancer with the angiogenesis inhibitors, bevacizumab and sorafenib. She flies from Florida to Washington D.C., every two weeks for the treatments. Her tumors have shrunk in stages and stabilized. "I have finally got to the point of seeing chemotherapy as my ally, not my enemy. Every time I see the drugs going in, I consider them warriors going into battle."

CCR connections is now available online: http://home.ccr.cancer.gov/connections

Web Sites with More Information about CCR

Center for Cancer Research
http://ccr.cancer.gov

Office of the Director
http://ccr.cancer.gov/about/OfficeDirector.aspx

Our News
http://ccr.cancer.gov/news

Office of Training and Education
http://ccr.cancer.gov/careers/OfficeEducation.aspx

Patient Information on Cancer and Clinical Trials

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

How to Refer a Patient
http://bethesdatrials.cancer.gov/health-care-professionals/index.aspx

NCI Cancer Information Service
http://www.cancer.gov/aboutnci/cis
1-800-4-CANCER (1-800-422-6237)

Understanding Cancer Series
http://www.cancer.gov/cancertopics/understandingcancer

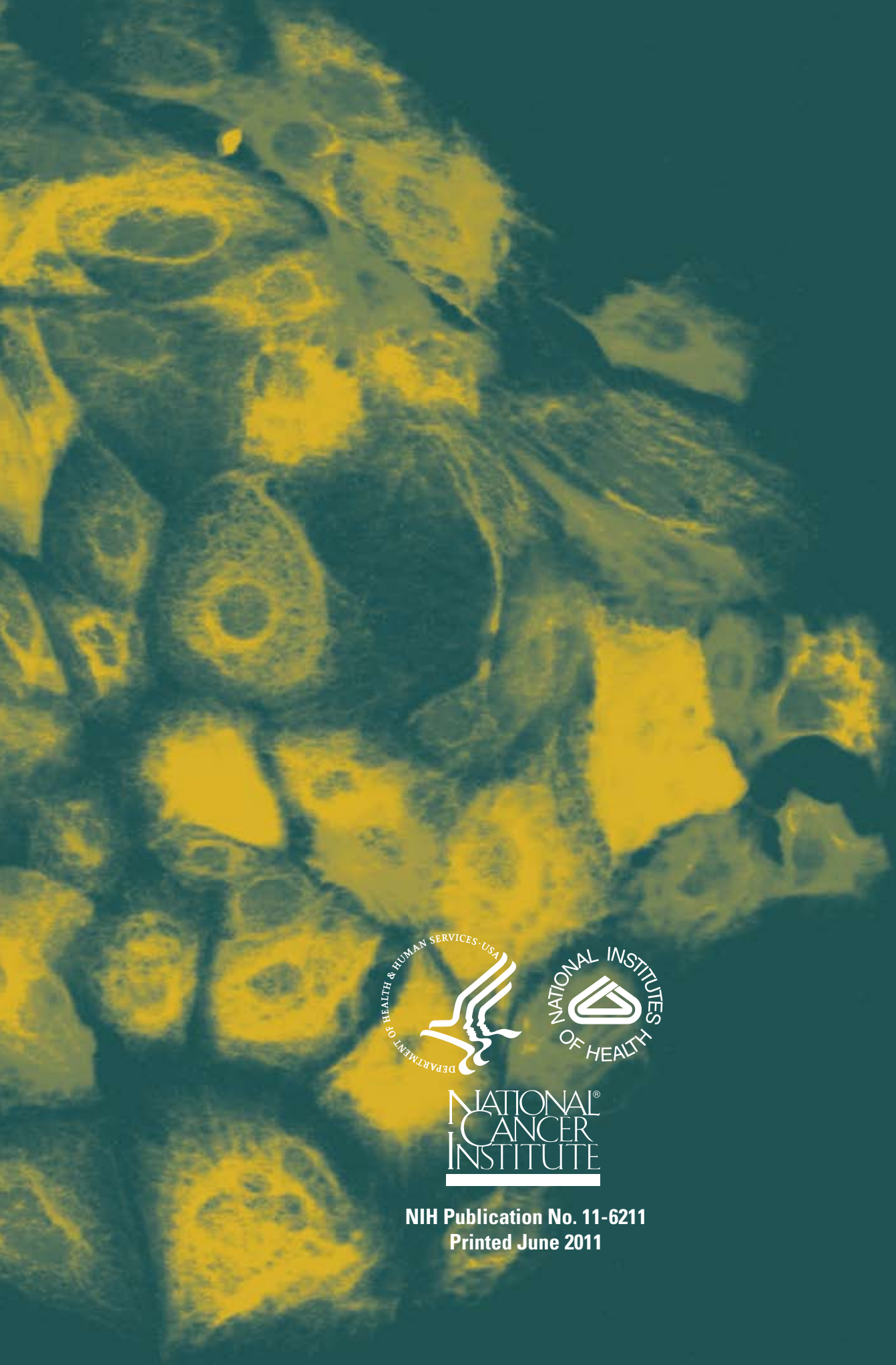
CCR Clinical Cancer Trials in Bethesda, MD
http://bethesdatrials.cancer.gov

Additional Links

National Cancer Institute (NCI)
http://www.cancer.gov

Working at NCI
http://www.cancer.gov/aboutnci/working

National Institutes of Health (NIH)
http://www.nih.gov



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