

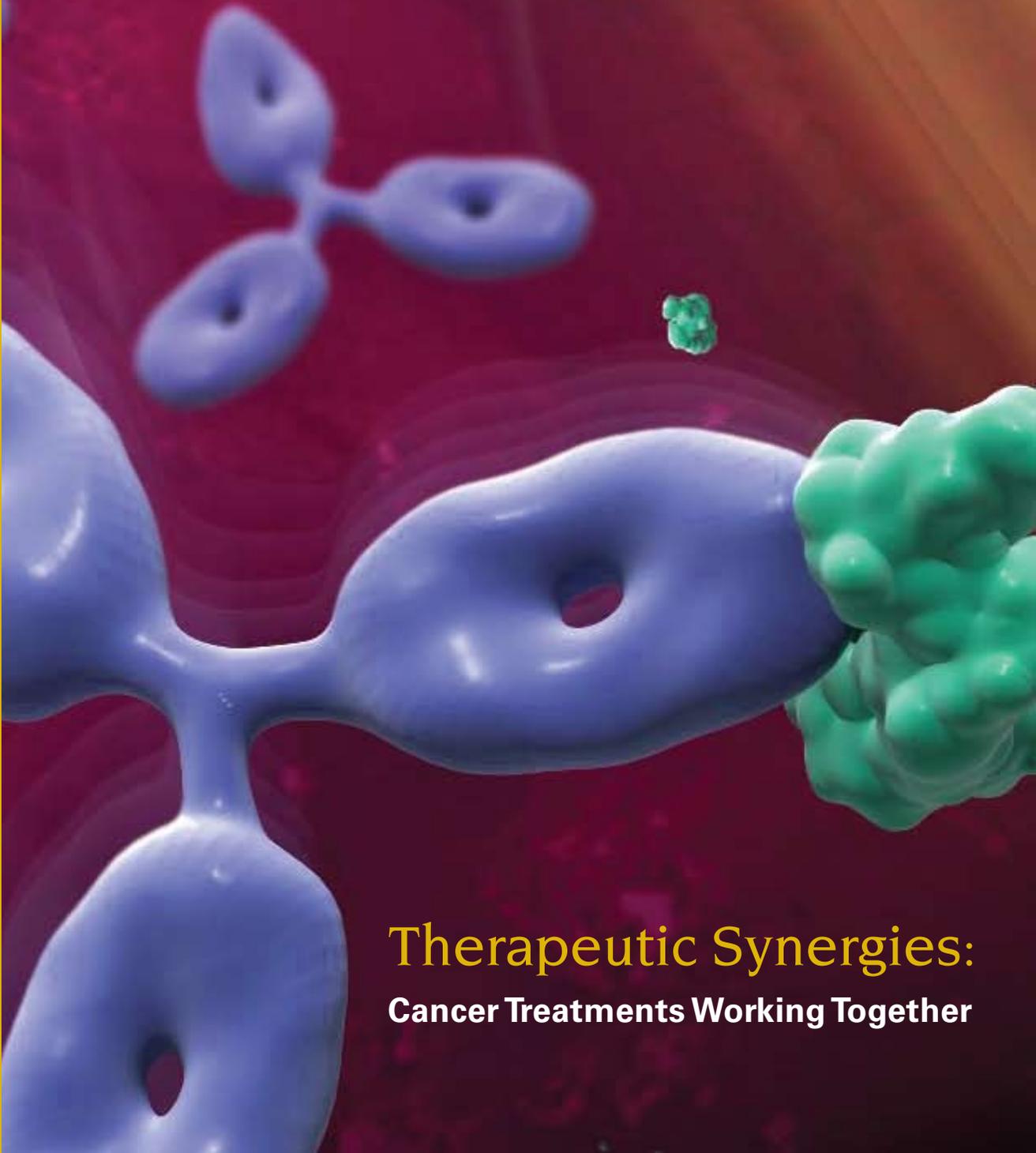
National Cancer Institute

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# CCR connections

CENTER FOR CANCER RESEARCH

[ccr.cancer.gov](http://ccr.cancer.gov)



**Therapeutic Synergies:**  
**Cancer Treatments Working Together**

U.S. DEPARTMENT  
OF HEALTH AND  
HUMAN SERVICES  
National Institutes  
of Health

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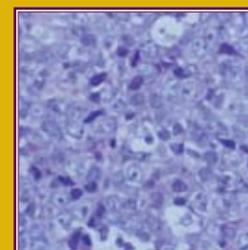
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We invite your comments and suggestions about *CCR connections*.  
Please email your feedback to [tellccr@mail.nih.gov](mailto:tellccr@mail.nih.gov).

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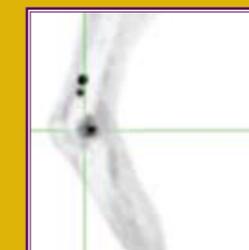
FEATURE



Therapeutic Synergies in the Fight Against Cancer

28

IN THE CLINIC



Multiple Approaches to Myeloma

**Center for Cancer Research**

The mission of CCR is:

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



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

**Contributors:**

- L.M. Bennett, Ph.D.
- D. Kerrigan, M.S.
- K. Martin, M.S.
- S. Fox, B.A., B.S.W.
- A. Cline
- J. Crawford, Ph.D.
- N. Giannosa, M.P.H.

# Customizing Cancer Care

*This year marks the 10<sup>th</sup> anniversary of the completion of the draft sequence of the human genome. At the White House event celebrating this landmark achievement in biomedical research, President Bill Clinton remarked, "With this profound new knowledge, humankind is on the verge of gaining immense, new power to heal. Genome science will have a real impact on all our lives—and even more, on the lives of our children. It will revolutionize the diagnosis, prevention, and treatment of most, if not all, human disease."*

Ten years later, the impact of genome sciences is yet to be widely felt in mainstream healthcare, but it is readily apparent at the forefront of clinical research, particularly in the realm of cancer. At CCR, several investigators are using genomic and genetic information on an unprecedented scale to understand and treat different forms of cancer.

As we learn in "Pediatric Tumors Made Personal," Javed Khan, M.D., is launching a multicenter trial for pediatric solid tumors in which clinical researchers will guide the treatment of individual patients with relapsing cancers using information obtained from the comprehensive analysis of their particular cancer genomes.

Meanwhile, in another wing of the Clinical Center at NIH, Ola Landgren, M.D., Ph.D., is running the first natural history study of precursor diseases that lead to multiple myeloma. As he describes in "Multiple Approaches to Myeloma," Landgren and his colleagues are studying the genetic and molecular signatures that define progression from precursor to full-blown disease with the ultimate aim of stopping multiple myeloma before it starts.

Diagnostic and prognostic power is also a goal for Tom Misteli, Ph.D., and his Research Fellow, Karen Meaburn, Ph.D., who have recently reported that the spatial position of genes in the nucleus may reflect their cancerous state. Their work is described in the article, "Everything



Robert Wiltrout, Ph.D.

in Its Right Place," and we also hear directly from Dr. Meaburn in our "In Conversation" series about her hopes for turning this observation into a prognostic tool for breast cancer.

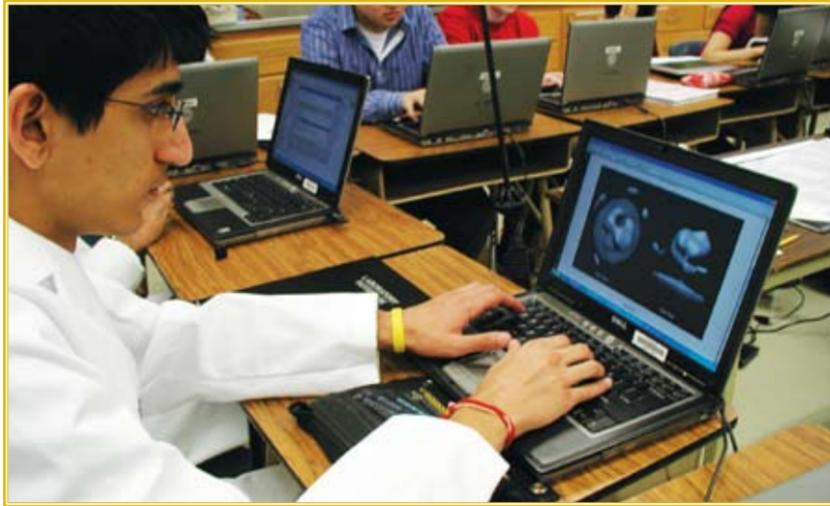
Of course, knowing what has changed in a particular cancer may not only be a tool for diagnosis but also a target for treatment. Natasha Caplen, Ph.D., is working with a number of investigators to use the power of functional genomics to look for new targets and analyze molecular interactions of existing drugs. In "The Art of Silence," we are treated to a sampling of the studies that are under way in her laboratory using RNA

interference to systematically disrupt individual genes in order to study their function and response to therapy.

Depending on your perspective, ten years can seem an eternity or the blink of an eye. For clinicians and patients, cures can never come quickly enough. But as biomedical researchers, we can also view our progress with amazement. Ten years after the genomic revolution was formally declared, we are actively translating the most basic biological information contained in our DNA into tools for the diagnosis and personalized treatment of cancer.

# An Accelerated Program

Students from Thomas Jefferson High School for Science and Technology help accelerate HIV structural biology research.



(Photo: L. Gaudreault)

Sid Bhatia at the Thomas Jefferson High School for Science and Technology connects remotely to the Biowulf computer cluster at NIH.

The NCI has had a long history of bringing students into the lab, but has only opened its doors to high school students relatively recently. “I have been at the NCI for almost 10 years,” stated Sriram Subramaniam, Ph.D., Head of the Biophysics Section of the Laboratory of Cell Biology at CCR. “In the last seven to eight years, about 70 students have been associated with my lab. Of these, about 20 were high school students, starting with one high school student whom I took on somewhat reluctantly about six years ago.”

But that reluctance has since vanished. Dr. Subramaniam’s lab now regularly taps into the pool of talented high school students in the area to accelerate his research in HIV structural biology. Last year, Subramaniam, Senior Research Fellow Martin Kessel, Ph.D., and their team worked together with Larry Gaudreault, a senior science teacher at the Thomas Jefferson High School for

Science and Technology in Northern Virginia, to carefully select six highly motivated high school seniors for a joint project. This school was ranked the best public high school in the nation in 2007, 2008, and 2009 by *U.S. News & World Report*.

The surface structure of the HIV virus particle contains one of the most important targets in HIV/AIDS vaccine research. Only a single protein is expressed on the surface of every HIV virus—an oligoglycoprotein, informally called a spike. This spike makes contact with CD4 receptors on T cells, and this interaction is critical for HIV to enter and infect a cell. Dr. Subramaniam’s lab is focused on visualizing the structure of this spike and how it varies across different strains in order to understand why some strains can be neutralized by antibodies and others cannot. Understanding these variations structurally is critical to finding an effective HIV vaccine.

“We’re looking at multiple states of the same virus, how the structure changes among different viruses, and the spectrum of HIV viruses in individual patients,” said Dr. Subramaniam. “We had a very large computational problem: The throughput of data was high enough that we could generate a large amount of data, but the bottleneck was converting that data into useful information. And that is where the students come in.” The students work remotely, using a very powerful computer resource at NIH called the Biowulf cluster—a collection of thousands of processors that’s the focal point of most of NIH’s computational needs—to extract structural differences from thousands of images.

The project has indeed accelerated progress in the Subramaniam lab, and at the same time, the students get a great deal out of it, too. “It would not be successful without the team effort of our entire lab and our collective approach to science,” noted Dr. Subramaniam. “The process of finding an effective way for the students to participate in our research actually sharpens our own efforts, making this a win-win partnership by accelerating our research program, while also making it exciting for the students as they work alongside senior graduate students, postdocs, and NIH scientists to contribute to NIH’s scientific mission.”

To learn more about Dr. Subramaniam’s research, please visit his CCR Web sites at <http://ccr.cancer.gov/staff/staff.asp?Name=Subramaniam> and <http://electron.nci.nih.gov>.

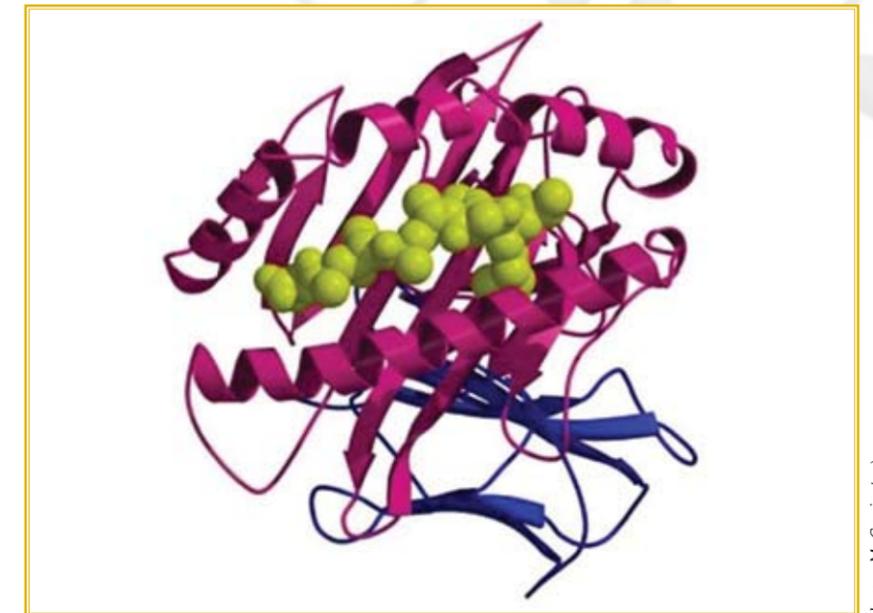
# High Marks for Destruction

Genetic variations boost HIV-killing immune response to slow disease progression.

Throughout the history of the AIDS epidemic, a few lucky people have avoided full-blown onset of the disease despite being exposed to HIV. Host genetic variation appears to play a major role in slowing disease progression in HIV-infected patients. In particular, individuals with naturally occurring variants of certain human leukocyte antigen (HLA) genes appear to take years longer to develop AIDS and die of complications of the disease. HLA genes encode cell surface proteins that present antigen—in this case, from HIV—to lymphocytes for destruction.

In a paper published in the November 22, 2009 issue of *Nature Genetics*, a team of researchers led by Mary Carrington, Ph.D., Head of the Immunogenetics Section at the Laboratory of Experimental Immunology at CCR, demonstrated that high levels of HLA cell surface protein HLA-C are associated with lower viral loads and slower progression of HIV to AIDS.

The researchers looked at variants in a region known to associate with levels of HLA-C gene expression and also to have one of the strongest genome-wide associations with the level of HIV in the blood during early infection: a region located 35 kilobases upstream (-35) of HLA-C. They genotyped nearly 1,700 HIV-positive individuals to determine which -35 variant they carried. They found that individuals with a variant called -35TT expressed HLA-C cell surface protein at low levels compared to individuals with a variant called -35CC. Furthermore, individuals with the -35CC variant—and therefore greater cell surface expression of HLA-C



(Image: M. Carrington)

The HLA molecule (magenta) presents a pathogen peptide (green spheres) to the immune system.

—had much lower levels of virus in the blood, better protection against HIV, and slower progression to AIDS.

The fact that the -35CC variant correlates with high HLA-C protein levels and lower levels of virus present in blood after HIV infection suggests that the genetic variation makes it easier for the immune system to kill cells infected with the virus. “If you have more HLA-C on the cell surface, the immune system’s T cells are going to recognize that infected cell much better than if the cell had low levels of HLA-C expression,” said Dr. Carrington. “The ones with high expression are going to make it very clear to the immune system that this cell is infected and needs to be destroyed.”

A better understanding of this type of genetic variation could help in the development of vaccine- or immune-based therapies that could delay or

even prevent the development of AIDS. But the researchers will first need to elucidate the mechanism underlying their observations. “So what we’re working on now is to figure out what is directly causing the difference in level of expression of HLA-C,” said Dr. Carrington. “The next step is to determine whether that mechanism could be manipulated in a way that we can turn low expression HLA-C alleles into high expression HLA-C alleles. But until we understand the mechanism, we have no way of knowing how to approach that question.”

To learn more about Dr. Carrington’s research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=carrington>.

# CliffsNotes for Your DNA

*Changing the chromatin binding domain of lens epithelium-derived growth factor changes how HIV reads the host genome and where it inserts its DNA.*

"Let's imagine that the human genome is the text of a very important book on how to build a human being," said Stephen H. Hughes, Ph.D., Chief of the HIV Drug Resistance Program Retroviral Replication Laboratory at CCR. There are many different cell types in the human body, and each of the different cell types contains the same DNA sequences, the same set of instructions. The generation and proper maintenance of the different cell types requires that each cell knows which parts of the "book" to read.

In humans, and all other eukaryotic organisms, genomic DNA is organized into chromatin. Annotations on the chromatin help define which parts of the genome a cell needs to read, and one of the important types of annotation are chemical modifications of the tails of histone proteins in the chromatin. In

collaboration with researchers at the Rockefeller University and the Dana-Farber Cancer Institute, Hughes and his colleagues have found a way to redirect the integration of HIV-1 DNA via annotations and modifications to the chromatin.

"When we began working on this project, we knew, from the work of others, that HIV DNA did not integrate randomly, but preferred to integrate into the bodies of expressed genes," said Dr. Hughes. "We wanted to gain control over where HIV DNA integrates to make retroviral integration safer as a tool for gene therapy, and to develop a new method to investigate the chromatin organization and annotation."

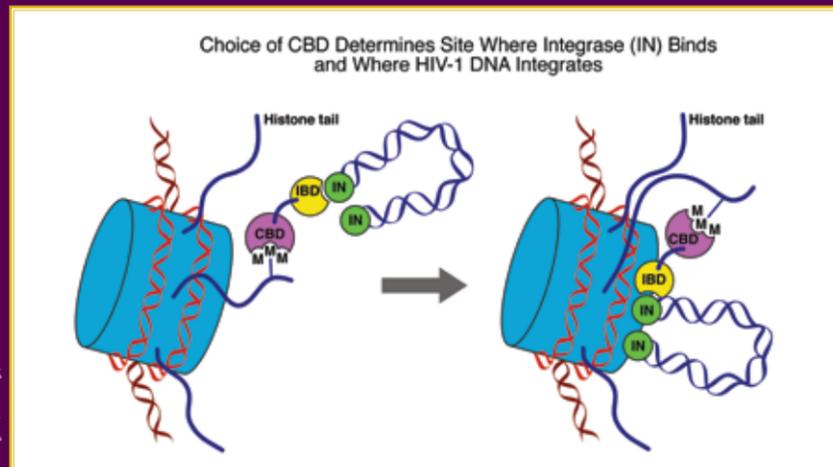
HIV DNA integrates into actively expressed genes because the integration machinery binds to a host protein called lens epithelium-

derived growth factor (LEDGF). LEDGF is a two-part protein made up of the chromatin binding domain (CBD), which binds to the host cell's chromatin, and the integrase binding domain (IBD), which binds the viral integration machinery. Thus, LEDGF acts as a tether linking the viral integration machinery to the host cell chromatin; the distribution of HIV integration sites reflects the distribution of LEDGF on host cell chromatin.

In the February 1, 2010 issue of *Proceedings of the National Academy of Sciences*, Dr. Hughes and colleagues reported that the integration site preference of HIV-1 can be changed by creating LEDGF fusion proteins in which the CBD is replaced by other CBDs that bind to different sites on host cell chromatin. These fusion proteins direct HIV integration to sites where the new CBDs bind.

"What it shows is we're not required simply to accept the distribution of HIV integration that nature provided; we can rewrite the rules," explained Dr. Hughes. This is important because HIV is a candidate virus for human gene therapy, and the researchers want to make sure that the viral DNA is inserted in safe places in the genome. Integration near an oncogene can activate the oncogene, causing cancer. Conversely, distribution of the binding sites for novel CBDs can be determined using HIV integration as a tool to mark the sites in the host genomes. This provides a powerful new tool for probing chromatin structure and function.

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

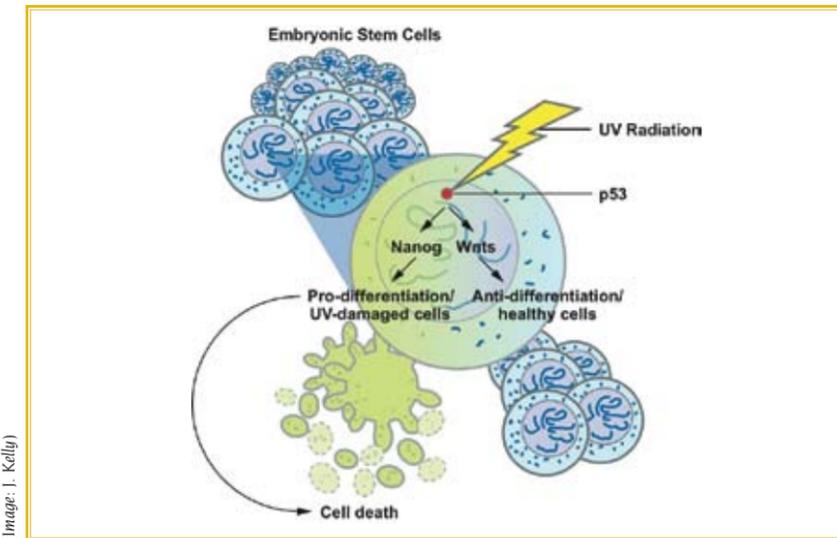


HIV-1 DNA (blue strands) integration into host DNA (red strands) depends on the composition of the chromatin binding domain (CBD).

(Image: J. Kelly)

# The Dual Effects of p53 on Differentiation

*The p53 protein promotes differentiation and activates Wnt-mediated anti-differentiation in mouse embryonic stem cells.*



(Image: J. Kelly)

The transcription factor protein p53 in UV-damaged embryonic stem cells both promotes differentiation and activates Wnt-mediated anti-differentiation.

DNA damage can have serious consequences in any cell. When it occurs in embryonic stem cells, however, the consequences can be even more devastating since the resulting mutations will be passed on in all subsequent cell divisions. It is therefore critical for a cell to have several sensor mechanisms in place to detect and hopefully repair such damage. One such sensor is p53, a transcription factor protein and so-called tumor suppressor that monitors cells for damage from many types of environmental stress. When damage occurs, p53 activates specific pathways to assess the damage and prevent its spread by inhibiting cell division or causing the cell to undergo programmed cell death.

Although the functions of p53 have been extensively researched in adult cells, they are largely unknown in embryonic stem cells. Jing Huang, Ph.D., Head of the Cancer and Stem Cell Epigenetics Section at CCR, along with

Postdoctoral Fellow Kyoung-Hwa Lee, Ph.D., and their colleagues recently published a report in the December 14, 2009 issue of *Proceedings of the National Academy of Sciences* that identifies a mechanism used by p53 to control the fate of mouse embryonic stem cells upon DNA damage.

To determine the genes affected by p53 in mouse embryonic stem cells, the scientists identified the p53 binding sites on cellular DNA using a genome-wide approach called chromatin immunoprecipitation-based microarray (ChIP-chip). They compared the binding of p53 in normal cells and in cells with DNA damage from adriamycin, an anti-cancer drug, and found that, when DNA damage occurred in embryonic stem cells, p53 strongly enhanced the expression of genes associated with the Wnt signaling pathway. The researchers measured the ability of p53 to activate Wnt signaling in cultured mouse embryonic stem cells

and found that the cells secreted specific Wnt proteins into the culture media that inhibit differentiation of surrounding cells. If the cells were modified to remove p53, Wnt production was diminished.

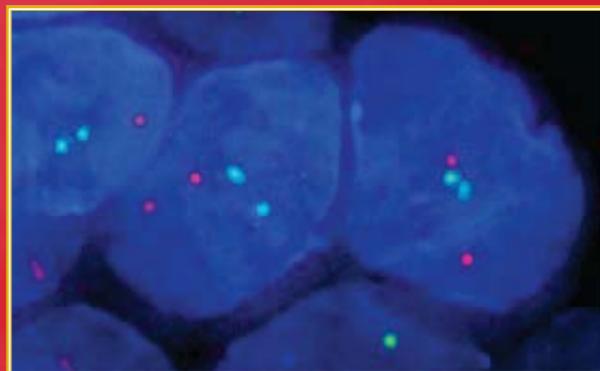
The direct connection of p53 to Wnt-mediated suppression of cell differentiation was puzzling at first since p53 is known to promote differentiation as a means of removing unhealthy cells from the stem cell pool. But the researchers concluded that p53 is essentially using its ability to both promote and inhibit the differentiation of embryonic stem cells to perform its role as a stress sensor, monitoring its cellular environment and reacting accordingly. When damage occurs, p53 does indeed remove unhealthy cells from the stem cell pool by promoting programmed cell death or differentiation. At the same time, p53 activates the Wnt pathway to inhibit the differentiation of surrounding, healthy embryonic stem cells to maintain a population for the development of the organism.

The next step is to determine whether mutations in p53 may restore its control of Wnt signaling, not only in embryonic stem cells but also in adult cells. Overactivation of Wnt is tumorigenic in certain somatic cells, so p53-activated Wnt signaling could become oncogenic. "If we can understand how p53 can be converted from a tumor suppressor to an oncogene," said Dr. Huang, "perhaps we can target the p53 mutant to fight cancer."

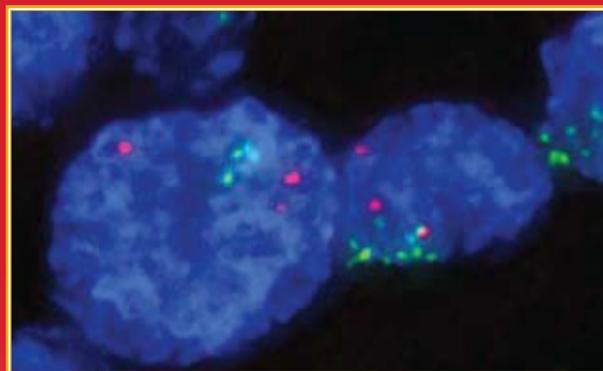
*To learn more about Dr. Huang's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=jinghuang>.*

# Everything in Its Right Place

Researchers identify genes that undergo spatial repositioning in breast cancer cells.



(Image: K. Meaburn)



(Image: K. Meaburn)

Genes imaged using FISH in normal breast cells (left), and repositioned genes imaged in cancerous breast cells (right).

Understanding how gene abnormalities affect tumor formation and progression is important for tracing the mechanisms of disease and for developing diagnostic tools. Although a great deal of research has focused on the genetic mutations that drive cancer development, they are not the only signs of genetic havoc. A gene's spatial position may also be affected in certain cancers. Researchers have recently discovered that the spatial positioning patterns of genes within a cell nucleus may offer a new diagnostic strategy to distinguish cancerous from normal breast tissue.

"The reason why we started working on this is because we didn't know the mechanism for gene movement, which is a big question in the field," said Tom Misteli, Ph.D., Head of the Cell Biology of Genomes Group at CCR. "Everyone knows that certain genes change their position, but no one knows how. We still don't know why these things move, but they do. And that's all that matters for diagnostic purposes."

In a study published in the December 7, 2009 issue of the *Journal of Cell Biology*, Karen Meaburn, Ph.D., Dr. Misteli, and colleagues identified several genes

whose spatial position within the cell nucleus is altered in breast cancer when compared to normal tissue. The researchers used fluorescent *in situ* hybridization (FISH), a technique used to detect and localize specific DNA sequences, to visualize 20 genes in a set of 11 normal human breast tissue samples and 14 invasive cancer tissue samples, to determine if they occupy distinct intranuclear positions. They found eight genes that were frequently repositioned in cancer tissues and determined the repositioning events did not simply reflect genomic instability because repositioning did not correlate with changes in the number of gene copies in the cell.

The altered position of a single gene, *HES5*, which affects biological pathways that have been implicated in cancer, allowed identification of invasive breast cancer tissue with nearly 100 percent accuracy. Only a minority of tested genes underwent significant repositioning in a given cancer tissue, suggesting that repositioning is gene-specific and does not reflect a large-scale alteration in how the genome is organized within the nucleus. Furthermore, the scientists found

that several combinations of two or three genes allowed identification of cancerous tissues with low false-negative and false-positive rates.

This approach has advantages over current standard breast cancer diagnostic tests in that it gives a quantifiable readout and reduces human error. "This could be a useful first-line molecular test. Nowadays, breast cancers are diagnosed by pathologists, and this is very much based on their experience and on their background. In contrast, this would be a molecular test that you can actually quantitate very accurately."

The next step for Dr. Misteli and colleagues is to validate their approach on a larger number of samples and see how accurate this method could be for diagnostic purposes. If successful, this method of cancer diagnosis would not be limited to breast cancer, but could someday be applied to distinguish other types of tumors.

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

# In Conversation: Research Fellow Karen Meaburn, Ph.D.

**CCR:** Karen, congratulations on your recent publication in the *Journal of Cell Biology*.

**Karen:** Thank you. We are very excited to discover that the physical positions of certain genes within the nucleus are altered in breast cancer cells—the finding opens up so many questions, both about the basic biology and translational potential.

**CCR:** What led you to the discovery?

**Karen:** We've known from the literature that genes and even whole chromosomes are in different positions depending on the situation, for example, depending on gene expression levels in cells or differentiation status. Given the dramatic changes in gene expression that occur in cancer, we thought that if we studied several cancer genes, we may find some that have also moved position.

**CCR:** And how are you planning to follow up on this work?

**Karen:** Our data is only from a small set of patients at this point, but we're hopeful that it might form the basis of a diagnostic test. We are currently expanding our study to hundreds of tissue samples, and we'll also be looking at cancers from other tissues than breast.

We also want to know if gene positioning is the same in primary tumors as compared to metastatic cancer. The answer could give us a very interesting window into the nature of metastasis and the way cancer evolves.

**CCR:** Judging from your publication record, your time in the Misteli lab has been very successful. Tell us a bit about your personal experience here.

**Karen:** I met Tom Misteli at a conference that was held at Brunel University in London, where I was studying genome organization as a doctoral student with Joanna Bridger. Moving to Tom's laboratory for my postdoctoral work seemed like a natural fit. Tom's lab isn't micromanaged—he trusts you to be independent and get on with things. At the same time, it's a very supportive environment and has a lot of resources available.

In June, I will have been in the laboratory for five years and will be staying on for another two years as a research fellow.

**CCR:** And has there been any downside to your time here?

**Karen:** I can't think of any. The campus is a great environment for cancer research, and there are also lots of opportunities to collaborate within the Institute. For example, Tom's lab has a long-term collaboration with Stephen Lockett's group in Frederick to develop the imaging software that we need to analyze changes in genome organization.

In fact, about the hardest thing has been being so far away from my home in the U.K.

**CCR:** Is your family supportive of your work?

**Karen:** Oh yes. None of them are scientists—in fact, I have a twin brother



(Photo: V. Roukos)

Karen Meaburn, Ph.D.

that is an accountant. They've always appreciated that I found something I love, but they didn't always understand the importance when I was doing very basic biology. Now that we are talking about diagnostic applications, I think they see the value more clearly. In fact, just before I came to the NIH, my mother was diagnosed with breast cancer so the connection is much more immediate for our family.

**CCR:** Do you think your interest in basic biology has shifted to more applied science?

**Karen:** Not really, although my interest in translation has grown, I see the basic and translational aspects as two sides of the same coin. We need to understand the biology to treat the disease, and we can learn a lot about the biology by studying the disease and its response to treatment.

# Recent CCR Awards



(Photo: B. Branson)

Robert Wiltrout, Ph.D.

## 2010 FLC Laboratory Director of the Year Award

Robert H. Wiltrout, Ph.D., Director of CCR, was honored with the 2010 Federal Laboratory Consortium for Technology Transfer's (FLC) Director of the Year Award on April 29, 2010, in Albuquerque, NM. This competitive and prestigious award is presented annually to directors who have made outstanding contributions supporting technology transfer activities at their federal laboratory for the previous year, and recognizes both the excellence of the recipient's efforts and the facility's technology transfer program.

Dr. Wiltrout views the many CCR/NCI technology transfer successes as a team effort that is driven by the ingenuity, perseverance, and commitment of the Center's researchers and their network of collaborators in government, industry, and academia. All of these collaborators work closely together with NIH's technology transfer professionals to accelerate research progress against cancer and AIDS/HIV. Under Dr. Wiltrout's leadership, the CCR/NCI has made significant advances in building strong scientific partnerships with public and private institutions and strives to continue to bring new scientific discoveries to the market place.

Under the direction of Dr. Wiltrout, CCR has continued to see substantial technology transfer growth, including in the last year alone: 275 active clinical trials, more than 126 active Cooperative Research and Development Agreements with industry, and 120 new commercial licenses. Additionally, CCR's technologies can be found in over 200 licensed products, including several FDA-approved products that were dependent upon contributions from CCR laboratories: AIDS test kit, Fludara, Videx, Hivid, NeuTrexin, Taxol, Vitravene, Velcade, Zevalin, Kepivance, Prezista, and Gardasil.

## 2010 European Association for Cancer Research Young Scientist Award

Brid Ryan, Ph.D., M.P.H., a Cancer Prevention Fellow in the CCR Laboratory of Human Carcinogenesis working as part of the Ireland-Northern Ireland-National Cancer Institute Cancer Consortium, was awarded the 2010 European Association for Cancer Research Young Scientist Award for her work on asymmetric division of DNA in lung cancer. Dr. Ryan's cancer research career began with her undergraduate work at University College Cork in Ireland and continued during her doctoral studies at St. Vincent's University Hospital in Dublin. She came to NCI in 2007 and continues to work under the mentorship of Dr. Curt Harris.

## Nicolaus Copernicus Medal

Zbigniew Dauter, Ph.D., Chief of the Synchrotron Radiation Research Section of the Macromolecular Crystallography Laboratory at CCR, was awarded the Nicolaus Copernicus Medal—the highest distinction awarded by the Polish Academy of Sciences. The award recognizes Dr. Dauter's contribution to the development of protein crystallographic methodology, in particular in the areas of phasing methods and macromolecular structure at ultimate resolution. Dr. Dauter developed the technique of quick halide soaks ("dauterization") of protein crystals, the exploitation of weak anomalous signals, and the use of single-wavelength anomalous dispersion (SAD) phasing.

## 2010 National Public Service Award

American Society for Public Administration and the National Academy of Public Administration  
For innovative and sustained contributions to public service and science education  
**Kenneth H. Kraemer, M.D.**  
Dermatology Branch

## 2010 SER-CAT Outstanding Science Award

Southeast Regional Collaborative Access Team  
For the structure of ERA in complex with the 3' end of 16S rRNA: implications for ribosome biogenesis  
**Xinhua Ji, Ph.D.**  
Macromolecular Crystallography Laboratory

## 2009 Trisociety Award

Society for Leukocyte Biology, International Society for Interferon and Cytokine Research, and the International Cytokine Society  
For lifelong contributions to cytokine and chemokine research  
**Joost Oppenheim, M.D.**  
Laboratory of Molecular Immunoregulation

## Elected to Association of American Physicians

**Mark Udey, M.D., Ph.D.**  
Dermatology Branch

## Harvard Health Letter Top-10 Health Story 2009

MicroRNA Expression, Survival, and Response to Interferon in Liver Cancer  
**Junfang Ji, Ph.D., et al.** NEJM, Vol. 361, No. 15:1437-1447. October 2009

## Wick R. Williams Memorial Lecture Award

Fox Chase Cancer Center  
For pioneering characterization of multiple genetic predispositions to kidney cancer and, with his colleagues, the cloning of the offending genes  
**W. Marston Linehan, M.D.**  
Chief, Urologic Oncology Branch

# Staff News at CCR



(Photo: M. Spencer)

## Tim Greten, M.D.

Tim Greten joins CCR's Medical Oncology Branch. He received his medical training at Christian Albrechts University, in Kiel, Germany, and did both his internship and residency in Munich and Hannover in that same country. He is board certified in Gastroenterology, Hematology/Oncology, and Internal Medicine. Before coming to CCR, Dr. Greten was a Principal Investigator at the Medical School in Hannover, Germany. His major research interest is the effect of tumor-specific immune responses as well as tumor suppressor mechanisms on tumor development and treatment, while his clinical interests are in hepatobiliary cancer.



(Photo: M. Spencer)

## Ji Luo, Ph.D.

Ji Luo joins CCR's Medical Oncology Branch. He received his Ph.D. and completed his postdoctoral training at Harvard University. Luo's research focuses on using RNAi and other functional genomics approaches to understand the mechanisms of cancer and to identify new cancer therapeutic targets. A major effort of Luo's research will be to identify therapeutic approaches for tumors that are driven by the *Ras* oncogene.



(Photo: M. Welch)

## Philipp Oberdoerffer, Ph.D.

Philipp Oberdoerffer joins CCR's Mouse Cancer Genetics Program. He obtained his Ph.D. in Genetics and Immunology under the supervision of Dr. Klaus Rajewsky at the University of Cologne, Germany. He then joined Dr. David Sinclair's group at Harvard Medical School, first as a National Space Biomedical Research Institute (NSBRI) Investigator and later as a Leukemia and Lymphoma Society Special Fellow. He is interested in studying the molecular link between DNA damage, chromatin, and aging.



(Photo: M. Welch)

## Shalini Oberdoerffer, Ph.D.

Shalini Oberdoerffer joins CCR's Mouse Cancer Genetics Program. Shalini obtained her Ph.D. in Immunology under the supervision of Dr. Jean-Pierre Kinet at Harvard Medical School. She then joined the laboratory of Dr. Anjana Rao at the Immune Disease Institute, Harvard Medical School, where she studied global shifts in alternative pre-mRNA splicing during the process of lymphocyte development. Her research focuses upon the regulation of alternative pre-mRNA splicing in the context of the immune system.



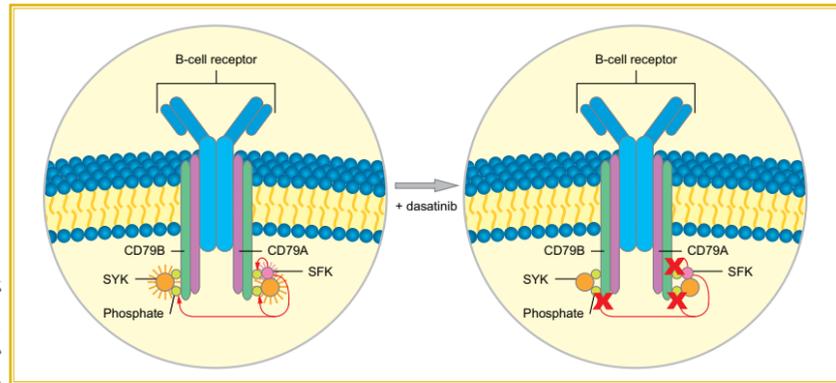
(Photo: R. Baer)

## Joel Schneider, Ph.D.

Joel Schneider joins CCR as Chief of the newly established Chemical Biology Laboratory. Working with Dr. Jeffery Kelly, he received his Ph.D. in Organic Chemistry from Texas A&M University and then went on to the University of Pennsylvania School of Medicine, Department of Biochemistry and Biophysics, where he was a George W. Raiziss Fellow with Dr. William DeGrado studying protein design. In 1999, he began his independent career at the University of Delaware as Assistant Professor of Chemistry and Biochemistry and was promoted to Associate and then Full Professor in 2009 with a secondary appointment in Materials Science and Engineering. He currently serves as Editor-in-Chief of *Biopolymers: Peptide Science*, the journal of the American Peptide Society.

# Sending the Right Signals

Mutations in B-cell receptor signaling pathways identify new molecular targets for the most common type of non-Hodgkin's lymphoma.



(Image: J. Kelly)

Dasatinib inhibits key signaling pathways in the activated B cell-like subtype of diffuse large B-cell lymphomas.

Diffuse large B-cell lymphomas (DLBCL), the most common type of non-Hodgkin's lymphoma, causes about 10,000 deaths every year in the United States, even though about half of all patients are cured with current regimens. There are different subtypes of DLBCL that vary biologically and have significantly different rates of patient survival following chemotherapy, with the activated B cell-like (ABC) subtype being the least responsive to current therapies. So Louis M. Staudt, M.D., Ph.D., Head of the Molecular Biology of Lymphoid Malignancies Section at CCR, and his team set out to find why patients with this subtype have such unfavorable outcomes and how treatment of this disease can be improved.

When a normal B cell recognizes a foreign substance, B-cell receptors (BCR) on the cell surface activate signaling pathways that trigger cell proliferation and survival. Mutations in signaling pathways have been

found in many types of cancer cells, and previous research has suggested that abnormal BCR signaling might contribute to the development of lymphomas. However, there wasn't any direct genetic or functional evidence to support this theory.

In the January 7, 2010 issue of *Nature*, Dr. Staudt and his colleagues reported a mechanism that promotes cell survival for lymphomas of the ABC subtype of DLBCL cells, thus identifying potential new targets for treatment of the disease. The team used a new approach—an Achilles heel screen—in which they used a technique called RNA interference to inactivate genes in ABC DLBCL cells and test their necessity for proliferation and survival. They determined critical points in the BCR signaling pathway that affect the survival of these lymphoma cells and found that interfering with several individual components of this pathway caused lymphoma cells to die. Thus, they came to the conclusion

that ongoing BCR signaling (chronic active signaling) is necessary for cell survival of the ABC DLBCL subtype.

The researchers then looked for mutations in DLBCL tumors in genes that encode these signaling pathway components and found that about 20 percent of ABC subtype tumors had mutations in a BCR signaling component known as CD79B. The mutations increased BCR signaling by blocking a braking process that normally turns off the pathway in response to inhibitory signals. "These mutations we found in the cancer were very juicy, in a way," said Dr. Staudt. "They hit critical amino acids responsible for B-cell receptor signaling, which clearly told us that this receptor was functionally mutated in these lymphomas. That was a genetic smoking gun that the B-cell receptor was important."

This study sets the stage for testing agents that target components of the BCR signaling pathway as new therapeutic strategies for DLBCL. In fact, the researchers have already found that dasatinib, a drug that is approved for the treatment of chronic myelogenous leukemia, could turn off BCR signaling by inhibiting the activity of a protein called BTK, thereby killing ABC subtype DLBCL cells that exhibit chronic active BCR signaling.

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

# Pediatric Tumors Made Personal

A mixed collection of relatively rare but often deadly pediatric tumors are collectively known as small round blue cell tumors (SRBCT) for precisely the reason one might imagine. Examined under a microscope after routine processing, bone marrow biopsies from cancers including neuroblastoma, Ewing sarcoma, rhabdomyosarcoma, and lymphoma appear as small, blue, and round cells. Despite some distinguishing molecular markers to guide them, oncologists can, on occasion, find it hard to diagnose these tumors specifically. Javed Khan, M.D., Head of the Oncogenomics Section of CCR's Pediatric Oncology Branch, has been using genomic approaches to study pediatric cancers for several years. He is now poised to launch an ambitious multicenter project to use comprehensive genomic data to guide the individualized treatment of children with advanced solid tumors.

## Tapping Gene Expression

Khan is a strong believer in the power of genomic information to guide solutions to the riddles of cancer. A pediatric oncologist who trained in Cambridge, England, Khan first came to the NIH on a hematology/oncology fellowship that involved translational research at the National Human Genome Research Institute (NHGRI). Jun Wei, Ph.D., was also at the NHGRI and moved with Khan to CCR when he became Head of the Oncogenomics Section in 2001. At the time, the NHGRI was heavily involved in developing microarray technology to analyze gene expression. "Those were very heady, exciting days," remembered Khan. "Working with

pediatric solid tumors, we were one of the first to use microarrays to find a cancer diagnostic."

In 2001, Khan, Wei, and their colleagues published a paper in *Nature Medicine* in which they demonstrated that relatively small numbers of genes could be used to distinguish four different SRBCTs. In the paper, they used artificial neural networks, a computational technique in which the correct method for finding a solution evolves through a training process. A set of microarray data from identified tumors is used to train the network to recognize patterns in the data that uniquely correspond to each tumor type. Once the network is trained in this way, it

can use the rules it learns to predict new cases.

"The advantage of our method," explained Khan, "is that it allows you to analyze multiple cancers and generate a score that reflects confidence in any particular diagnosis." It is, for example, easily adaptable to a Web site format so that physicians could load microarray or other gene expression data from their own patients to obtain diagnostic information. In fact, Khan and his colleagues have a patent on their method, which a San Diego-based diagnostic company, AltheaDx, is developing into just such a product for pediatric cancers.

## Reading the Whole Genome

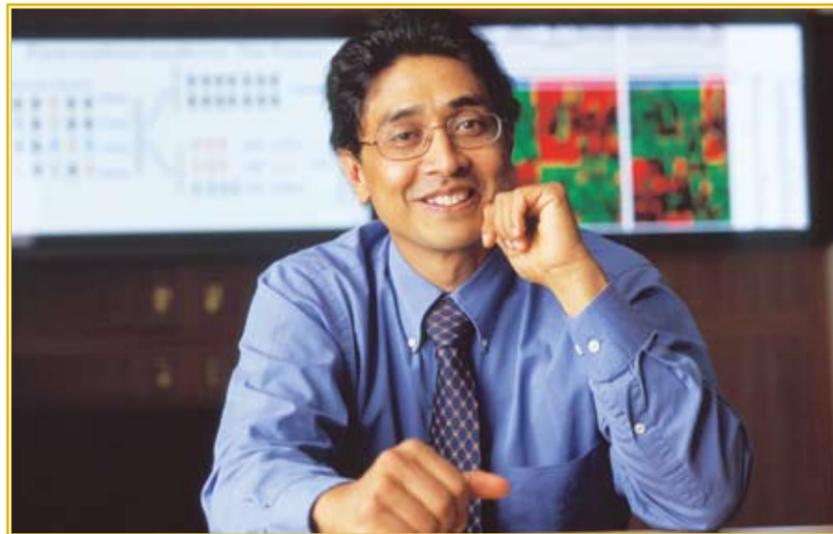
“The end game for me is personalized therapy,” said Khan, “in other words, being able to use genomics to diagnose cancers and to distinguish those who will survive on existing therapies (prognostication). And in the midst of studying all those genetic alterations, find ones that are the key targets for therapeutic intervention in advanced disease.” To search for genetic changes that might be driving these cancers, Khan and his team rely on multiple strategies.

Microarrays measure the expression of genes that are being actively transcribed from only a subset of the entire genome—the transcriptome. These data give you important information about changes that occur during RNA transcription and processing. Although he has firsthand experience with the diagnostic value of gene expression data, when it comes to stratifying disease progression, defining targets, and predicting outcomes, Khan’s first bet is on looking at the DNA directly. DNA sequence information does not tell you which genes are expressed at a given time, but it does tell you directly which genes have been mutated.

“To distinguish one cancer from another, the differences [in gene expression] are quite large,” explained Khan. “But to distinguish survival outcomes for one type of cancer, the differences are often much smaller. So it becomes much more of a challenge to distinguish prognostic signatures using gene expression data.”

The problem with RNA is largely a practical one. The molecules

“The end game for me is personalized therapy”



(Photo: R. Baer)

Javed Khan, M.D.

themselves are simply much more dynamic. “If you take a tumor sample out and you don’t freeze it immediately and then wait an hour, the expression profile can be profoundly altered. Also, tumor cells that are hypoxic at the center of a tumor may have a very different profile from cells in the periphery of the mass. DNA doesn’t change. RNA does.” Khan noted that although there are several published prognostic gene expression signatures for breast cancer or neuroblastoma, for example, there is very little overlap between each of the gene sets for a given cancer. Thus, to validate these signatures for prognostic purposes requires prospective clinical trials in which sample handling and analysis are stringently controlled with standard operating procedures.

As a result of incredible advances in DNA sequencing technology over the last decade, it is no longer impossible to think about sequencing the whole cancer genome of an individual cancer. “Where it’s going is next generation sequencing,” said Khan. “The human genome project sequenced the first human genome in 15 years. Now you can do a whole genome in about a month, which is still too long in terms of using it to make therapy decisions. But, you

can sequence all the protein-coding genes—the exome—within a week.”

With exome sequences in hand, it is still a long and laborious process to identify the mutations that might be critical to tumor growth and survival. The sequence from the tumor must be compared to the patient’s germline DNA and also to published sequence data to find mutations that are specific to the cancer. From one tumor, a hundred functional mutations might emerge and many of these are probably passenger mutations resulting from an unstable genome that are not critical to cancer progression. Comparing mutations across tumors can help to narrow the field, as can analyzing the pathways that might be compromised by individual mutations.

In addition to the transcriptome and the exome and whole genome sequencing, Khan and his colleagues are also interested in applying next generation sequencing to analyzing epigenetic changes in the DNA (methylation) and miRNA profiles. Both have been shown to be important in different models of cancer, and drugs have been developed that specifically impact epigenetic states (e.g., HDAC inhibitors); however, therapeutic strategies to target miRNA changes are still in their infancy.

It is no longer impossible to think about sequencing the whole cancer genome of an individual cancer.

## Hitting the Target

James Taylor, M.D., currently serves as a Staff Physician and Postdoctoral Fellow at the National Heart Lung and Blood Institute (NHLBI). He began collaborating with Khan’s team when he was a CCR Fellow in a laboratory just down the hall. Although his primary research interest these days is in monogenic diseases and sickle cell anemia in particular, as a hematologist, he has seen plenty of SRBCTs.

“When I am on clinical service in hematology, I look at bone marrows all the time and am often called upon to make the diagnosis in the middle of the night.” So, Taylor knows first hand how difficult a diagnosis of rhabdomyosarcoma or neuroblastoma can be when based only on what you can observe under a microscope. “That [*Nature Medicine*] paper was really important from a diagnostic standpoint,” he noted.

But what was of mutual interest to him and Khan and subsequently

became the subject of their collaboration was one gene in particular, among the many that showed altered expression patterns predictive of disease. “One of the big hits in that paper was the discovery of high expression of *FGFR4* in rhabdomyosarcoma.” *FGFR4* codes for a particular receptor subtype of fibroblast growth factor (FGF). When FGF activates its receptors, it activates a molecular signaling cascade within the cell that ultimately stimulates growth. Khan and his colleagues have shown that *FGFR4* is overexpressed in rhabdomyosarcoma and that it is particularly highly expressed in an aggressive subtype called alveolar rhabdomyosarcoma. “So from a clinical standpoint, it made sense that *FGFR4* might be a good diagnostic marker,” noted Taylor. “But what nobody knew is whether this gene did anything [to promote disease].”

Taylor and Adam Cheuk, Ph.D., a Postdoctoral Fellow in Khan’s laboratory, led a study to analyze the

role of *FGFR4* in rhabdomyosarcoma. They sequenced the gene in available tumor samples and discovered that the gene was mutated and that the mutations seemed to cluster at a site on the molecule that was critical to its function as an enzyme. They were then able to show that the mutation actually enhanced the activity of *FGFR4* in cells. “I think that’s the most exciting part of this,” said Taylor. “A lot of genetic studies report mutations, but Javed and his group went into the lab to prove that these were functional mutations.”

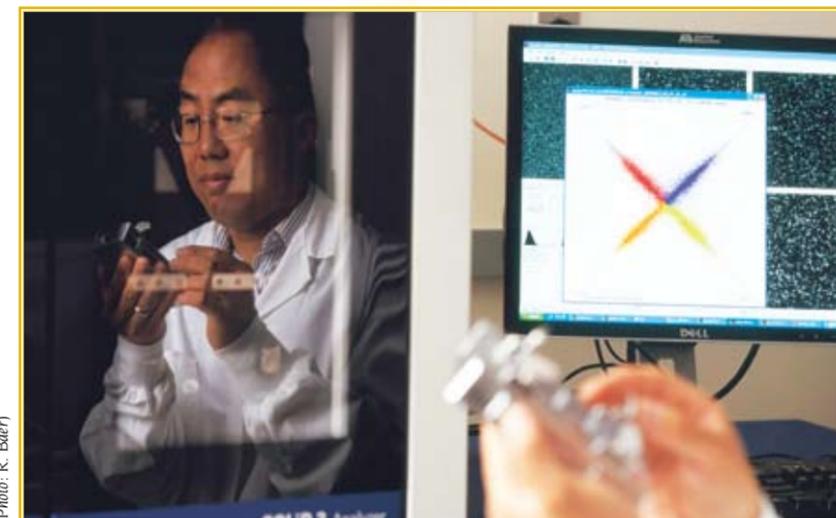
But that is far from the end. The next step for Khan is to bring FGFR inhibitors to patients with these mutations. “There actually is an FGFR inhibitor in Phase 2 clinical trials—the company has contacted us and we are getting hold of the drug. We’re also making a therapeutic antibody against the protein.”

## A Protocol for Personalized Medicine

“That’s the paradigm,” said Khan about the *FGFR4* work. “First, find a gene that seems key to the particular cancer, then find the mutations. Establish that the mutations promote the cancer phenotype by activating the gene to promote growth or metastasis in cellular models. And then find—or make—an inhibitor to administer with chemotherapy.”

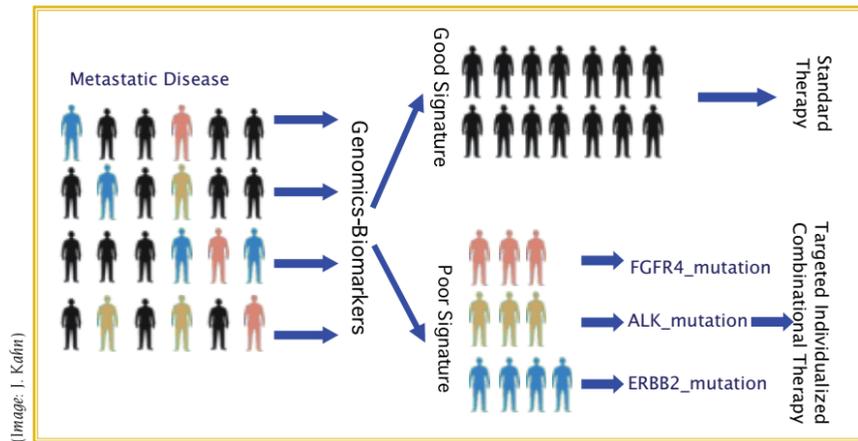
Khan predicts that each gene like *FGFR4* that is discovered for a particular tumor type will only be responsible for a smaller fraction, say 10-20 percent of the cases. “This is where personalized medicine will come in.”

In a multicenter collaboration that includes the Translational Genomics Research Institute (TGen), Helen DeVos Children’s Hospital, and the Vermont Cancer Center, Khan is developing a protocol that will make personalized medicine for pediatric tumors a reality. In the first phase, all admitted patients



(Photo: R. Baer)

Jun Wei, Ph.D.



The goal of personalized medicine is to treat each patient with the best possible therapy.

will have samples taken before initial standard-of-care treatment. If they relapse, the patients will have new biopsies taken. “Often, when they relapse, it’s because the cancer has changed and evolved. The relapsed cancer is not the same cancer they started with,” explained Khan.

The researchers will then do a comprehensive analysis of each cancer genome including gene expression microarrays, look for increased expression of certain proteins, and sequence the exome and the transcriptome to see whether they can identify a molecular therapeutic target. If a particular target is found and there is an ongoing clinical trial that involves an inhibitor of that target, the patient will be enrolled into that trial. Otherwise, the researchers will investigate whether there are any FDA-approved drugs active against the identified target that might be effective.

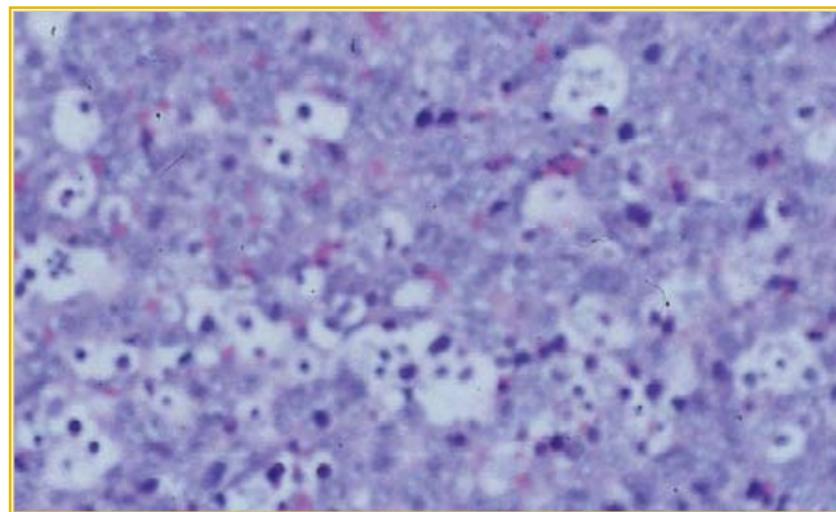
“We know 60-70 percent of these patients with high-stage disease will relapse after standard treatment,” explained Khan. Normally, after relapse, without molecular markers to guide them, choice of clinical trial for advanced disease is something of a shot in the dark. “This is a way of personalizing the choice of clinical trial.”

### The Need for Drugs

Khan is optimistic about the timeframe for developing the individualized analysis of cancer at a molecular

level. “Within the next couple of years, researchers will have catalogued all the mutations. There are groups around the world doing this for all kinds of cancers.” He believes the genomic analysis of individual tumors will be standard practice in clinical trials within five years. Where he is more cautious, however, is in the timeframe for delivering personalized cures. “The biggest problem is that there are only approximately 260 FDA-approved drugs that target a known human protein. So you’re not necessarily going to have the drugs even when you know which mutations to target.”

Khan wonders if federally funded programs to produce anti-gene inhibitors rapidly, using antibody or



Small round blue cell tumors can be difficult to diagnose. This case of rhabdomyosarcoma was originally diagnosed as lymphoma.

“Often, when they relapse, it’s because the cancer has changed and evolved.”

cell-based technologies, might be one answer. Not one to sit idly by while others solve the next problem, Khan is deploying some of his own resources towards developing therapies. He has a small group in his laboratory working on a class of inhibitors called peptide nucleic acids that can bind to DNA or RNA and stop transcription or translation. He also has a postdoctoral fellow working on aptamers—molecules that may be able to target specific markers on cancer cells and deliver chemotherapeutic agents directly to them.

“Developing those inhibitors for known mutations—that’s going to be on my 10-year plan.”

To learn more about Dr. Khan’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=khan>.

(Image: J. Kahn)

# The Art of Silence

*The transition from biology to technology is never as easy as it sounds, and taking technology out of the hands of artisans for widespread use adds another level of difficulty. Natasha Caplen, Ph.D., Head of the Gene Silencing Section in CCR’s Genetics Branch, first recognized the gap between understanding in principle and implementing in practice as a Postdoctoral Fellow working on gene therapy for cystic fibrosis. One of the first disease genes cloned, cystic fibrosis genes still elude therapeutic attempts to replace them with functional copies. While working at the National Human Genome Research Institute on gene delivery methods, Caplen was first exposed to RNA interference—RNAi—and was among the first to generate the phenomenon in mammalian cells. Less than 10 years later, RNAi is a much valued technique for studying gene function, but its nuanced execution still demands an artisanal approach.*

“We’ve all been stunned by how RNAi is being adapted.”

“In March 2001, when I first observed the effect of RNAi in mammalian cells on the 10<sup>th</sup> floor of Building 10, I ran around the lab saying ‘They aren’t green any more!’” Caplen often tells this story in seminars of how she first silenced a reporter gene that encoded green fluorescence protein (GFP). “For 18 months, I looked at very dead mammalian cells because if you put double-stranded RNA into cells, they trigger immune responses and die. But I thought there had to be a way.”

RNAi is a gene silencing mechanism that was first identified in nematode worms where it operates to modulate gene expression. Double-stranded RNA (dsRNA) is processed

in cells to form small interfering RNAs (siRNAs) that contain 21-23 nucleotides. These siRNAs direct the breakdown of gene transcripts that contain complementary sequences and thereby silence gene expression. Although dsRNA provokes mammalian immune responses, Caplen and her colleagues discovered that siRNAs can be used directly as an experimental tool in mammalian cells.

“I don’t think anyone at the time could have really realized what we were letting loose into the world,” said Caplen. “We’ve all been stunned by how RNAi is being adapted—the imaginative ways people have used this basic mechanism and the implications that it has.”

In fact, three years later, in 2004, Caplen was recruited to CCR because it had rapidly become clear that no large cancer research center could be without expertise in RNAi. Since then, she has embarked on several collaborations with investigators studying diverse models of cancer who want to use RNAi technologies.

### Any Individual Gene

Thomas Ried, M.D., Head of the Cancer Genomics Section, has been studying colorectal cancer (CRC) for several years with the goal of understanding changes that happen early in the transformation from normal epithelium through dysplasia or polyps into full blown carcinoma. “Our interest is to identify the dynamics of genomic and transcriptomic changes that occur in early tumorigenesis and are responsible for the change from a benign dysplasia to a carcinoma.”

“We have done a lot of thinking as to what it means to silence a single cancer gene.”

Over the course of their studies, Ried and his colleagues have uncovered several genes that are consistently upregulated in CRC tumors and cell lines. “It is very reasonable to believe that they are necessary for growth and viability of these cells,” said Ried. “And of course, if you identify genes that are exclusively expressed in cancer but not normal colon, you can assume that they are viable targets.” But, reasonable hypotheses and assumptions are not proof. RNAi seemed like one obvious strategy to silence these genes and establish their roles in supporting CRC.

“We have done a lot of thinking as to what it means to silence a single cancer gene,” said Caplen. When an investigator comes to



(Photo: R. Baer)

Natasha Caplen, Ph.D.

her with a project, she wants to be sure that she can match the best technologies with the best assays and analysis tools. In terms of RNAi resources, this means analyzing the architecture of the transcripts produced by any one gene as well as the potential for unwanted interactions with other nucleic acid elements. John Weinstein, M.D., Ph.D., former Head of the Genomics and Bioinformatics Group at CCR, and Mike Ryan, Ph.D., worked with Caplen to create Web sites that help determine how well a siRNA sequence will line up with and thereby silence different gene isoforms and, more recently, identify potential off-target interactions of siRNAs. “It’s those kinds of tricks that can help you identify sources of any inconsistency in your data.”

Although there are newer and fancier methods for building RNA molecules to silence genes that the lab keeps abreast of, synthetic siRNAs serve Caplen and her colleagues well for most applications. “Building experience and tools to interpret

the data and using the right assay has been more of our focus than building more RNAi resources per se,” said Caplen.

Because Caplen works predominantly in cell lines that model cancers, she also wants to make sure that the model is as faithful a reflection of the tumor biology as possible. “These experiments can get very big very quickly, so you really want to know that what you have is modeling the question well,” explained Caplen. She often asks her collaborators for additional characterization of the cell lines they are using.

“The fidelity of cancer cell line models has been a concern for many years,” said Ried. “However, we have found that both the genomic aberration profile, which is very specific for certain tumors, and the transcriptome profile of our cell lines match the tumors very well. So my confidence has actually grown for using tumor cell lines.”

An exciting outcome of silencing experiments is to look at several snapshots in time of changes in gene expression across the entire

molecular network as a result of a single inactivation event induced by RNAi. “If you perturb gene X and look at a whole transcriptome level at what happens 10 hours later, 24 hours later, etc.,...now you can start to use systems biology to model the pathways that are really being affected over time in a way that needs no assumptions about the role of the gene you silenced.

“That gives you a lot of information,” said Ried. “The challenge will be doing the right bioinformatic analysis—nobody knows exactly what that is. But this approach should allow us to identify the functional space in which a single gene operates. The gene expression signature of the knockdown also might tell us whether inhibiting a combination of genes might give

an additive effect rather than just targeting the same pathway.”

### Many, Many Genes

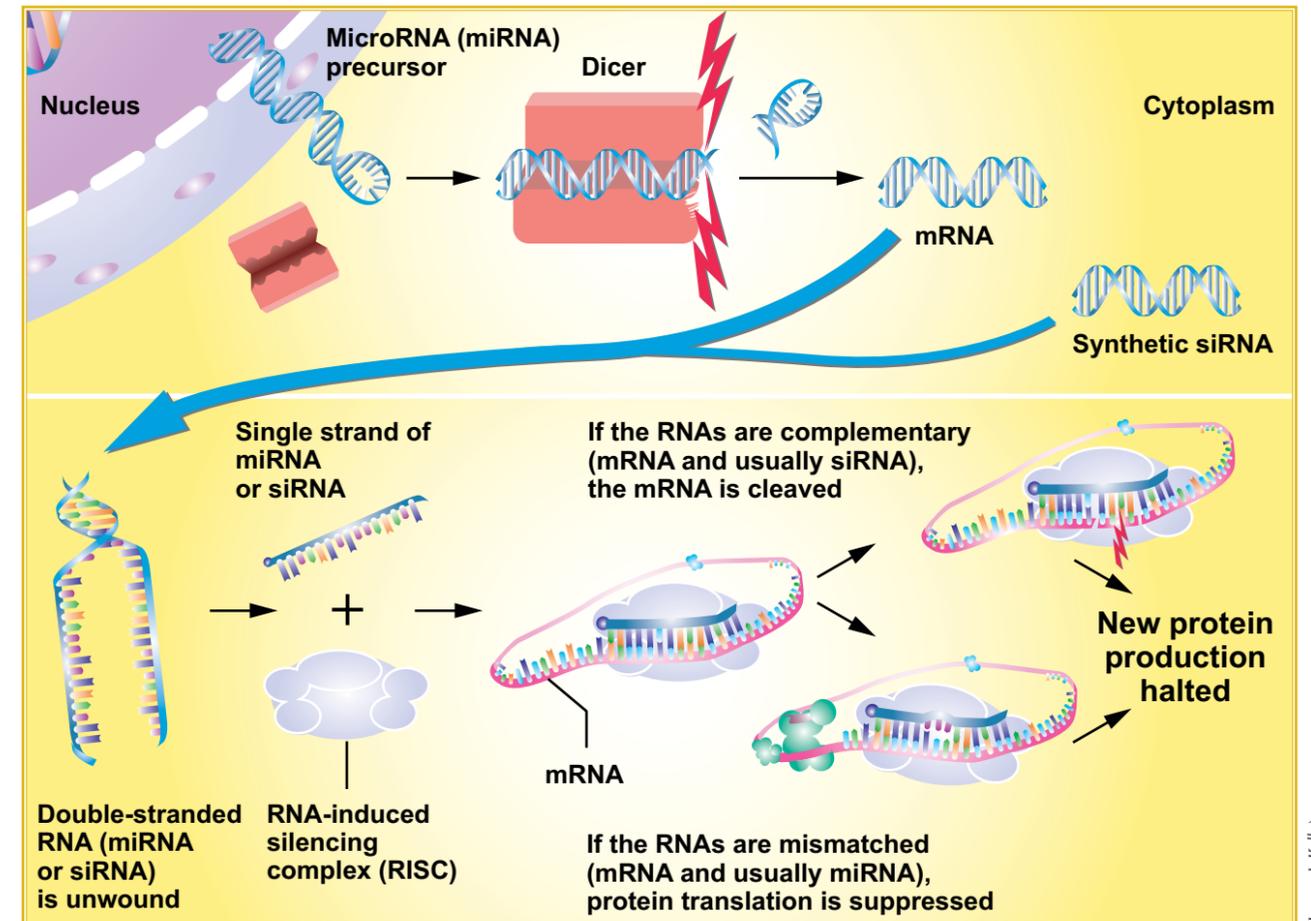
Anita Tandle, Ph.D., Staff Scientist in the Radiation Oncology Branch under the direction of Kevin Camphausen, M.D., is also trying to find gene targets in cancer cells, but she is looking for genes that might sensitize glioblastoma stem cells to the effects of radiation.

“Most of these gliomas are managed by surgery and radiation, but the median survival can be counted in months. We want to see if we can identify proteins or genes that make these tumors more sensitive to radiation.”

The Camphausen group, in collaboration with Philip Tofilon, Ph.D., at the Moffitt Cancer Center,

has developed a glioblastoma stem cell line. “The proportion of cancer stem cells is very small in a tumor, but they are responsible for resistance that develops to radiation and chemotherapy,” explained Tandle. She and her colleagues want to identify genes that confer such unique viability on these specialized cells and ultimately discover ways in which to make them vulnerable to radiation.

“We have relatively large libraries of siRNAs, in which you run your assay in 384 well plates just like drug screens,” explained Caplen. “We can run one screen a week in which we ask a specific question.” For example, which genes affect the growth of a glioblastoma stem cell? To answer this question, the researchers use four siRNAs per gene that they silence and the readout is either cell



(Image: J. Kelly)

To induce gene-specific inhibition of protein production, synthetic small interfering RNAs (siRNAs) exploit aspects of the naturally occurring RNAi mechanism that includes the control of gene expression by microRNAs (miRNAs).



(Photo: R. Baer)

Kristen Gehlhaus, M.H.S.

proliferation or cell death. Kristen Gehlhaus, M.H.S., a Biologist in Caplen's lab, has been handling the RNAi screen for this project.

Unlike other cancer cell lines, glioblastoma stem cells grow as neurospheres and are actually quite tricky to work with. "They don't grow as monolayers, plus they are much slower growing than other tumor cell lines," explained Tandle.

"This is a great example of true team science," noted Caplen. "We bring the RNAi expertise and each collaborator brings their particular cancer biology expertise."

Caplen's group has used the same siRNA libraries to look at growth of breast cancer cell lines for another collaboration. Each collaborator has been focused on his own model of cancer, but Caplen sees a third angle of investigation that her lab will pursue independently—the differences in screening data across cell lines. "We're going to run with those differences," said Caplen. "In the same experiment, there is the potential to look at basic mechanisms for how a gene promotes the tumorigenic process as well as looking at pathways that

you can target therapeutically. It is a lot of work following up on either of those results, but at least you have a rich source of leads."

### Drugs, Too

Ultimately, Tandle and Gehlhaus will run their screens to look for genes that confer survival on glioblastoma stem cells after they have been irradiated. Caplen's group has been using RNAi screens with increasing frequency to study the genes that modulate the response to specific interventions. Most often, these interventions are drugs.

Stanley Lipkowitz, Ph.D., Senior Investigator in the Laboratory of Cellular and Molecular Biology, is interested in a family of receptors that trigger cell death: the tumor necrosis factor (TNF) family. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of that family that is currently in clinical trials as a chemotherapeutic agent. It turns out that most breast cancers are not responsive to TRAIL, but Lipkowitz and his colleagues have shown that a subset of breast cancer cells—the so-called triple negative tumors—are sensitive to

TRAIL. "We don't know why, despite having studied several candidate genes," said Lipkowitz. So, they decided to try an RNAi screen, looking at roughly 1000 genes.

In addition to RNAi they gave cells TRAIL at  $IC_{50}$ , i.e., the concentration at which exactly half of the cells would die. The screen will give them information about genes that either makes these cells more resistant or more sensitive to TRAIL. The screen is now complete and for Lipkowitz, "the hard part begins."

"The drug studies have worked out very well," said Caplen. "We have had one published and a number of others that are coming out." In collaboration with Yves Pommier, M.D., Ph.D., Caplen's team has identified genes that affect the mechanism of action for camptothecins, a venerable class of anti-cancer drugs. And they have just begun a collaboration with Beverly Mock, Ph.D., trying to study how two different pathway inhibitors interact in cancer cells to produce synergistic effects on cell death.

### Finding the Balance

RNAi, of course, is much more than just a tool for manipulating experiments. It is also a fascinating field of biology that has been transformed in the last decade. RNA molecules are involved in directing cellular activity at a number of levels and, in cancer research, the hottest new members are microRNAs (miRNA), very short molecules that modulate whole networks of genes. "The great unknown is how to find efficient and sustaining ways of linking miRNAs with their dominant targets in specific settings." When they are not honing their RNAi technologies, Caplen and her team are busy investigating the secrets of RNAi biology. The challenge for Caplen is in creating the right mix of projects in an environment whose structure is different from a traditional laboratory. During

a recent site visit, one outside examiner said of her laboratory, "It would be almost impossible to replicate anywhere else."

Caplen is justifiably proud of her unique niche and the important role it plays in developing not just the biology and technology of small RNA molecules, but also the next generation of team scientists.

"The first thing I tell every post-doc joining the lab is to make sure they are aware that this is a team science environment," said Caplen. "There is a continual balance between their own projects and working with other groups."

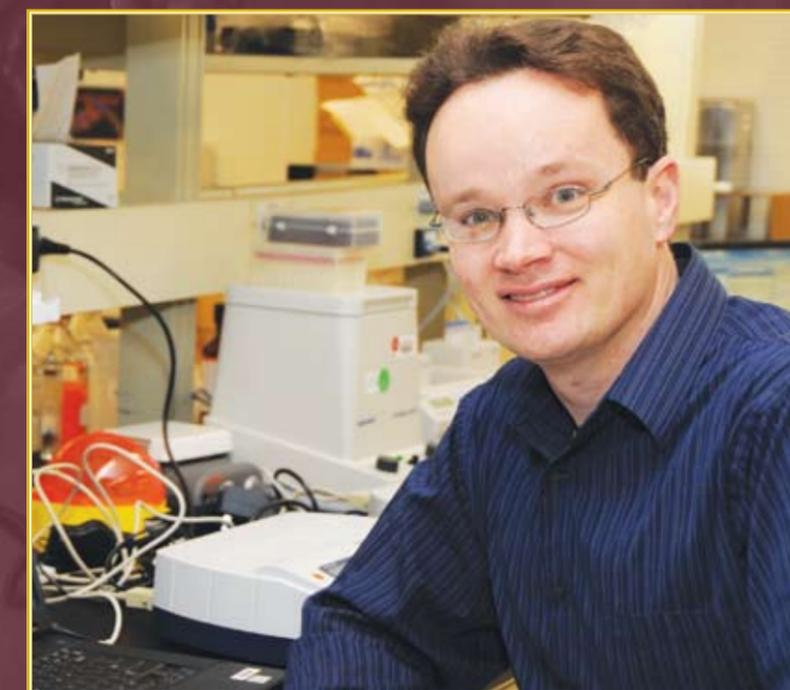
Scott Martin, Ph.D., came to the Caplen laboratory after his doctoral work developing small molecule probes for biological systems. "I became more interested in biology and decided to immerse myself in cancer biology." He found the Caplen laboratory an ideal interface between his prior training and newfound interests. "RNAi made sense as an extension of my work developing reagents to alter molecular activity in cells."

Martin found a great deal of freedom in the lab. "You can pursue whatever you want as long as it is RNAi related. I was interested in drug sensitization; other people were off investigating miRNA biology." Although he engaged in many rewarding projects during his four years in the lab, he is most proud of discovering genes that sensitize breast cancer cells to camptothecins.

Martin has recently joined the NIH Chemical Genomics Center (NCGC) to lead a new RNAi initiative (see "NIH Chemical Genomics Center Takes in RNAi"). "So, this is sort of full circle for me. I thought I was a jack of all trades kind of person and it would be difficult to find a perfect fit, but there I found it."

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

# NIH Chemical Genomics Center Takes in RNAi



(Photo: R. Baer)

Former CCR Fellow Scott Martin, Ph.D., now leads the RNAi screening initiative at NCGC.

A new RNAi screening facility based in the NIH Chemical Genomics Center will serve intramural investigators across the whole of NIH. "The goal is to provide access to genome-wide RNAi screening, primarily in human cells," explained Scott Martin, Ph.D., who is leading the project.

"We envision this as being a highly collaborative type of process, starting with discussions with investigators to help them get an assay that addresses the biology that they are interested in," said Martin. The project would then have to be approved by a trans-NIH committee, headed by Natasha Caplen, Ph.D.

Once approved, the Center will actually perform the screening and provide real-time feedback. At the end of the project, the investigator will be provided with a list of hits and all the data associated with activities in the screen.

So far, the infrastructure is in place and pilot studies are ongoing. "It was a huge advantage to build this on top of the physical and informatics infrastructure that the NCGC has in place to do large chemical compound screens," noted Martin. They hope to be fully operational by 2011.

# Therapeutic Synergies in the Fight Against Cancer

*Surgery, radiation, and chemotherapy are the mainstays of oncology, composing most of the first-line standard of care for virtually all cancers. As newer strategies are introduced into the therapeutic arsenal, particularly for earlier stages of disease, they are almost always tested in addition to, rather than instead of, the standard of care. Not only are these newer strategies proving effective in combination with the older methods, but surprisingly strong synergies are emerging among them. Several CCR investigators are finding ways to exploit these synergies for the benefit of patients.*

## Vaccine Trials and Tribulations

"Cancer vaccines have had a hard time over the last several years," remarked James Hodge, Ph.D., Senior Scientist in the Laboratory of Tumor Immunology and Biology. Hodge described a series of failed Phase 3 clinical trials for cancer vaccines as monotherapies against late-stage disease that forced companies like Cell Genesys and Therion Biologics out of business. One reason these trials failed, Hodge believes, is that cancer vaccines are probably least effective as a last resort against late-stage disease. Studies have shown that the immune response is

blunted in patients that have recently undergone chemotherapy or have had several courses of chemotherapy prior to receiving a vaccine.

As the safety of cancer vaccines has been established over time, however, clinical researchers have been able to administer vaccines to patients closer to the time of diagnosis. In such patients, where a clear standard of care exists, the natural strategy is to test a new therapy in combination with existing care. But first, the safety of any novel combination must be established in preclinical models.

"Combining radiation and vaccine therapy in our preclinical models was a real eye-opener," said Hodge.

He and his colleagues designed an experiment in which the vaccine was essentially set up to fail on its own—they injected tumor cells into a mouse engineered to express the human tumor antigens targeted by the vaccine and then withheld the treatment until eight days later. As expected, neither vaccine alone nor radiation therapy alone was sufficient to reduce the large tumor burden achieved in the meantime. However, in those mice given a combination of radiation and vaccine, 60 percent were cured outright. "That's something we hardly ever see in preclinical models," said Hodge. "And it was all we needed to move forward into the clinic."

## Radiation and Immunotherapy—The Theory

Radiation works by producing cellular damage that evokes programmed cell death. "The oxidizing radicals damage cancer cells, but also the normal tissues as well," explained Aradhana Kaushal, M.D., Staff Clinician in the Radiation Oncology Branch. "The idea is that the normal cells can repair themselves, but of course they are affected by radiation too, which is why we see side effects." Radiation oncologists work on trying to limit the effects of radiation to cancer cells, whether by physically constricting the beam of radiation to focus on a tumor mass or by co-administering compounds that will either enhance the vulnerability of tumors or reduce the vulnerability of healthy cells (see "Radiating Change," *CCR connections* 3(1)).

Cancer vaccines work by training the immune system to recognize and destroy cancer cells. Cancer cells have distinct molecular markers—antigens—that, when processed by specialized antigen-presenting cells (APCs), help the immune system to recognize and target the cells bearing them for destruction by cytotoxic T cells. APCs are drawn to diseased or damaged cells because the cells give off stress signals that let the system know that they need to be cleared away. It makes sense, therefore, that levels of radiation that may not be sufficient to kill cancer cells outright might be sufficient to boost signs of stress and disease that the immune system can recognize.

James Gulley, M.D., Ph.D., Head of the Clinical Trials Group in the Laboratory of Tumor Immunology and Biology, is enthusiastic about a trial he is currently running to combine vaccine therapy with a form of radiation therapy in patients with metastatic prostate cancer who have run out of proven therapeutic options. The radiation, in this case, is coming not from an external beam but from a compound, samarium-153, that contains a short-lived isotope.



James Hodge, Ph.D.

(Photo: R. Baer)

Samarium-153 is not very effective in killing tumor cells. Instead, it is given to patients to ease the pain of metastatic bone cancer. The drug accumulates in areas of the bone that contain cancer and provides pain relief. Hodge has done the preclinical studies, demonstrating that the radiation delivered by samarium-153 has a similar immune-boosting effect as beam radiation in a cell culture model. Gulley is taking the work into the clinic, comparing tumor progression in patients who receive samarium-153 with or without their prostate cancer vaccine. "I am really quite intrigued by the results so far," Gulley said, cautioning however that the data are not yet mature for his randomized Phase 2 trial.

## Timing Is Everything

Chemotherapy is a blunt instrument, killing any cell that is dividing rapidly, including cells of the immune system. And unlike radiation, it

Chemotherapy and immunotherapy can also have synergistic effects.

is seldom focused on a particular tumor or organ and thus usually also disrupts the immune system. But Gulley and Hodge are finding that, if delivered correctly, chemotherapy and immunotherapy can also have synergistic effects.

A strong hint on the importance of timing in vaccine trials came from the results of a human trial in which patients with prostate cancer that did not respond to hormone therapy were initially given either of two treatments—an experimental prostate cancer vaccine or an FDA-approved androgen receptor

antagonist, nilutamide. Untreated, these patients would likely develop metastases within a year. Neither treatment improved the odds much: patients who were randomized to receive the vaccine alone progressed similarly to those who received the nilutamide. “However, the interesting thing was that after six months, patients who had rising PSA [prostate-specific antigen] levels but no metastases visible on scans could add in the other treatment,” explained Gulley. Surprisingly, the patients that started out on vaccine first and then added nilutamide after six months had much slower disease progression and actually lived longer.

“It seems like the patients that start out with vaccines first do better on a subsequent therapy, said Gulley. “We have seen many anecdotal reports and retrospective subset analyses that support this finding.” The group also performed a trial in which patients with prostate cancer received vaccine alone or with the standard-of-care chemotherapeutic agent docetaxel. Both patient groups had similar results (the disease progressed after three months), but for patients who received vaccine alone and then were switched to the combination after initial assessment, progression on chemotherapy was delayed another three months.

“I attribute it to the fact that you are generating an immune response that can be around for a long time. Most of the time, you think about treatment as only effective around the time of administration, but vaccines can exert continued effects on growth for much longer,” said Gulley.

Jeffrey Schlom, Ph.D., Chief of the Laboratory of Tumor Immunology and Biology, pointed out that several factors may be involved in early vaccination. “If you get the vaccine on first, the immune system may be responding with circulating T cells, but they may not be strong enough to kill on their own. If you give radiation

## “Maybe you do better if you get the vaccine first.”

or chemotherapy, it could act as a boost or change the cancer cells to make them more susceptible.” The team is getting ready to open a large randomized cooperative group trial to specifically compare the effects of docetaxel given alone with the effects of docetaxel given after a series of vaccinations. “This will be the first prospective study evaluating the concept that maybe you do better if you get the vaccine first.”

### Don't Forget the Blood Supply

Vaccines, of course, are not the only rising stars in the world of anti-cancer strategies. Ever since the pioneering work of Judah Folkman, M.D., the tumor blood supply has been an important target for cancer research. William Dahut, M.D., Clinical Director of CCR, has studied angiogenesis drugs in prostate cancer for many years.

“We started with thalidomide, which probably has some anti-angiogenesis properties,” remembered Dahut. “We showed in a small randomized trial that if we added thalidomide to docetaxel, it improved survival over docetaxel alone.” When bevacizumab (Avastin) came along, his group combined it with thalidomide as an addition to the standard of care in a single-arm Phase 2 study. “We had probably the highest response rate of any trial in that population. In 90 percent of the patients, PSA levels fell by 50 percent and the time to cancer progression was about 18 months, which was pretty much equal to the overall survival time historically.” The team has since replaced thalidomide with a related drug, lenalidomide, which has a better side effect profile, and are conducting additional trials. “We have treated about 11 people so far and I think virtually everyone has responded.”



Jeffrey Schlom, Ph.D., and James Gulley, M.D., Ph.D.

(Photo: R. Baer)

“Anti-angiogenesis—I’m not even sure what that means,” noted Dahut. “You don’t necessarily see blood vessels disappear. Usually, they are called that because they interfere with things like VEGF, which are shown to be involved in angiogenesis. But it is less clear that’s why these drugs have activity. They could be improving drug permeability, for example.” Regardless, the consensus seems to be that, in most cases, angiogenesis inhibitors work best in combination with other agents. Although initially studied on their own, they were mostly found to have minimal activity in solid tumors, with kidney cancer being the notable exception.

### One Big Happy Family?

Vaccines may soon join angiogenesis inhibitors in commercial triumph. Hodge, Gulley, Dahut, and their colleagues are optimistic because of the recent launch of a revolutionary prostate cancer vaccine, custom-made for each individual patient. Dendreon is the first company to receive FDA approval for a cancer vaccine. Their vaccine—Provenge—also targets prostate cancer, but is designed against a different antigen associated with the cancer. “It will open the floodgates,” predicted Hodge, who is keen to try their own vaccine in combination with Provenge.

“Combinations are where we’ve seen our strongest clinical effects,” noted Hodge, even though the mechanisms may not always be completely clear. In both preclinical and clinical observations, the team has noted, for instance, that their vaccine—which is designed to elicit an immune response to a particular antigen found on prostate cancer cells—in combination with radiation elicits a much broader immune response (i.e., to multiple antigens) than expected. “That was the tumor itself educating the immune system about which antigens are most important.” This antigen cascade is



William Dahut, M.D., confers with a patient.

(Photo: R. Baer)

not only a tool to help the researchers discover better antigen targets, but also may allow the immune system to recognize heterogeneous tumors and distal metastases on its own.

“We look at vaccines as part of an immunologic platform, which involves using other immune stimulators in combination,” concluded Schlom. “But we also look at this as a program in immuno-oncology where vaccines are integrated with standard oncology or new oncology drugs.”

Recently, Hodge and his colleagues were invited by the journal *Molecular Biosystems* to write an article in which they speculated about the potential synergy between immunotherapy, radiation, and angiogenesis inhibitors. “We haven’t tried that combination,” explained Hodge. “But bevacizumab is very quickly working its way into the standard of care—for instance, in colorectal cancer.” So it is worth exploring how all the

players might work together. The responses they have received from the community to the article have been gratifyingly positive.

To learn more about Dr. Hodge’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=hodge>.

To learn more about Dr. Gulley’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=gulley>.

To learn more about Dr. Schlom’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=schlom>.

To learn more about Dr. Dahut’s research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=dahut>.

# The Culture of Research

*Samuel Broder, M.D., joined the NCI's Metabolism Branch (now part of CCR) in 1972, became Head of the Clinical Oncology Program in 1980, and was appointed NCI Director by President Ronald Reagan in 1989. He subsequently moved from government research into the private sector. Broder is currently Vice President and Chief Medical Officer at Celera, a company that first came to prominent public attention for its part in the race to sequence the human genome, and which now has a strong focus in personalized medicine. Twenty-five years after his pivotal contributions to the discovery of the first HIV-1 antiretrovirals, Broder reflects on the impact of his early discoveries and the research environment within the NCI that made them possible.*

Twenty-five years ago, in collaboration with industry, my team at NCI developed the first series of drugs that were active against HIV-1. We were a small group, but we had seen the lethal and terrifying effects of AIDS on patients and we wanted to do something to provide tangible and immediate relief.

I was fortunate to work with brilliant colleagues, including Hiroaki "Mitch" Mitsuya and Bob Yarchoan. The NCI also had a strong investment in basic science that proved of central importance to HIV pathology. At the time, NCI had a constant and almost unique commitment to search for viral

causation of cancer and to discover retroviruses in particular. NCI also had a longstanding research interest in the relationship between immunodeficiency diseases and cancer.

And yet, as important as the people and the resources are that make up a research organization, I believe its success also primarily lies in its culture. In addition to its scientific focus, the intramural program's research culture itself—one that encouraged taking intellectual risks to advance the forefront of knowledge, one that encouraged a strong relationship between

bench and clinical work, and one that encouraged interaction with industry—was key to our success.

## Pushing the Limits

In 1962, Arthur C. Clarke wrote in an essay titled "Hazards of Prophecy: The Failure of Imagination," that the only way of discovering the limits of the possible is to venture a little way past them into the impossible. That boundary, of course, is constantly shifting with advances in technology and insight. But, it is very important to have a research culture that allows you to cross that boundary in attempts to push the outer limits of the possible, without misconstruing it as a kind of failure. If pushing limits is not encouraged, then you will end up with people devising very conservative, tried-and-true research agendas, and it will be very difficult to shift the therapeutic paradigm for difficult-to-treat, lethal diseases.

Today, AIDS is a chronic manageable disease, with over 30 FDA-approved drugs available for its treatment. The death rate due to HIV has plummeted since the mid-1990s. At the same time, we still don't have a way of eradicating the virus from the body.

Many people said, at the time that we were starting our research, that we didn't know enough, that the basic

science had not advanced enough for us to consider developing treatments. But, if we had waited for the technology to mature to the point that we could cure the disease before we took what we knew into the clinic, then we would still be waiting.

To some degree, the real value of the NCI intramural programs should be to push that envelope between what is possible and what is not possible. And to try to do things that other institutions just can't do for many reasons, particularly in crossing the laboratory-to-clinic divide.

## Wholeness of Motion

In my opinion, one of the most important features of the NCI intramural program is the tradition of locating research laboratories both physically and intellectually next to clinical wards. This juxtaposition strongly encourages physician-investigators to translate knowledge from their own research laboratories into clinical wards and back to the laboratory, without an intermediary hand-off to another group. It means that all the expertise required to solve a problem remains with that problem from start to finish. NCI has seen this model pay off over and over again.

We discovered a set of drugs, dideoxynucleosides—one of which was AZT—that showed activity against widely divergent HIV-1 isolates in tissue culture. They inhibited viral replication at much lower concentrations than necessary to cause toxic effects on target T cells. We also had a likely mechanism of action (inhibition of reverse transcriptase) and an understandable, intuitively obvious relationship between the structure and activity of these compounds, which proved important to the further development of this class of drugs.

Without reliable animal models of the disease at the time, we essentially moved from tissue culture to patients, enrolling the first AIDS patients at the NCI. Ironically, we saw positive effects in the very first study that we did. The effects sometimes didn't last because,

as we now know, no single agent could reliably work against HIV for long periods. But we laid the groundwork and illuminated a path that other agents could follow. Against prevailing wisdom, we proved that it was possible to treat pathogenic retroviruses like HIV-1 with antiretroviral therapy.

When I later became Director of the NCI, I was committed to facilitating translational medicine while recognizing that it is not possible to simply replicate the intramural program in other places. Under my tenure, the specialized programs of research excellence (SPORE) were founded, and I think they had a tremendous impact because they allowed people to form interdisciplinary research programs, including collaborations with industry, under coordinated leadership. One of my "secret" plans was to force people in institutions to work together and have that ability to move from lab to clinic even if they weren't originally focused that way. People would tell me that the mere act of putting SPORE applications together did that, even if they weren't funded.

We are moving into an era where there is an increasing separation of people doing basic research and people doing translational research, an era in which we lack the wholeness of motion that takes you from bench to bedside. This separation may create efficiencies

of specialization, but I believe it comes with a cost hidden in the transition.

## The Private Sector

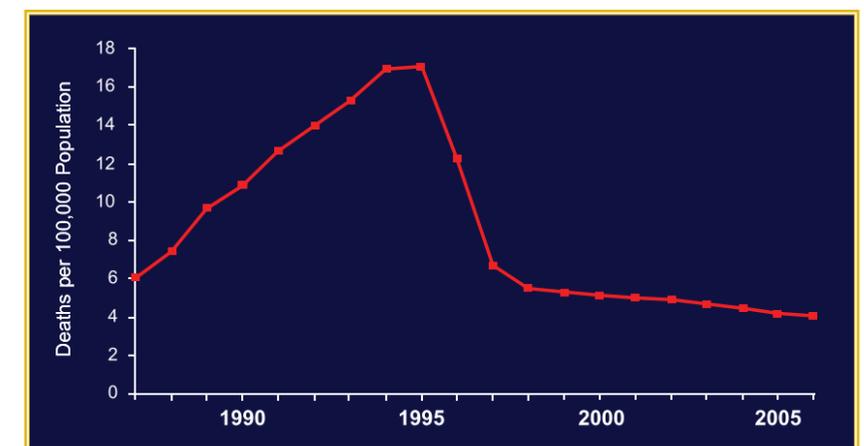
The other thing that NCI did much better than most other government research institutes at the time we were working on HIV was to encourage collaboration with industry. This was quite critical to our work and happened at a time before the Federal Technology Transfer Act of 1986 created a means to facilitate collaboration between government researchers and industry. Early on in our HIV research, I realized we would need partners in the private sector to develop drugs rapidly, and we were lucky enough to have a connection with scientists at Burroughs Wellcome who were keen to work with us.

Of course, I have since made my own transition from government into the private sector, and I have learned a great deal from both sides. I would like to see more cross-pollination between government and the private sector, and not just in the form of standard collaborations. I think we need to do more to encourage people from academic centers and biotechnology companies to take positions of relatively high rank in the NCI and then go back to the private sector again. The flow of new ideas and brainpower would be a benefit to all.



(Photo: S. Broder)

President Ronald Reagan visits the Broder laboratory at NCI.



(Image: S. Broder)

Trends in annual age-adjusted rate of death due to HIV disease in the United States, 1987-2006. (Figure adapted from the Centers for Disease Control: <http://www.cdc.gov/hiv/topics/surveillance/resources/slides/mortality/>).

# Multiple Approaches to Myeloma

A native of Sweden, Ola Landgren, M.D., Ph.D., trained as a hematologist at the Karolinska Institute in Stockholm, where he also took advantage of the strong medical database resources in Scandinavia to study cancer at a population level. Initially intending to spend only two years at NCI, Landgren opted to remain as an Investigator in the Medical Oncology Branch of CCR because of the unique opportunities to take his observational findings back into the clinic and address his longstanding interest in multiple myeloma. His recent finding that multiple myeloma is consistently preceded by an asymptomatic precursor state—known by the acronym MGUS—has opened a unique window of opportunity for studying the progression of this fatal disease.

Multiple myeloma is a cancer that affects plasma cells in the bone marrow. These cells normally play a critical role in adaptive immunity by producing the antibodies that target infection and disease. In multiple myeloma, genetically aberrant plasma cells proliferate and produce excess antibody or antibody fragments, which show up clinically as M proteins (monoclonal gamma-globulins) in blood and sometimes urine.

In otherwise healthy individuals, normal plasma cells constitute less than five percent of the cells in healthy bone marrow. However, in multiple myeloma patients, abnormal plasma cells typically account for 10 percent or more of all cells. These cells can also circulate in the bloodstream and accumulate in bone marrow at sites far removed from the original source of the aberrant cells. This abnormal accumulation eventually results in damage to the bones and surrounding tissue, and the term

“multiple myeloma” comes from the scattered bone lesions that are observed in later stages of the disease. Resulting damage eventually includes kidney failure, recurrent infections, abnormally high calcium levels in the blood, and anemia. At this time, it remains incurable.

## Catching it Early

In cancer, early diagnosis is quite often the difference between life and death. Catching cancer before it starts, of course, is the best possible situation. However, in most cases, by the time patients come to our attention clinically, the cancer is well rooted in the body.

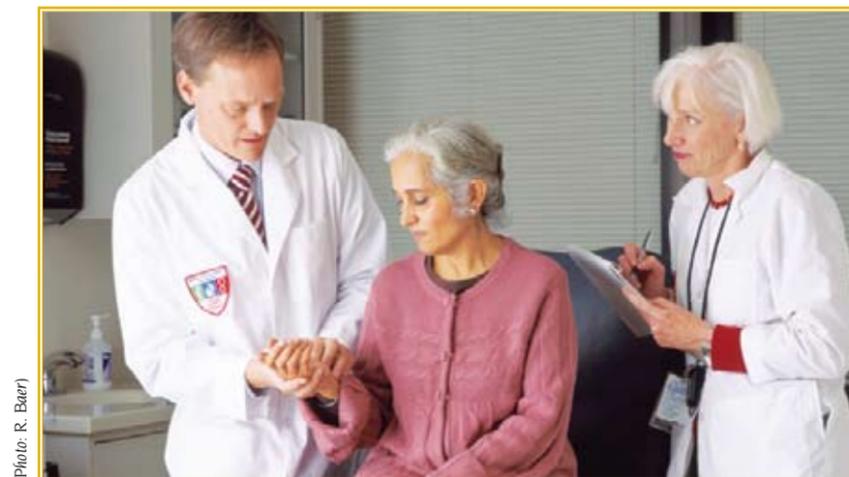
In the case of multiple myeloma, we know that there is a related condition called monoclonal gammopathy of unknown significance (MGUS). The name comes from the M proteins that are found in the serum in the absence of any disease pathology. In fact, MGUS is present in approximately three percent

In cancer, early diagnosis is quite often the difference between life and death.

of the general population above the age of 50. There are no symptoms associated with MGUS—it is usually diagnosed when abnormal M-protein levels turn up during diagnostic tests performed for other reasons (see “The Doctor-Patient Relationship,” page 32). We also know that for people with MGUS, the risk of developing multiple myeloma is significantly increased relative to the general population.

From the time I was working in Sweden, I have been fascinated by the existence of this precursor disease with a high risk of transformation. Using the unique population-based medical history databases that exist as part of universal health care in Scandinavia, we were able to identify over 4,000 MGUS patients and over 14,000 first-degree relatives of these patients. Equally important, we were also able to identify individuals and their relatives that were well matched to our patient population in important characteristics to serve as controls. In that study, which we published last year in the journal *Blood*, we found that MGUS is about three times as common in families as compared to controls, which indicated to us that susceptibility genes and/or shared environmental influences are involved in the disorder. We have since shown that the risk of these diseases varies in different populations.

In fact, although the link between MGUS and multiple myeloma has been known for some time, it has never



(Photo: R. Baier)

Ola Landgren, M.D., Ph.D., and Mary Ann Yancey, R.N., examine a patient with multiple myeloma. Yancey is the lead research nurse for the Multiple Myeloma Section at CCR.

been established whether MGUS is a required stage in the development of multiple myeloma or just one of many paths to the disease. From the beautiful work of John Shaughnessy's laboratory at the University of Arkansas, we know, for example, that multiple myeloma is at least seven molecularly distinct disease subtypes and that some of these entities are relatively more indolent or aggressive. And we've done some preliminary work that indicates that, on average, African Americans have a better prognosis than Caucasians, which seems to be a reflection of the fact that they are more prone to the more indolent subtypes of multiple myeloma.

We were able to look at the relationship between MGUS and multiple myeloma longitudinally using an extraordinary NCI resource: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial that has charted the cancer histories of over 77,000 participants since its inception in 1992. These individuals, who were all cancer-free at the beginning of the trial, had blood work done every year for up to six years and have been followed for up to 10 years. From this trial, we identified 71 individuals who developed multiple myeloma over the course of the study and went back to the freezer to examine each of their blood samples.

In 100 percent of cases, we found MGUS abnormalities prior to the multiple myeloma diagnosis.

## Tracking the Transformation

Although a simple finding, this unyielding relationship between multiple myeloma and MGUS has enormous implications. Suddenly, we have a population that we can hone in on and state with confidence that all cases of multiple myeloma will arise from it. Another key finding in our PLCO-based study is the fact that about 50 percent of the MGUS patients had a steady increase in M-protein levels prior to the development of multiple myeloma, while the other 50 percent had a stable M protein and yet they developed myeloma. Thus, a stable M-protein level over time is not a reliable marker to rule out multiple myeloma progression. There is no doubt we need better markers.

This unyielding relationship between multiple myeloma and MGUS has enormous implications.

We are taking several parallel approaches to address the need for better predictors of progression. For example, using stored blood samples of patients with MGUS and multiple myeloma, we are screening for biomarkers that signal progression. Also, newer imaging methods may give us insights into the course of the disease. We are currently developing a protocol that will take advantage of contrast agents to enhance imaging by positron emission tomography/computed tomography (PET/CT) and magnetic resonance (MR). Using these techniques, we will study patients with MGUS and newly diagnosed multiple myeloma in order to establish better clinical markers of progression. In this study, we will correlate our imaging results with traditional skeletal surveys and with several molecular biomarkers.

We have also just opened the first natural history study of myeloma precursor disease here at the NIH Clinical Center and we are actively seeking patients for this important study. We are enrolling people with MGUS and smoldering multiple myeloma (SMM) and following them for up to five years. SMM is a high-risk precursor disease defined based on higher levels of M protein (>3 g/dL) or higher levels of plasma cells in the bone marrow (10 percent or more), or a combination. We will collect blood, bone marrow, and urine samples at multiple time points. The aim is to define molecular signatures for progressors versus non-progressors. At the moment, we don't have any molecular markers that definitively distinguish between MGUS, SMM and multiple myeloma—

We want to do much more than just discover markers of these diseases.

the diagnoses are based on clinical criteria.

Of course, we want to be able to identify the patients with MGUS that will go on to develop multiple myeloma. If you are diagnosed at the age of 40 and you live to the age of 90, that's 50 years of living with a one percent risk of transformation per year. For such an individual, the lifetime risk of developing multiple myeloma is 50 percent—essentially the same as flipping a coin. We need to identify the molecular signals that will allow us to predict individual risk scores with much greater accuracy.

But we want to do much more than just discover markers of these diseases. Our natural history study has the potential to provide novel biomarkers for the clinic and, at the same time, to uncover biological mechanisms of transformation. Ultimately, it will allow us to define new targets for early treatment of high-risk MGUS/SMM cases.

### No Cell Is an Island

There are a lot of molecular candidates that we can follow in our patient studies. And they don't just come from

studies of the plasma cells themselves. Disease progression in multiple myeloma is related to both intrinsic changes of plasma cells and the influence of the microenvironment—the bone marrow stromal cells, angiogenesis, and immunologic factors. MGUS and multiple myeloma cells appear to produce an abnormally broad superfamily of immunoreceptors that, when signaled by multiple factors, support sustained proliferation.

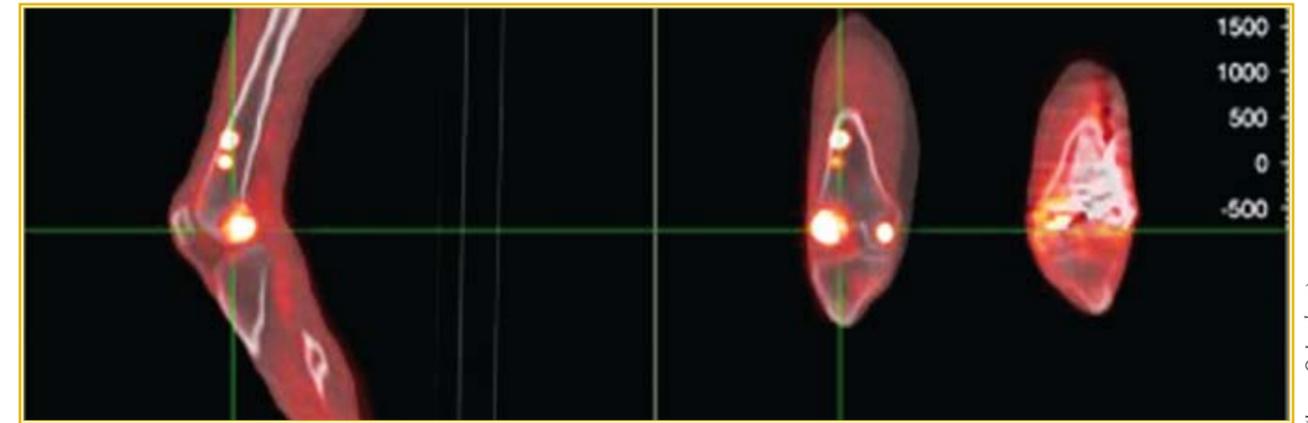
In our natural history study, we will be screening a broad range of markers. For example, we will look at gene expression profiles as well as cytokines and chemokines, either secreted by tumor cells or the environment, that have been reported as being important for myeloma progression. We are also looking at circulating proteasomes (molecular complexes that degrade proteins inside cells and that are often overproduced in cancer cells) and factors that are secreted in the bone marrow that are known to promote tumor proliferation. But there will surely be more possibilities as the mechanisms of pathogenesis are better understood.

In a collaborative project including Michael Kuehl, M.D., Head of CCR's Molecular Pathogenesis of Myeloma Section, Pamela Gehron Robey, Ph.D., and Arun Balakumaran, M.D., Ph.D., at the National Institute of Dental and Craniofacial Research, and Adriana Zingone, M.D., Ph.D., at CCR's Multiple Myeloma Section, we are working on a mouse model of multiple myeloma. Dr. Robey developed a xenograft method

to induce human bone marrow stromal cells to produce small bone formations (ossicles) under the skin of these mice. We have been able to inject human myeloma cells into the ossicles. We are still working to further develop and validate this model, but our aim is to jump between our discovery work using human samples from biobanks, our prospective trials, and our mouse studies. For example, the mouse models may reveal disease mechanisms with signatures that we can look for in human samples. And in a complementary way, our human studies may suggest drug targets that we can test in our mouse models. The mouse model has potential to help us develop novel drugs and gain a better understanding of myeloma pathogenesis.

### Caught Is not Cured

Currently, patients diagnosed with multiple myeloma below the ages of 65-70, and without major comorbidities, are typically given an immunomodulatory agent (thalidomide or revlemid) and/or a proteasome inhibitor (bortezomib), in combination with steroids (dexamethasone). After courses with these drugs, stem cells are typically harvested and returned (autologous transplant) to the patient after treatment with high-dose melphalan. There is currently some debate and ongoing research on the need for autologous transplant/high-dose melphalan treatment as a consolidation in all patients. For patients above the ages of 65-70, who cannot



(Image: O. Landgren)

Bone lesions in the right distal femur of a patient with multiple myeloma, identified with 18F-FDG-PET/CT imaging.

tolerate autologous transplant/high-dose melphalan treatment, there are currently two FDA-approved treatment approaches. They are melphalan and the steroid prednisone in combination with either thalidomide or bortezomib.

None of the approved myeloma drugs are without toxic side effects and in my opinion, therefore, it is far too early to start treating patients with MGUS with currently available therapies. However, for SMM patients, the average risk of transformation reaches 50 percent within only five years; for SMM patients with certain adverse clinical features, the risk is 70-80 percent at five years of follow-up. The current standard of care for SMM patients is basically an aggressive "watch and wait" strategy until multiple myeloma is diagnosed. Based on small numbers, prior research has not supported early intervention with standard multiple myeloma chemotherapy regimens and there are theoretical reasons to be concerned that such intervention might paradoxically encourage the development of more aggressive myeloma clones.

Using novel approaches that are not based on conventional multiple myeloma therapy, we are currently developing a protocol to treat patients with SMM and hopefully delay or prevent progression to multiple myeloma. For example, in collaboration with Richard Childs, M.D., Ph.D., at the National Heart, Lung and Blood Institute, we are

We see multiple lines of investigation coming together to help define and treat multiple myeloma at all stages.

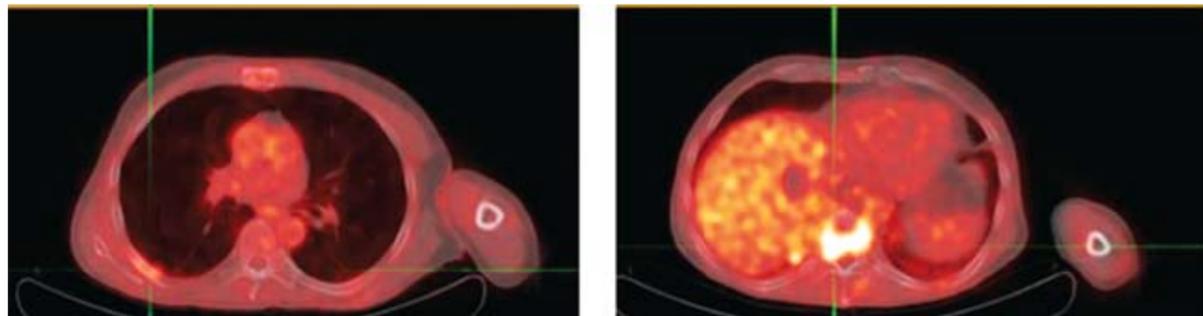
building on evidence that suggests that the innate immune system—and in particular, natural killer (NK) cells—may be fighting multiple myeloma. For this particular trial, we will be trying to encourage the activity of NK cells with a biologic, but we are also exploring other targeted strategies that include both immune-based and small-molecule approaches.

At the other end of the treatment spectrum, we are working on novel molecularly targeted therapies based on what we know about the signaling abnormalities that develop in refractory and/or relapsed multiple myeloma patients. For example, the MEK/ERK pathway is important in several tumor types, including multiple myeloma. Christina Annunziata, M.D., Ph.D., and Louis Staudt, M.D., Ph.D., in CCR's Medical Oncology and Metabolism Branches, have screened myeloma cell lines and found genetic alterations that lead to activation of the MEK/ERK pathway. Furthermore, it turns out that the osteoclasts—cells that secrete growth factors into the microenvironment in which myeloma cells proliferate—are

also impacted by inhibition of MEK in a way that might decrease myeloma proliferation and survival. This and other evidence has led us to a Phase 2 clinical trial in collaboration with the South East Phase II consortium led by Steven Grant, M.D., for the treatment of refractory multiple myeloma with an oral drug that inhibits MEK signaling.

It is really a very exciting time for our work. A lot of the research that we have built up over the years seems to be coming to fruition and we see multiple lines of investigation coming together to help define and treat multiple myeloma at all stages. Of course, there is still so much that we don't know. When my 11-year-old daughter heard me talking about MGUS recently, she asked me, "What comes before the precursor?" And that's a very good question. The answer is probably another precursor.

To learn more about Dr. Landgren's research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?profileid=14864>.



(Image: O. Landgren)

Bone lytic lesions in a patient with multiple myeloma, visualized with PET/CT imaging.

# The Doctor-Patient Relationship



(Image: O. Landgren)

*A physician chanced upon his own diagnosis of MGUS, and now must wonder whether he will develop multiple myeloma.*

Jim M. practices internal medicine in the Washington, DC area. One day, he was running lab tests on his own blood—the reasons aren't important—and found that the total protein levels were highly elevated. Concerned, he began to run a series of tests to isolate the source of the excessive proteins. Using serum protein electrophoresis, he discovered higher-than-normal levels of M protein. In fact, as it turned out, the initial lab tests he had done on his total protein levels were erroneous and they were not elevated over the normal range. But the finding of excess M protein (a small percentage of total protein)—and with it, the diagnosis of MGUS—remained.

"It was a sheer random event," explained Jim. "There must be thousands of people walking around with MGUS and unaware of it." Jim knows that at his age—64 years—approximately three percent of the population has MGUS and that a small fraction of those will progress to multiple myeloma. "It's a terrible disease, but it is rare."

MGUS has no symptoms and no treatment. It is typically diagnosed when blood tests are done for other

reasons. "We've got tons of patients—several hundreds in our hospital facilities alone—that have MGUS," noted Jim. "The only thing to do is watch and watch and watch."

"Having a diagnosis of MGUS is a nuisance, a pain in the neck," said Jim. "It's a predisposition—no different from the people walking around with skin moles that may suddenly become a severe form of melanoma." Vigilance—in this case, frequent testing—is the only available tactic and there is currently no way of knowing whether the disease is transforming into outright cancer or any way to prevent that transformation from happening.

After his diagnosis, Jim happened to be talking with a hematologist colleague who had recently returned from the 2009 Annual Meeting of the American Society of Hematology. The colleague had heard that Dr. Ola Landgren was studying MGUS, enrolling individuals with MGUS in a prospective trial to uncover markers and mechanisms of transformation.

"The number of people in hematology that are actually doing research on this subject is miniscule because they are focusing on how

to cure multiple myeloma, not how you prevent something with a low likelihood of transformation," explained Jim. That's why he is enthusiastic about the work Dr. Landgren is undertaking. "I am most interested in the possibility of understanding why these cells change and begin to produce abnormal proteins."

"Ultimately, the question is, can you go in there and stop it," concluded Jim, noting that there aren't too many diseases where you can pick out precursors and could therefore intervene early. "Ideally, you would take some medicine and the next time you came in, there would be no abnormal proteins and a bone marrow biopsy would show no abnormal cells. We may be very far from that, but at least we are asking the questions."

*CCR's Understanding Targeted Therapies for Multiple Myeloma, an animated tutorial that explains some of the new approaches to treating this cancer, can be viewed at <http://www.cancer.gov/flash/targetedtherapies/multiplemyeloma/main.html#>.*

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>

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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