

National Cancer Institute

VOLUME 2, No. 1 | 2008

# CCR connections

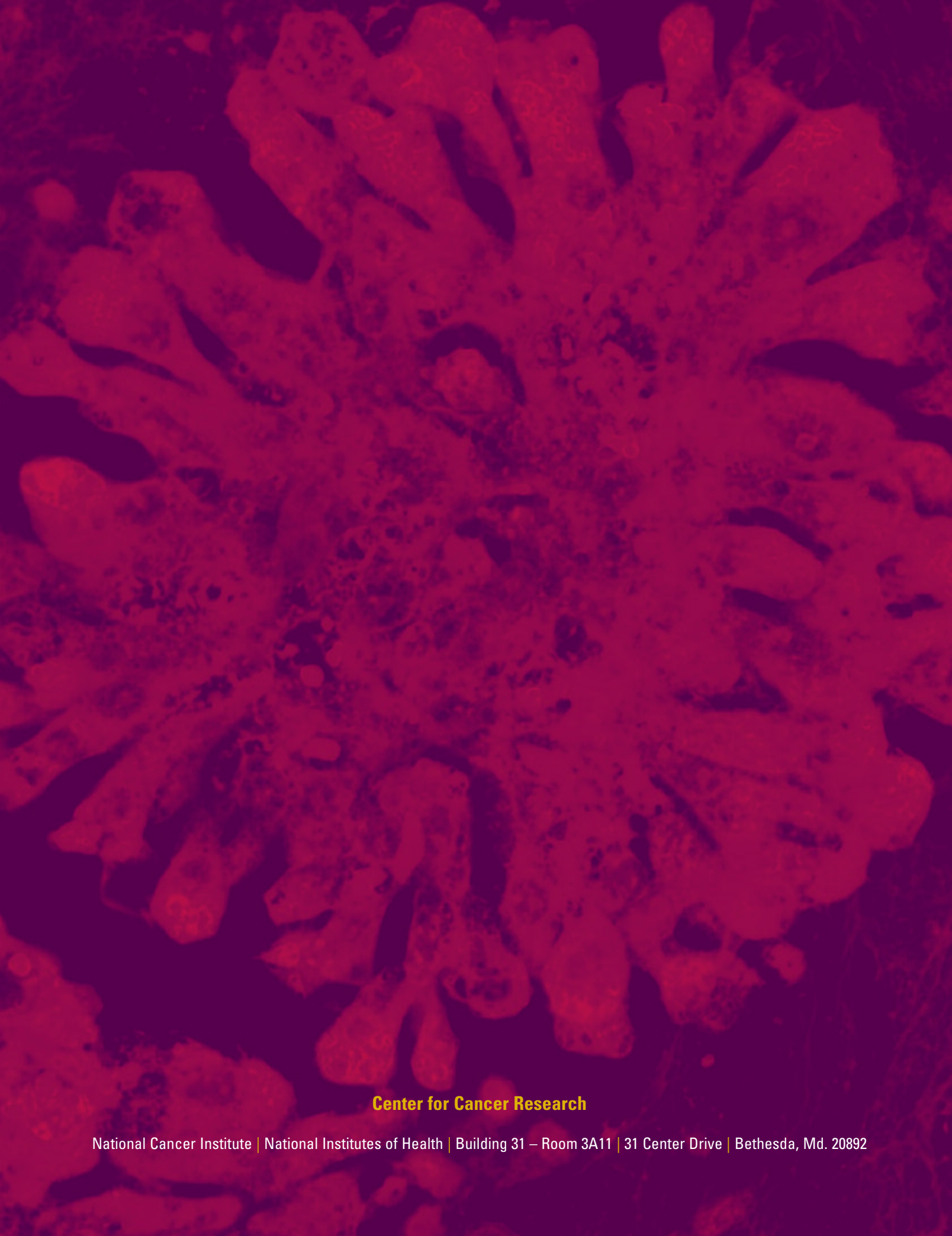
CENTER FOR CANCER RESEARCH

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

## Joining Forces

Complementary approaches are piecing together the mysteries of chromosomal translocations and cancer

U.S. DEPARTMENT  
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**Center for Cancer Research**

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

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<http://ccr.cancer.gov/about/default.asp>

Our News

<http://ccr.ncifcrf.gov/news/default.aspx>

Office of Training and Education

[http://ccr.nci.nih.gov/careers/office\\_training\\_education.asp](http://ccr.nci.nih.gov/careers/office_training_education.asp)

## **Patient Information on Cancer and Clinical Trials**

Open NCI Clinical Trials

<http://www.cancer.gov/clinicaltrials>

How to Refer a Patient

<http://bethesdatrials.cancer.gov/professionals/refer.asp>

NCI Cancer Information Service

<http://cis.nci.nih.gov>

1-800-4-CANCER (1-800-422-6237)

Understanding Cancer Series

<http://www.cancer.gov/cancertopics/understandingcancer>

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## **Additional Links**

National Cancer Institute (NCI)

<http://www.cancer.gov>

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<http://www.cancer.gov/aboutnci/working>

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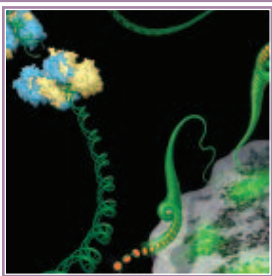
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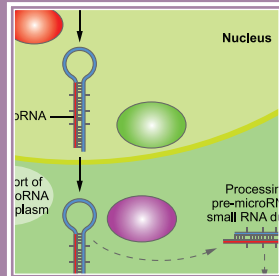
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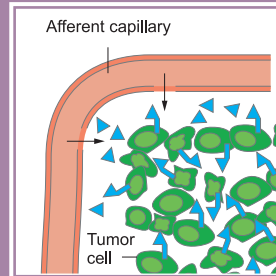
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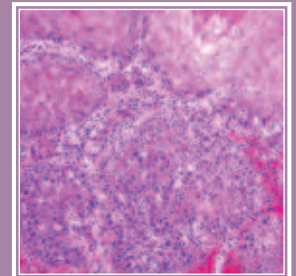
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FEATURE



No Longer Skimming the Surface of Skin Cancer



The mission of the CCR is:

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

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# A Different Kind of Cancer Center, a Unique Collaborative Team

*The sheer complexity of human biology, both normal and disease-related, continues to amaze and often baffle us as we struggle to unravel it so that we can reduce the burden of suffering and death that results from cancer. Yet understanding cancer biology is only half the battle: We must actively and rapidly translate that knowledge into clinically meaningful applications. Reaching that goal requires a strategic interdisciplinary and multidisciplinary effort to bridge critical basic science with clinical care.*

The National Cancer Institute's own clinical cancer program, under the direction of the Center for Cancer Research (CCR), sits squarely at this intersection of exploding basic cancer biology knowledge and new cancer treatments. Although we may not have the same name recognition as other national cancer centers, which are primarily large volume, full-service cancer treatment hospitals and clinics, we are in fact the largest cancer-focused clinical research center in the world. But we are not a traditional cancer care center that happens to do research: Our sole mission is to perform patient-intensive clinical research aimed at translating the explosion of new scientific insights into new approaches to prevent, diagnose, and treat cancer.

One of our greatest strengths is our unmatched broad integration of basic and clinical scientists. This combination is particularly powerful at NCI, where basic laboratories adjoin clinical wards. Thanks to this tight collaboration of researchers across disciplines and settings, we are uniquely able to knit together insights from preclinical disease models with science-based approaches to human clinical

treatment. This ability is especially true in understudied cancers, although our findings often have relevance to many cancers and even other diseases. Our collaborative model allows us to identify new cancer therapies, both single agents and combinations, and bring them rapidly through early development and clinical testing and, if successful, partner with extramural collaborators to ensure that they are quickly brought to widespread trials and hopefully into clinical practice. Our structure also uniquely enables us to find novel ways to detect cancer early and even prevent it, largely through building powerful new imaging technologies that allow us to "see" cancer even in its initial stages.

But perhaps the most important collaborative partners of all, the ones who truly make the NCI cancer clinical research center unique from all others, are the patients who come to us. They come here seeking to actively participate in research with our basic and clinical scientists to drive understanding of their cancer and, ultimately, to find an effective treatment—if not for them, then for others. It is hard to describe the selflessness—the nobility—of those

who participate in the NCI clinical trials that are performed at the National Institutes of Health Clinical Research Center here in Bethesda, Md. We are proud to work with them every day, and their hope and focus underlie ours. If—when—we succeed in our mission, it is clear that the lion's share of the credit must go to them.

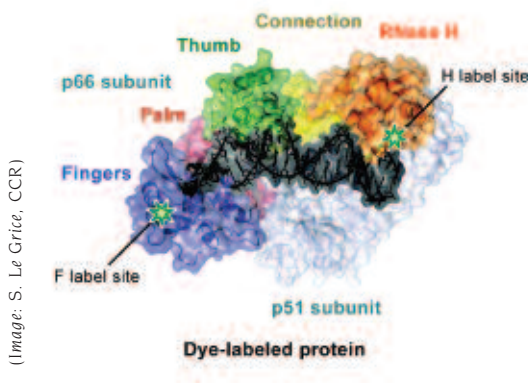
## **Lee Helman, M.D.**

Scientific Director for Clinical Research  
Center for Cancer Research

(Photo: R. Baer)



# Reverse Transcriptase: When Function Follows Direction



The direction in which HIV's reverse transcriptase binds to the viral genome determines whether the enzyme reads the RNA or degrades it. This knowledge has shed new light on the mechanisms by which antiviral drugs called non-nucleoside RT inhibitors act against HIV.

Getting into a host cell's genome can be a tough job for HIV. Before it can be integrated, the virus' single-stranded, RNA-based genome must undergo a complete transformation into double-stranded DNA, a multistep process that holds much room for error. This transformation is where HIV's multifunctional reverse transcriptase (RT) comes into play. RT does three things: transcribes the viral RNA (vRNA) into a single, complementary DNA (cDNA) strand; destroys the original vRNA; and uses the new cDNA as a template for a complete double-stranded viral DNA (vDNA). These three activities require that RT behave in two seemingly contradictory ways: as a polymerase and as an RNase H.

While the structural sites of RT's polymerase and RNase H activities are well known, the parameters that determine when and how each site is engaged are not. How does RT decide whether to enter polymerase or RNase H mode?

Using combinations of DNA- or RNA-based primers and templates, the groups of Harvard University's Xiaowei Zhuang, Ph.D., and Stuart Le Grice, Ph.D., Head of CCR's RT Biochemistry Section, have found that RT's function is controlled by its orientation.

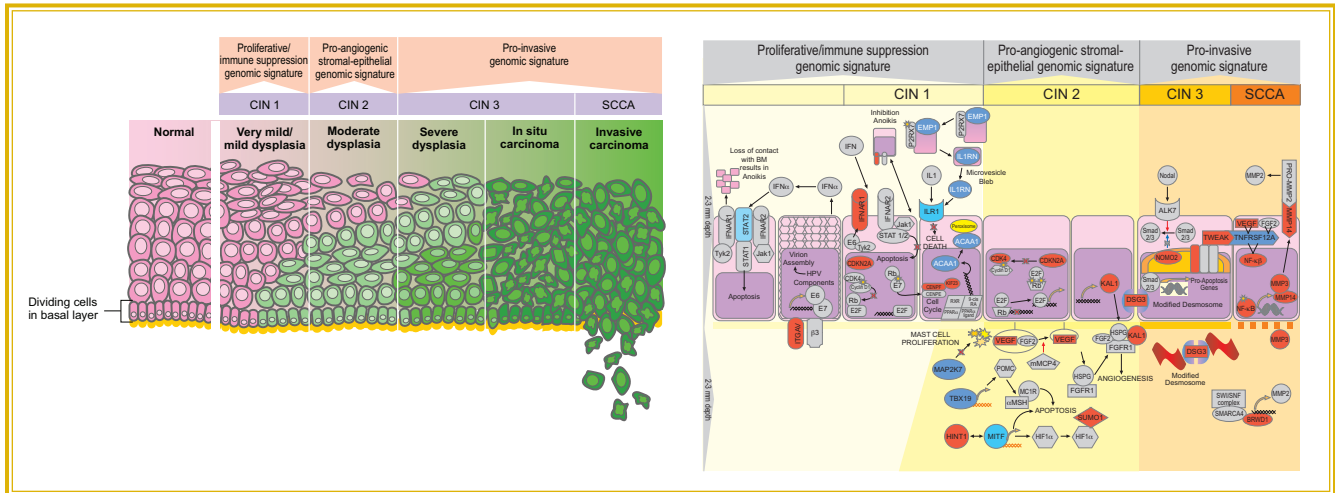
In this system, when presented with a DNA primer bound to an RNA or DNA template—a scenario analogous to the production of a cDNA or vDNA strand—RT positions itself in a way that favors its polymerase site. However, when RT finds a short RNA primer bound to a longer stretch of DNA—which to the enzyme looks like a newly transcribed cDNA strand bound to its original vRNA template—it binds in the opposite orientation, promoting its RNA-template-degrading RNase H activity.

Remarkably, the researchers also found that when RT sees a substrate that is amenable to both of its functions, such as polypurine tracts (vRNA sequences that in nature serve as primers for reverse transcription of the HIV genome), the protein flips spontaneously between orientations, potentially helping it maximize the efficiency with which it transcribes and degrades the viral RNA. Exposure to non-nucleoside RT inhibitors (NNRTIs)—a class of potent and clinically approved anti-HIV drugs that bind to RT's polymerase site—significantly altered RT's flipping behavior, forcing the enzyme to adopt its RNase H-promoting orientation.

These data, published in the May 8, 2008, issue of the journal *Nature*, provide significant insights into the structural biology underlying the reverse transcription process in HIV and lend new evidence to the observation that NNRTIs can, in certain circumstances, prevent the synthesis of DNA by reversing enzyme orientation.



# Taking Time-Lapse Genomic Snapshots of Cervical Cancer



(Image: J. Kelly)

Striking shifts in gene expression at different time points in the process of cervical cancer development, from human papillomavirus (HPV) infection to invasive carcinoma, may help reveal how HPV goads cervical cells into becoming cancerous and why the process of carcinogenesis varies so much from woman to woman.

Researchers and physicians have known for over a quarter of a century that human papillomavirus (HPV) infection underlies the vast majority of cervical cancers. While the approval of the first HPV vaccine<sup>1</sup> in 2006 was a breakthrough in cervical cancer prevention, important questions remain. How does the virus fuel cervical cancer development and why do the developmental steps vary from woman to woman?

Not all women who contract HPV develop cervical cancer. In many, the series of precancerous changes triggered by the virus, called cervical intraepithelial neoplasia (CIN), progresses to a certain point and simply stops. In those women who do develop cancer, the process can take mere months or go on for years.

Answering the how and why would be easier if researchers had a time-lapse series of genomic snapshots spanning the cellular progression from viral infection to invasive malignancy. In the August 1, 2007, issue of *Cancer Research*, a multi-institutional team of

scientists including CCR's David Gius, M.D., Ph.D., Head of the Molecular Radiation Oncology Section, unveiled just such a comprehensive photo series. Working with Janet Rader, M.D., and colleagues at Washington University School of Medicine in St. Louis, Mo., Gius examined gene expression within the cervical epithelium (the layer of cells that HPV infects and where cervical cancer originates) and the underlying stroma (normal cells that form a developing tumor's microenvironment) at different histological time points in cervical cancer development.

The team found striking shifts in gene expression that correlated closely with the progressive changes seen under the microscope as the cervical epithelium transforms. Their results suggest three distinct genomic phases in the development of cervical cancer. First, an immunosuppressive phase, characterized by genes linked to the establishment of HPV infection and immune system evasion. Second, a pro-angiogenic phase, where the precancerous cells "talk" to the nearby stroma and encourage the production of factors fostering blood vessel growth, possibly corresponding to the "angiogenic switch" proposed a decade ago by the late Judah Folkman, M.D. Finally, a pro-invasive phase, associated with genes that promote the breakdown and invasion of neighboring healthy tissues.

Together these results constitute an *in vivo* model of cervical cancer development that both provides a window on the evolution of cervical cancer and highlights a number of genes with prognostic and therapeutic potential.

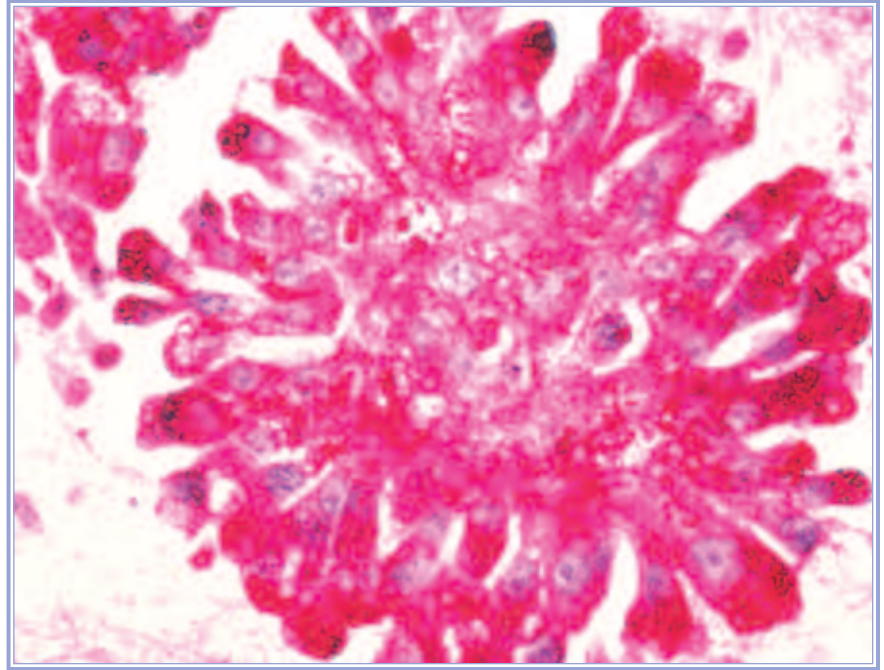
<sup>1</sup>See "A Victory against Cancer: 25 Years in the Making," CCR Connections, Vol. 1, No. 1.

# New Clues to Blocking Metastasis in Sarcoma

Cancer's wanderlust can be its most fatal feature. Metastasis—the spread of cancer cells to sites far from the original tumor—is the cause of death for approximately 90 percent of cancer patients. It is not a simple, one-step process: To metastasize, cancer cells must escape the original tumor, enter and exit the bloodstream or lymphatic system, and establish themselves in a new target organ or tissue. All told, the process requires the activation of several genes and the deactivation of several others.

Findings from a team of CCR researchers suggest that for at least some sarcomas (a group of cancers of the connective and supportive tissues, such as bone, cartilage, fat, muscle, or blood vessels), cancer cells may get the green light to metastasize not because of genomic changes but because a protein important for suppressing this process is tagged for destruction. This insight has come about because the CCR researchers—led by Allan M. Weissman, M.D., Chief of the Laboratory of Protein Dynamics and Signaling, and Chand Khanna, D.V.M., Ph.D., of the Pediatric Oncology Branch—chose to study not genomic mechanisms of metastasis but rather the fate of the proteins produced from these genes.

Weissman and Khanna focused their attention on gp78, one of many proteins whose job it is to mark other proteins with



(Image: L. Liotta, CCR)

Suppressing a metastasis-suppressing protein, called KAI1, by tagging it for degradation by the proteasome may be one factor in a sarcoma cell's development of the ability to spread.

a small protein called ubiquitin. The cell normally uses this molecular tag to flag excess or damaged proteins for destruction by a complex cellular machine called the proteasome.

Using RNA interference (RNAi) in an animal model of sarcoma, the researchers turned down gp78 expression, finding that metastasis was similarly reduced. Looking at gp78's interactions, they found that it tags a protein called KAI1—one of about 10 known metastasis suppressors—for degradation by the proteasome. In their laboratory sarcoma models, the team found that reducing levels of gp78 caused KAI1 levels protein to rise and the survival of metastatic sarcoma cells to diminish. Subsequently, turning gp78 expression back up reversed the situation: KAI1 was tagged more for destruction more frequently, and metastatic cancer cells with the rejuvenated gp78 expression had better survival.

To establish the clinical relevance of their findings, the scientists looked at archived clinical samples of sarcoma tumors. There, too, they found evidence for an inverse relationship between the two proteins: When levels of gp78 were low, KAI1 levels were high, and vice versa.

The structure of gp78 makes it a potential candidate for targeted therapeutics. Spurred on by the findings, the CCR team, as well as other scientists, is now beginning to look for similar relationships between gp78 and metastasis in other cancers. The results also open the door to exploring the use of available proteasome inhibitors, such as bortezomib (Velcade®), to treat metastatic cancers. Bortezomib is already successfully used to treat the blood cancer multiple myeloma and has been widely studied in other cancers.

# Recent CCR Awards

**American Society for Public Service and National Academy of Public Administration National Public Service Award**

~ For excellence in public service  
Peter M. Blumberg, Ph.D.  
Laboratory of Cancer Biology and Genetics

**American Association of Immunologists Public Service Award**

~ For advancing immunological research related to aging  
Richard J. Hodes, M.D.  
Experimental Immunology Branch

**American Society of Investigative Pathology Chugai Award**

~ For excellence in mentoring and scholarship  
Elaine S. Jaffe, M.D.  
Laboratory of Pathology

**American Medical Association Dr. Nathan Davis Award**

~ For outstanding government service  
W. Marston Linehan, M.D.  
Urologic Oncology Branch

**Service to America Medal**

~ For selection as Federal Employee of the Year

**Novartis Prize for Immunology**

~ For excellence in immunology research

Douglas R. Lowy, M.D.  
John T. Schiller, Ph.D.  
Laboratory of Cellular Oncology

**American Cancer Society's Medal of Honor**

~ For basic research

**Eugene J. Van Scott Award and Phillip Frost Leadership Lecture**

~ For innovative therapy of the skin

Douglas R. Lowy, M.D.  
Laboratory of Cellular Oncology

**Society for Melanoma Research Senior Researcher Award**

~ For development of murine melanoma models

Glenn Merlino, Ph.D.  
Laboratory of Cancer Biology and Genetics

**Twelfth Annual Keio University Medical Science Prize, Japan**

~ For development of AIDS drugs  
Hiroaki Mitsuya, M.D., Ph.D.  
HIV and AIDS Malignancy Branch

**Weizmann Institute of Science Sergio Lombroso Award**

**American Society of Clinical Oncology Statesman Award**

**Abbott Laboratories Award in Clinical and Diagnostic Immunology**

~ For distinguished achievement in cancer research

Steven A. Rosenberg, M.D., Ph.D.  
Surgery Branch

**American Association of Immunologists-Dana Foundation Award in Human Immunology Research**

~ For significant and sustained achievement in immunology research  
Thomas A. Waldmann, M.D.  
Metabolism Branch

**Academy of Sciences of the Czech Republic Jaroslav Heyrovsky Honorary Medal for Merit in Chemical Sciences**

~ For contributions to the studies of protein structure

Alexander Wlodawer, Ph.D.  
Macromolecular Crystallography Laboratory

**Stanford Medical Research Scholarship Award**

~ To fund research and training in Tumor Immunology and Biology

Jorge Caballero, B.S.

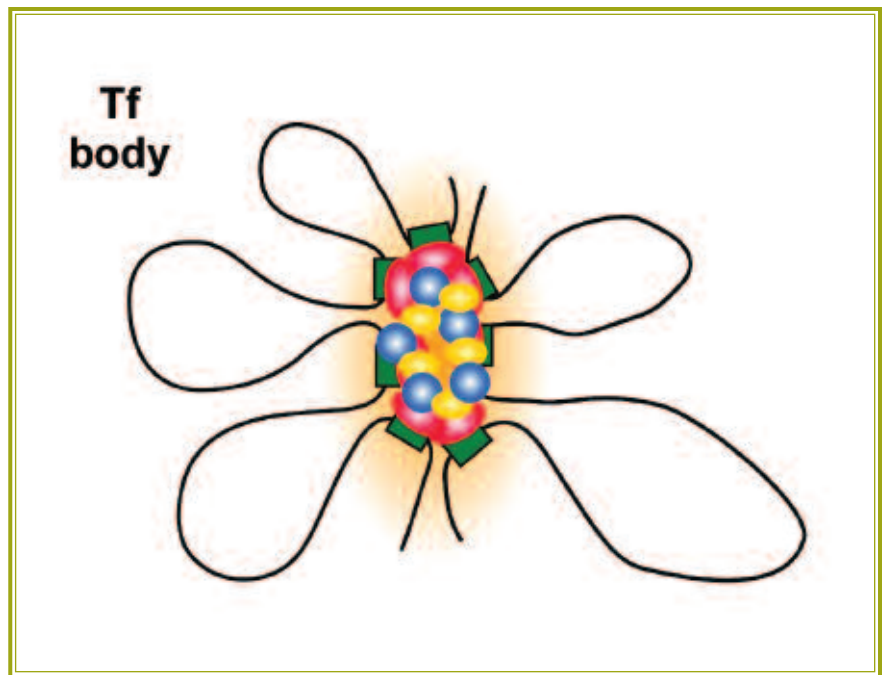
Laboratory of Tumor Immunology and Biology

## CCR Summer Student Was an Intel Semifinalist

Sappho Z. Gilbert, a summer student in Maria Tsokos, M.D.'s Pediatric Tumor Biology and Ultrastructural Pathology Section of the Laboratory of Pathology, was one of 40 finalists in the highly competitive Intel International Science and Engineering Fair for 2008. This competition for high school students awards approximately \$1 million in scholarships each year and recognizes outstanding students nationally. Gilbert's project was entitled, "Survivin Correlates with Prognosis and Inhibits Drug-induced Apoptosis in Ewing Sarcoma Family Tumors."

By silencing and clustering Tf2 retrotransposons (green), proteins of the CENP-B family (red), themselves the remnants of ancient transposons, regulate the movement and placement of these mobile genetic elements and help maintain the organization and integrity of the genome.

(Image: S. Grewal, CCR)



## Transposon, Regulate Thyself

*The neighborhood around the genome is not a quiet place. Rather, it has all of the activity of a city at rush hour: Chromosomes ravel and unravel, DNA unzips while proteins zoom in for transcription or replication, and RNAs zoom out for translation into proteins.*

Adding to the frenzy are genes that just cannot sit still. Called transposable elements or transposons, these “jumping genes” move about the genome with abandon. Generally considered to be the remains of ancient viruses that merged into our DNA far back in our evolutionary past, transposons exist in two distinct classes: DNA transposons and RNA-based retrotransposons.

With their ability to randomly cut- or copy-and-paste themselves into just about any genomic location, transposons can be both boon and bane. They can increase genome size and complexity, providing fertile ground for the development and evolution of new traits. Or they can wreak genomic havoc by disrupting genes critical for normal cellular functions, such as proliferation and apoptosis.

Because they can have such widespread and powerful effects, the genome needs to keep the activity of transposons under tight control. In the January 24, 2008, issue of the journal *Nature*, Laboratory of Biochemistry and Molecular Biology Senior Investigator Shiv Grewal, Ph.D., Postdoctoral Fellow Hugh Cam, Ph.D., and

their colleagues reported the results of studies in yeast revealing the existence of a powerful mechanism by which the genome may regulate how and where transposons move and act.

This surveillance system centers on the action of a group of proteins homologous to a human protein family called the CENP-B family. According to the Grewal team’s results, the CENP-B proteins—themselves derived from the remnants of past transposons—seek out and silence a group of retrotransposons called the Tf2 family. The CENP-Bs also appear to be able to corral these retrotransposons into clusters, called Tf bodies, which may facilitate the proteins’ surveillance tasks and also have wider implications for genome organization, gene regulation, and response to environmental stresses.

Taken together, the scientists’ results suggest that the genome has, over time, tamed some of these “jumping genes” and redirected them into roles where they regulate their own kin, thereby helping to maintain genome integrity.

# Finding What's Real, Functionally Speaking

Not all mutations are created equal. Where some can be silent, having no discernable effect on a cell, tissue, or organ, others can be profoundly disruptive.<sup>1</sup> Deciding which category a mutation fits into is often the subject of significant amounts of experimental work and debate, particularly when the gene or genes in question could potentially raise or lower the risks of developing diseases like cancer.



(Image: S. Burkett, CCR)

**The functional consequences of mutations in *BRCA2* (represented here in a multispectral karyotype displaying a translocation between chromosomes 4 and 13) and other genes can now be better and more rapidly understood thanks to a new *in vitro* validation system based on mouse embryonic stem cells.**

Such is the case for the DNA repair genes *BRCA1* and *BRCA2*. Mutations in these genes can significantly increase a woman's risk of developing breast cancer. While sequencing-based tests can help women assess their risk and make appropriate decisions about preventive treatment, the functional impacts of nearly 1,900 identified *BRCA1* and *BRCA2* mutations on the genes' protein products remain unclear. Tying mutations to functions would help improve breast cancer risk assessments; however, such functional studies are both labor- and resource-intensive.

CCR's Shyam Sharan, Ph.D., Head of the Mouse Genetics Program's Genetics of Cancer Susceptibility Section, and Research Fellow Sergey Kuznetsov, Ph.D., have developed an *in vitro* functional assay system capable of accurately and relatively rapidly measuring the effects of *BRCA2* mutations. The pair, together with Pentao Liu, Ph.D. (a former CCR Research Fellow now at the Wellcome Sanger Trust Institute), published a report on their system in the July, 2008, issue of *Nature Medicine*.

The system measures a mutation's functional impact using a knockout-and-replace strategy. The researchers replaced both normal *Brca2* gene copies in mouse embryonic stem cells (ESCs) with copies of human *BRCA2* containing particular mutations of interest. Measurements of the cells' growth, survival, and sensitivity to agents (chemicals, radiation, etc.) that cause DNA damage indicated which mutations had no effect and which kept *BRCA2* from compensating for the loss of *Brca2*.

Using this system, Sharan, Kuznetsov, and Liu examined the functional impacts of 17 catalogued *BRCA2* mutations. Their findings validated the known effects of 13 of the mutations and gave the first evidence of the impacts of four others whose functional impacts had not been characterized previously. The researchers noted that with their system, between three and five mutations can be assessed on a two- to three-month timeframe, and the system could potentially be used with any gene mutation that causes a phenotype in mouse ESCs, making this system a useful tool for genetic counselors. They caution, though, that the system needs to be validated further before it is ready for clinical application.

<sup>1</sup>For more information, see "Silent No More," CCR Connections, Vol. 1, No. 1.

# A Cancer Center Shines in Jordan

*The King Hussein Cancer Center (KHCC) is a story of transformation and purpose-driven aspirations. And CCR's Samir Khleif, M.D., is a central reason for its success.*

(Image: Courtesy of S. Khleif)



The KHCC treats more than 50 percent of Jordan's cancer patients. About one quarter of its patients come from outside the country, mainly Syria, Iraq, the Palestinian Authority, Sudan, Libya, Algeria, and Yemen.

In 2002, this medical center, located in Amman, Jordan, and called the Al-Amal ("Hope") Center, had significant quality and safety problems. A large percentage of its patients did not know they had cancer. It was not even called a cancer center.

That year, the monarch, King Abdullah, asked NCI to help his country develop a high quality cancer center. Khleif—an NCI oncologist, cancer vaccine specialist, and Palestinian-American with a medical degree from the University of Jordan—moved to Amman to become KHCC's Director General and CEO.

Today, remade at his hands and renamed for the late King Hussein, KHCC is the only comprehensive cancer center in the Middle East.

To transform the center, Khleif radically changed its culture and organization. The most important thing, he said, was implementing quality measures and building

teams among the staff, thereby engendering a sense of commitment to the center's mission. "It became something of a jewel, a source of pride for the country," he said.

Khleif's leadership motivated KHCC staff to "make significant sacrifices that went beyond their own self-interest," according to a case study by Duke University researchers published in November 2007 in *Globalization and Health*. "There was a sense of purpose or vision-driven efforts to attend to the needs of patients and mid- and lower-level hospital staff...in an effort to rapidly raise the standard of care at KHCC."

The results, in terms of morale, performance, and outcomes, have surpassed all expectations. Staff retention is high ("They feel they are accomplishing things. They've shown they can improve healthcare," noted Khleif). KHCC residents now outrank those of top-tier American medical centers on the American College of Physicians' annual

in-service exam (Jordan is one of only six countries outside of the U.S. and Canada that participate in this evaluation of resident performance). And in February 2006, KHCC received accreditation from the Joint Commission on International Accreditation (JCIA), a division of the U.S.-based Joint Commission on the Accreditation of Healthcare Organizations (JCAHO).

"We built a Western-style medical institution, introduced quality management in healthcare, and got JCIA accreditation, a very important accomplishment worldwide," Khleif said. "We established the first support groups, the first palliative care program, the first early detection unit, and the first smoking cessation clinic in the country. These actions changed the whole landscape of healthcare in the country."

Khleif returned to CCR and his laboratory in the Cancer Vaccine Branch the day after KHCC received JCIA accreditation. But his ties to Jordan remain strong. King Abdullah has asked for additional help to build a cancer and biotechnology institute. Khleif is again at the helm, traveling between Bethesda and Amman to guide the birth of a new hospital and laboratories for basic and translational research, a CCR in the desert.



(Photo: E. Branson)

Samir Khleif, M.D.

# Staff News at CCR

## announcements

(Photo: R. Baer)



### L. Michelle Bennett, Ph.D.

Bennett has been named Deputy Director of CCR. She trained at the University of Wisconsin and at the National Institute of Environmental Health Sciences. Since joining CCR in 2002 as Associate Director for Science, she has administered numerous initiatives, including CCR's strategic plan and the new collaborative Centers of Excellence. As Deputy Director, Bennett will focus on strategic planning, program integration, and scientific communications.

(Photo: E. Branson)



### Kevin Camphausen, M.D.

Camphausen has been appointed Chief of the Radiation Oncology Branch (ROB) where he previously served as Deputy Branch Chief and Acting Chief. Before joining NCI in July 2001, he studied the interaction of angiogenesis inhibitors and radiotherapy at Harvard Medical School. He envisions the ROB at the cutting edge of combined modality therapy with treatment strategies that merge radiation and molecular targeted agents.

(Photo: M. Spencer)



### J. Carl Oberholtzer, M.D., Ph.D.

Oberholtzer joins CCR as Chief of the Laboratory of Pathology. Oberholtzer originally came to the NCI in 2006 as Associate Director for Training. Before NCI, he was Associate Professor of Pathology and Laboratory Medicine, Director of the Division of Neuropathology, and Vice Chair of the Pathology Department at the University of Pennsylvania. He received his Ph.D. from the University of Pennsylvania and his M.D. from Jefferson Medical College.

## Newly Tenured CCR Scientists

### Jairaj Acharya, Ph.D.

Laboratory of Cell and Developmental Signaling

### Mirit I. Aladjem, Ph.D.

Laboratory of Molecular Pharmacology

### Javed Khan, M.D.

Pediatric Oncology Branch

## new tenure-track scientists

(Photo: M. Spencer)



### Itzhak Avital, M.D.

Avital joins the NIH Clinical Center's Surgery Branch as a Tenure-Track Investigator. He came to CCR in 2006 to launch a solid-organ cancer stem cell research program. Trained in surgery and surgical oncology at the Memorial Sloan-Kettering Cancer Center (MSKCC), Avital received his M.D. from New York University's School of Medicine.

(Photo: Courtesy of P. Kalab)



### Petr Kalab, Ph.D.

Kalab returns to NIH as a Tenure-Track Investigator in CCR's Laboratory of Cellular and Molecular Biology. Kalab, from the University of California at Berkeley, developed molecular imaging tools to study the RanGTP gradient in live cells. At CCR, he will investigate Ran-regulated mitotic functions and their overlap with misregulated pathways in cancer.

(Photo: M. Spencer)



### Udai S. Kammula, M.D.

Kammula joins the Surgery Branch as a Tenure-Track Investigator. Trained in surgical oncology at MSKCC and the Surgery Branch of the National Cancer Institute, his clinical work focuses on malignancies of the liver, pancreas, and gastrointestinal tract. His research focuses on tumor immunology and immunotherapy development.

(Photo: E. Branson)



### King F. Kwong, M.D., FACS

Kwong recently joined CCR's Thoracic Oncology Section as a Tenure-Track Scientist from the University of Maryland. His CCR research will focus on apoptosis in thoracic malignancies. Kwong received his M.D. from The George Washington University (GWU) in Washington, D.C., and he received training at both GWU and Washington University in St. Louis, Mo.

# Joining Forces

**Complementary approaches are piecing together the mysteries of chromosomal translocations and cancer**

*It has been almost 50 years since a “minute chromosome” was first identified in patients with chronic myelogenous leukemia (CML). This genetic abnormality, named the Philadelphia chromosome for where it was discovered, was the first genetic defect linked to cancer (Figure 1). Investigators initially believed that the Philadelphia chromosome resulted from the loss of genetic material. However, advances in cytogenetics over the next decade made it possible to view the true nature of this abnormality—the genetic material “missing” from chromosome 22 was not lost but “translocated” to chromosome 9.*

It is now known that this specific translocation, found in 95 percent of patients with CML, fuses a proto-oncogene (a normal gene with oncogenic potential) on chromosome 9 (*c-ABL*) to a site on chromosome 22 known as a breakpoint cluster region. This hybrid oncogene, *BCR-ABL*, produces a constantly activated mutant protein (BCR-ABL), which wreaks the genomic havoc in the cell that ultimately causes CML but which also is the target of imatinib mesylate (Gleevec®), the first FDA-approved treatment to target a translocation-specific fusion protein.

Since the 1970s, chromosomal translocations have been associated with several types of blood cancers, and they are recognized increasingly as key players in

solid tumors as well. Today, cytogenetic testing is performed frequently in the clinic to determine which chromosomal translocations are present in patient samples, helping facilitate diagnosis as well as treatment planning. But although we have come a long way in 50 years, we have yet to fully understand the molecular mechanisms behind these deadly chromosomal rearrangements and why they happen so often in the same chromosomal locations. Instead of treating the resulting cancer, can we prevent them from occurring in the first place? Researchers in the Center of Excellence in Chromosome Biology at CCR are putting together answers to these questions.



Since the 1970s, chromosomal translocations have been associated with several types of blood cancers, and they are recognized increasingly as key players in solid tumors as well.

### Taking a Global View

Tom Misteli, Ph.D., Head of the Cell Biology of Genomes Group in CCR's Laboratory of Receptor Biology and Gene Expression, arrived at NCI nine years ago with the task of building an imaging program. Within a few years, he gradually began to apply *in vivo* imaging techniques to chromosome biology and specifically to understanding how genome organization affects genome regulation (see "The Right Place at the Right Time"). He and his CCR colleagues are now using high resolution microscopy, live-cell imaging, and computer simulation to study the positioning of entire chromosomes and particular gene loci within the nucleus in order to understand how these arrangements change during normal and aberrant physiological processes (Figure 2).

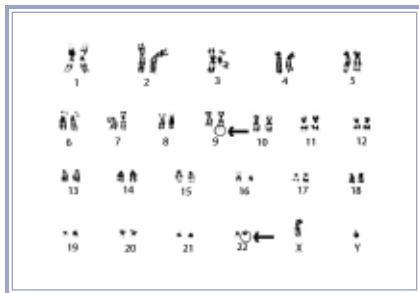


Figure 1: The Philadelphia chromosome—the result of a translocation between chromosomes 9 and 22 (circles)—is often found in the cells of patients with chronic myelogenous leukemia.

Far from being randomly scattered around the nucleus, chromosomes, sub-chromosomal domains, and individual genes are nonrandomly organized into discrete territories, or neighborhoods, within the nucleus. Although these entities may have preferred localizations, the patterns can change in response to the cellular environment. The distinct territory occupied varies with cell type, upon cell differentiation, and when cells exit the cell cycle, suggesting a link between positioning and genome function. The positioning is believed to influence gene expression programs as cells undergo changes throughout development and differentiation.

Positioning can determine direct interactions between genes, which in turn can regulate gene expression. In naïve T-helper immune cells, for example, when a specific region on mouse chromosome 11 (TH2) directly interacts with the *lfn* locus on chromosome 10, the locus is turned "off." When these cells then receive a signal to differentiate, the two regions separate, and the *lfn* locus turns back "on."

Chromosomal translocations occur when direct physical interactions go wrong, and these can be deadly. DNA double-strand breaks occur frequently throughout the genome during replication or as a result of DNA-damaging agents like radiation. What remains unclear is how the broken ends of two different chromosomes—which should not be allowed to "mingle" under normal circumstances—assemble and fuse together, forming a translocation.

Spatial mapping studies by Misteli's laboratory demonstrated that the breakage sites of several common translocations (e.g., Myc-Igh, BCR-ABL, and RAR-PML) are preferentially positioned in close proximity to each other in normal B lymphocytes prior to undergoing chromosomal rearrangement. This observation suggests that proximity may be a requirement for translocation.

### Taking a Closer Look

This proximity requirement suggests that broken DNA ends do not just flop around in the nucleus but are fairly limited in their

freedom of movement. To better understand these findings, Misteli teamed up with Andre Nussenzweig, Ph.D., Head of the Molecular Recombination Section at CCR's Experimental Immunology Branch. Nussenzweig and his colleagues (see "Making the Right Connections") explore chromosomal translocations at the molecular level—an approach that complements Misteli's "macro" view of chromosome biology—to focus on the cellular mechanisms of genomic stability and how defects in DNA repair and cell cycle checkpoints lead to chromosomal translocations and malignancies.

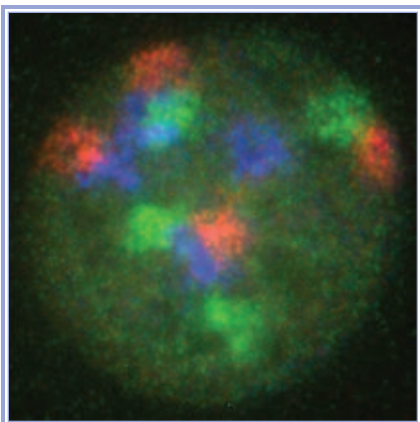
Misteli and Nussenzweig designed an experimental system that allowed them to introduce a double-strand break within the genome at will and then visualize the fate of each of the damaged DNA ends in living cells in real time. They found that while the broken chromosome ends do separate slightly from each other, their long-range motion is significantly constrained. This positional stability depends upon the presence of a specific DNA binding protein known as Ku80, a component of the nonhomologous end-joining (NHEJ) DNA-repair pathway. Data from Ku80 knockout mice generated by the Nussenzweig lab demonstrate that Ku80 is important for maintaining genomic stability—it forms an asymmetric ring around the two broken ends to align them for repair. Several follow-up studies are being conducted to further characterize this protein and the role it plays in DNA repair and chromosomal translocations.

Chromosomal translocations occur when direct physical interactions go wrong, and these can be deadly.

## Of Recombination and Translocation

The NHEJ pathway is a key pathway in normal immune cell development through its involvement in V(D)J recombination. This normal programmed recombination event occurs early in the life of developing B lymphocytes, resulting in the generation of cell surface receptors that can accurately identify a massive diversity of intruders and mobilize the immune system to respond. During recombination, the variable (V), diversity (D), and joining (J) gene segments are selected randomly and recombined to ultimately produce an antigen receptor gene. These receptors can even recognize microbes that an individual or his/her ancestors have never encountered, which explains how some individuals are naturally immune to new infections or viral strains.

By studying the process of V(D)J recombination, Nussenzweig and colleagues hope to gain invaluable insight into the mechanisms responsible for chromosome translocations. Aberrant chromosomal translocations between antigen receptor loci and proto-oncogenes are a hallmark of lymphoid cancers. These translocations are not random—they involve only a few oncogenes with recurrent breakpoints. Mature B-cell



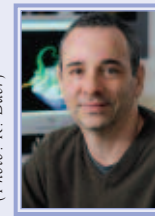
**Figure 2: The positioning of the 23 pairs of chromosomes within the nucleus (here labeled with red, green, and blue dyes) as a cell goes through the cell cycle may significantly influence gene expression.**

lymphomas, for example, typically involve fusions of antigen receptor loci with *BCL1* (mantle zone lymphoma), *BCL2* (follicular lymphoma), or *MYC* (Burkitt's lymphoma).

V(D)J recombination is initiated by the RAG-1/2 endonuclease, an enzyme that introduces specific double-strand breaks within the VDJ genes. The broken DNA strands are then fused together via the NHEJ pathway to form the antigen receptor gene. The strands are prevented from joining illegitimately by ataxia-telangiectasia mutated (ATM), a key enzyme that is activated by DNA double-strand breaks. ATM also prevents the propagation of cells with broken chromosomes by activating mechanisms that lead to cell cycle arrest, DNA repair, or apoptosis (cell death) (Figure 3). Mutations within the *ATM* gene result in the rare disorder ataxia telangiectasia (from which the gene name is derived), which is characterized by immunodeficiency and predisposition to lymphoid malignancies caused by chromosomal translocations near antigen receptor genes.

Nussenzweig's group discovered that ATM is also part of a system that prevents genetic damage from being passed on to a cell's offspring. In a recent study published in *Cell*, Nussenzweig and his brother, the Rockefeller University's Michel Nussenzweig, M.D., Ph.D., showed that when ATM is absent, chromosomal breaks created during V(D)J recombination go unrepaired, and checkpoints that normally prevent the damaged cell from replicating are lost. Remarkably, the cell divides, matures, and maintains the breaks, which can persist for more than five generations *in vitro* and approximately two weeks *in vivo*. Since ATM is mutated in a number of lymphomas, the new finding suggests to researchers that the lymphocytes could have been living with DNA damage for a long time, and that this damage likely plays a role in later chromosomal translocations that lead to cancer. This novel form of "delayed" genomic instability, which permits more time for long-range movement of chromosomal breaks, might

## Making the Right Connections



(Photo: R. Baer)

**Andre Nussenzweig, Ph.D.**  
Head, Molecular Recombination Section, Experimental Immunology Branch

Andre Nussenzweig takes

a different angle toward understanding how and why chromosomes break and rejoin and how they relate to cancer: that of a physicist (Ph.D. from Yale) and an expert in laser spectrometry. This background has, during his nearly 10 years with NCI/CCR's Experimental Immunology Branch, given him a unique perspective on the mysteries of cell nuclei. "Al Singer was crazy enough to hire me," he joked.

Nussenzweig "learned biology" during a postdoctoral stint at Memorial Sloan-Kettering Cancer Center, where he studied a mouse model of defective DNA repair. It was there that he first teamed up scientifically with his brother Michel, a Howard Hughes Medical Institute Investigator at the Rockefeller University. This familial collaboration continues to this day.



(Photo: R. Baer)

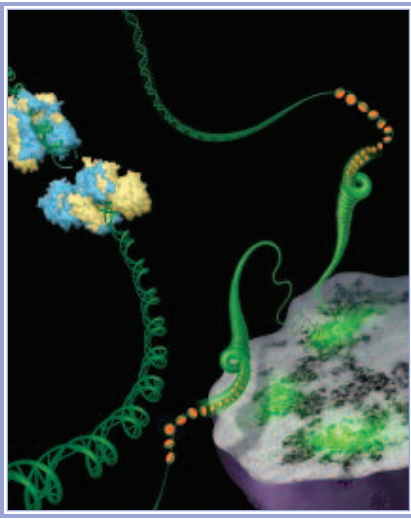
**Elsa Callen, Ph.D.**  
Visiting Fellow

For the past three years in Nussenzweig's lab, Elsa Callen has been working to understand the possible roles of DNA repair

proteins normally seen in diseases like lymphoma. Before joining NCI, Callen completed her Ph.D. at the Universitat Autònoma de Barcelona (Spain) at the end of 2004, where she studied Fanconi anemia, a devastating disease that can be caused by a set of genes involved in DNA repair. This work led her to seek further understanding of DNA repair mechanisms in Nussenzweig's lab.

"Working here has many advantages," said Callen, particularly in the availability of necessary resources and the collaborative atmosphere. "It makes it really easy to develop interesting, challenging, and high quality projects that would be almost impossible under other circumstances."

(Image: A. Nussenzweig, CCR)



**Figure 3:** If the protein ATM (blue and yellow) is mutated, double-strand DNA breaks can go unrepaired and result in genomic damage that can be passed on to daughter cells. If unchecked, this damage can promote the development of lymphomas and the rare disorder ataxia telangiectasia.

contribute to the oncogenic transformation of mature lymphocytes.

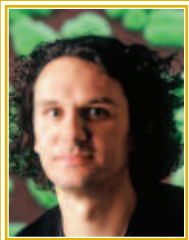
In a separate study published recently in *Nature*, a group of NCI scientists, led by Raffael Casellas, Misteli, and Nussenzweig, identified one mechanism by which ATM protects cells from passing on genetic aberrations to progeny cells. When mouse cells are exposed to a DNA damaging agent, the resulting double-strand breaks transiently inhibit RNA polymerase I (which is required for the synthesis of ribosomal RNA) via the ATM pathway. Thus, in response to chromosomal breaks, ATM shuts down ribosomal gene expression, which ultimately impacts the assembly of all cellular proteins.

This new finding suggests new avenues of research for understanding the

role of translocations in early disease development. Such insights into the structural and molecular defects that happen in the earliest stages of chromosome translocations have perfectly positioned CCR researchers to develop novel strategies for the early detection of cancer and, perhaps, to even prevent these genetic abnormalities from occurring in the first place.

## The Right Place at the Right Time

(Photo: R. Baer)



### Tom Misteli, Ph.D. Head, Cell Biology of Genomes Group, Laboratory of Receptor Biology and Gene Expression

Tom Misteli's focus when he joined NCI was on setting up a cellular imaging program, based on his pioneering efforts to visualize

gene expression and protein dynamics in living cells. However, he soon started looking at the behavior of whole chromosomes within their nuclei. "I always wanted to do work on chromosome positioning, but it just wasn't fundable outside of the NIH," said Misteli.

His success has attracted numerous research fellows. "We have 10 to 12 people at any time now, all extremely good at what they do," said Misteli. "In fact, every postdoc in my lab is better than I ever was."

Misteli received his Ph.D. from the University of London and the Imperial Cancer Research Fund, London, United Kingdom.

(Photo: M. Spencer)



### Karen Meaburn, Ph.D. Visiting Fellow

Karen Meaburn has been doing research in the Misteli lab for the past two and a half years on understanding chromosome and gene positioning, particularly in breast cancer. Her hope is that her work will help

lead to ways to detect breast cancer at its earliest stages.

(Photo: M. Spencer)



### Evi Soutoglou, Ph.D. Visiting Fellow

Evi Soutoglou has spent four years at NIH, first as an International Human Frontiers Science Program Fellow and now as a Visiting Fellow in the Misteli lab. She says that imaging work was an

"obvious" choice for her, and that the capabilities and research interests at NCI attracted her, particularly in working in the Misteli lab in understanding the stability of chromosome breaks.

"Tom is not afraid to try new things and brings the energy and resources to help us address difficult but interesting questions," said Soutoglou, who also cites the collaborative atmosphere provided by the lab and by her colleagues in other labs as unique aspects of the NCI intramural program.

Soutoglou received her Ph.D. in 2002 from the University of Crete's Institute of Molecular Biology and Biotechnology.

# One Man's Junk DNA Is Another Man's Treasure: MicroRNAs and the Next Big Thing in Cancer Prognosis

*Until very recently, scientists thought they had a pretty good basic model for how cells work. DNA was the storehouse of all information, the blueprint for life. Proteins were the building blocks: the bricks, mortar, and switches that actually made a living thing and also made it work.*

*According to the model, while RNA did a good job shuttling the DNA's instructions from the nucleus to the cytoplasm, it did not serve any larger purpose. And the long stretches of DNA that did not contain information for building proteins were unimportant.*

In 1993, scientists started to find the first hints that this long-standing model of how life works might not explain everything. Researchers studying *Caenorhabditis elegans* worms started finding evidence that short 18–25 nucleotide-long snippets of RNA produced from genes that did not encode protein might be bigger players in the workings of cells than had been realized. These snippets started turning up in a number of different species, showing remarkable conservation and suggesting that some fundamental piece of the cell was starting to make itself known (Figure 1).

Fast forward to the present day. The importance of these short RNA pieces—now dubbed microRNAs—as epigenetic regulators of cell development, survival, and disease is becoming ever clearer. And their popularity as a research topic has exploded. “The basic science of microRNAs is just fascinating,” said Curtis Harris, M.D., Chief of CCR's Laboratory of Human Carcinogenesis, who studies microRNAs as prognostic tools in cancer. “How the microRNA is processed from its gene, its function in normal cell

biology, in development, and in disease—since microRNAs are relatively newly discovered, all of these features are still being worked out.” A search of the PubMed database turned up 2,611 papers published on microRNAs since 2001, with 1,027 published in 2007 alone.

The links between microRNAs and cancer are also now well appreciated. “The cancer field has now become very excited by microRNAs, in part because they are so new,” noted Harris. Cancer researchers also have a clearer view of microRNAs as actors

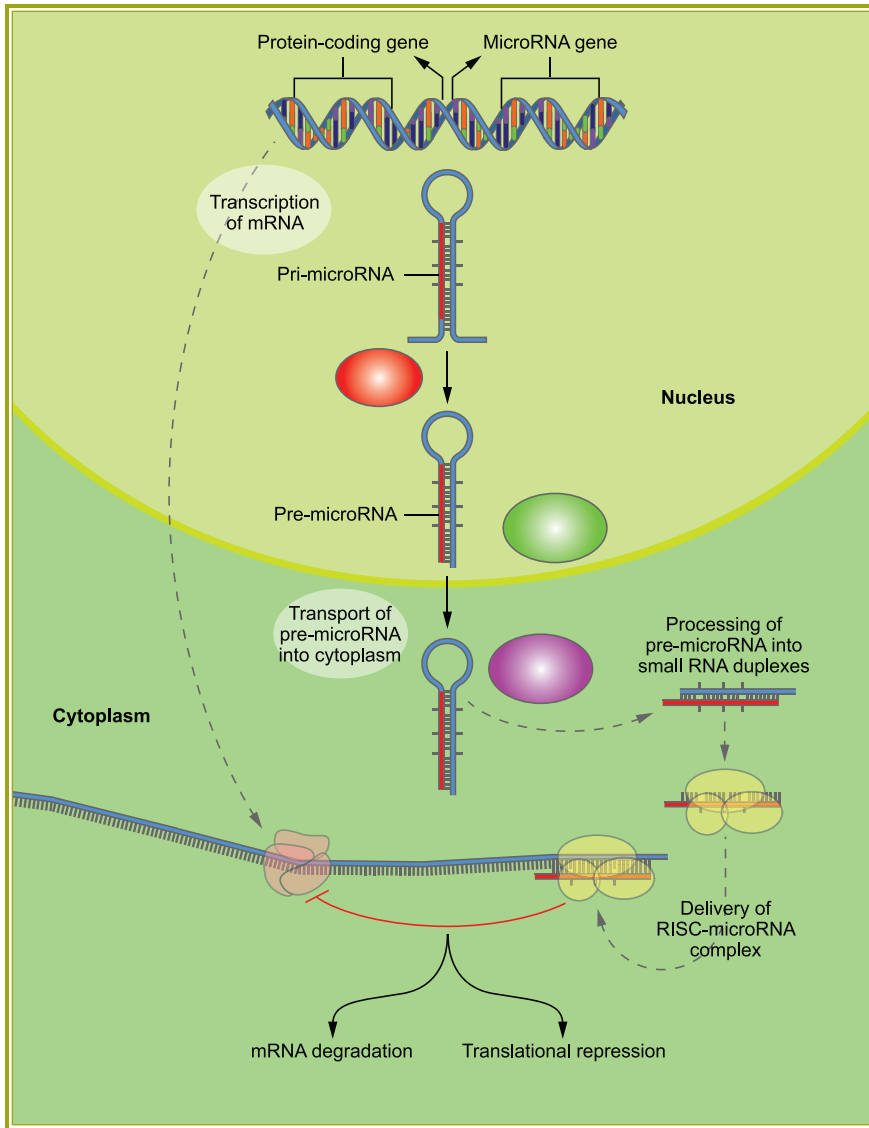
in carcinogenesis. Based on this knowledge, researchers like Harris are investigating how to turn our growing knowledge of microRNAs into clinical tools for cancer prognosis and therapy.

## Shooting the Messenger

MicroRNAs are like transcription factors for RNA. Just as transcription factors control a gene's transcription into messenger RNA, microRNAs control a messenger RNA's translation into protein. But instead of promoting gene expression, as transcription factors do, microRNAs impede it: MicroRNA-bound messenger RNAs do not get translated, effectively silencing the gene from which they were transcribed. “MicroRNAs bind to the messages of protein-coding genes, either changing the stability of that message or the translation of that message into protein,” explained Harris.

The number of microRNA genes tucked away in the genome is unclear, but by some estimates there may be as many as a thousand. Researchers estimate conservatively that about one-third of all

A search of the PubMed database turned up 2,611 papers published on microRNAs since 2001, with 1,027 published in 2007 alone.



(Image: J. Kelly)

**Figure 1: The roles of microRNAs—simple short pieces of RNA that do not encode protein—in the control of nearly all critical cellular processes have gained widespread and rapid appreciation.**

protein-coding genes may be controlled to some extent by microRNAs. They can have such widespread effects because they tend to act globally. “Because they are short and their ‘seed’ sequences [the first six nucleotides in a microRNA, which act as binding sites] are somewhat degenerate, they physically interact with messages that they don’t exactly match,” Harris noted. “So a single microRNA may target 10, 50, maybe even a 100 messages in different genes or pathways.”

### Drawing the Lines

The first paper suggesting a link between microRNAs and cancer, published in 2002 by a colleague of Harris, The Ohio State

University’s Carlo Croce, M.D., reported that a genomic region deleted in about half of all cases of B-cell chronic lymphocytic leukemia (B-CLL) housed genes for two microRNAs. The development of techniques for microarray and bead-based flow cytometry analyses of microRNA expression soon led to the discoveries of microRNA signatures unique to specific tumors and their cellular origins.

As the research on microRNAs and cancer has gone deeper, particular microRNAs have begun to stand out as potentially causative agents. For instance, microRNAs called miR-155 and the miR-17-92 cluster act like oncogenes, while miR-15a and miR-16-1 appear to function as tumor

suppressors. The genetic lesion Croce identified in B-CLL included the genes encoding miR-15a and miR-16-1.

“We are finding that microRNAs can serve a range of purposes in the context of cancer and cancer treatment,” said Harris. “They can tell us a lot about the basic biology of cancer and about what pathways are involved and what might be good targets for therapeutic development. One can, at least in preclinical studies, knock down the expression of a specific microRNA with an antisense strategy and see an anti-tumor effect. They can also be good clinical biomarkers, useful tools for diagnosis and, maybe, for predicting therapeutic outcome.”

### Translating Science Together

It was the role of microRNAs as developmental players in cancer, combined with Croce’s B-CLL paper, that gave Harris his entrée into the world of microRNAs. “When Carlo made what I think was a seminal observation that microRNAs were associated with cancer, that seemed to be a very exciting finding and one that I thought might have relevance to solid tumors.”

Having known Croce for some time, Harris contacted him and suggested that they work together to look at microRNA profiles in solid tumors. Joining forces, their laboratories produced, in early 2006, an examination of the microRNAomes (the total palette of microRNA expression within a cell) of tissues from a spectrum of solid tumors (e.g., lung, breast, stomach, prostate, and colon). “This paper was one of the first to indicate the extensive involvement of microRNAs in the pathogenesis of solid tumors,” reported Harris. It also suggested that microRNA expression could influence cancer development by controlling protein-coding oncogenes and tumor suppressors (Figure 2).

### Peeking into a Tumor’s Future

Apart from the questions of development and pathogenesis are those of progression. The ability to predict an individual’s clinical outcome or risk for recurrence has been an

enigmatic target for researchers but one that could have great benefit. Could microRNAs give clinicians insight into how a tumor will behave as time goes on?

Harris framed the prognosis problem in the context of lung cancer. “Frequently a surgeon operating on a patient with stage I lung cancer will tell them after surgery, ‘We got it at an early stage; hopefully we got it all.’ In fact, about half of these patients will have recurrence with distant metastases over a five-year period. If surgery isn’t sufficient, we bring in our armamentarium of adjuvant therapies, but because of the side effects, you don’t want to use them unless you think there is a good reason to.

“If you knew,” Harris continued, “which of these patients will have a good prognosis, and perhaps need less therapy and screening, versus those who will have a poor prognosis and need more therapy and need to be screened more frequently, you would have greater justification to offer particular adjuvant therapies to a particular patient. This need for better prognosis has fueled the whole biomarkers field. MicroRNAs, in this context, are a new class of biomarkers that will be informative not only when combined with clinical stage, but also with other biomarkers such as genomic or proteomic changes.”

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With this reasoning in mind, Harris, Croce, and their colleagues started narrowing their view of microRNA expression profiles to focus on prognosis. In 2006, they released a paper, authored by now-Harris lab alumnus Nozomu Yanaihara, M.D., Ph.D., revealing that the expression pattern of microRNAs in lung tumors correlated not only with tumor type, but also with prognosis. It was one of the first studies to tie microRNAs to a patient’s prognosis following surgery, independent of tumor stage.

### Broad Effects

“The same things we know about stage I lung cancer and the limitations of clinical staging can be said for stage 2 colon cancer,” Harris noted. “There is a similar need to identify those individuals who have a good or poor prognosis independent of stage. You

can get a good idea on a population level who is going to face recurrence and who is not, but on an individual basis, you have to be cautious when deciding who needs what therapy and how aggressively a patient needs to be treated.”

Knowing that they could predict lung cancer outcomes, Harris and Croce teamed up again to see if they could achieve the same results in colon cancer. The time and place for this study were ideal—Harris has studied colon cancer for a number of years and has a long-standing cohort of patients from whom he has collected tissue and detailed clinical and family histories. “In addition,” Harris said, “I knew of a cohort in Hong Kong that would provide us with a validation population that would allow us to confirm any results we found in our locally-based cohort. I wanted to make a diverse comparison by looking at two very different cohorts. If a typical U.S. population and, in this case, a typical Chinese population show the same result, it is more likely that your data will be generalizable to a broad range of people with colon cancer.”

And once again, Harris’ and Croce’s collaboration has proven fruitful. A new paper, released in January of this year and co-authored with Yanaihara by Harris laboratory Cancer Prevention Fellow Aaron Schetter, Ph.D., M.P.H., and Postdoctoral Fellow Jane Sohn, Ph.D., showed that microRNA profiles could be used to predict both prognosis and clinical outcome, another first in the microRNA world. In addition, they found that the levels of

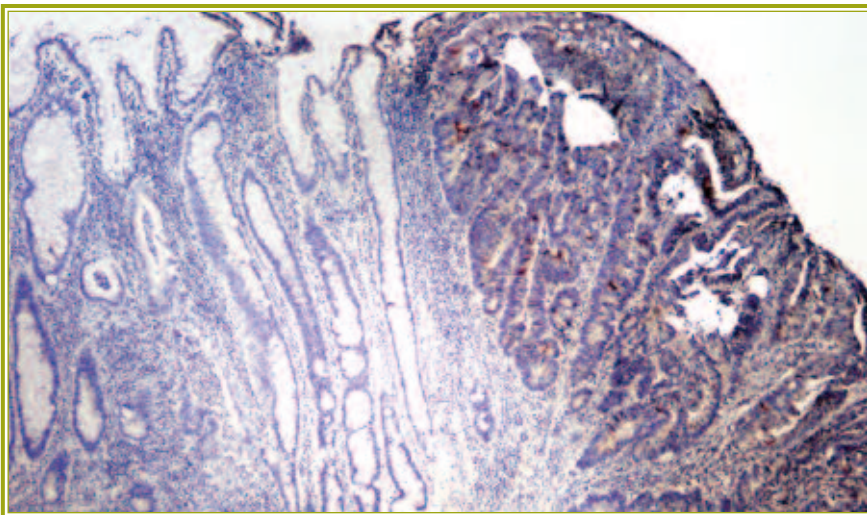
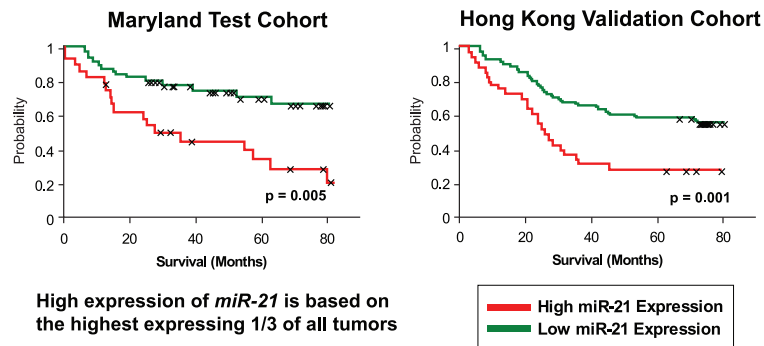


Figure 2: The overabundance of microRNAs like miR-21 in tumor tissue (right, adjacent to normal tissue expressing low levels of miR-21) may represent both a causative factor in colon and other cancers and an opportunity for the development of new prognostic tests and therapeutic agents.

(Photo: C. Harris, CCR)

## High *miR-21* Expression in Tumors is Associated with Cancer-Specific Survival in Both Cohorts



(Photo: C. Harris, CCR)

Figure 3: Comparisons of *miR-21* abundance in colon cancer tissues with patient outcomes reveal a striking correlation—the more of this microRNA that is present in patients' tumor cells, the worse their prognosis. The Harris lab conducted the first study of this link between colon cancer survival and microRNA expression.

a specific microRNA called *miR-21* also correlated with cancer stage: The later the stage, the more *miR-21* they found in the tumor cells. *MiR-21* is one of the microRNAs that have, over time, made an appearance in other cancers as well. Thus, there is a growing body of evidence to suggest that it might play a fundamental role in the progression of colon and other cancers (Figure 3).

Which means *miR-21* may also fit the bill as a therapeutic target. “While surgery is the first-line treatment of colon cancer,” Harris noted, “if there is evidence of metastases, one still gets the best response overall by using fluorouracil-based therapies, which are nearly 50 years old. We need novel options for colon cancer patients, and maybe targeted microRNAs could be a good one.”

## Looking to a MicroRNA-Based Future

“We are only five or so years separated in time from the first suggestions that microRNAs could be involved in cancer,” said Harris. “In that short time, we have come very far, and in my opinion, microRNAs are going to be very significant biomarkers for diagnosis and prognosis in a number of cancers. I also anticipate that microRNAs may be useful clinical targets, which is a longterm goal for us to determine.”

But he is the first one to declare that there is a great deal more work to be done. “I’d like to see our prognosis results replicated in a number of different populations, so that we can see how broad they are,” mused Harris. “Ours was the first report on the use of microRNAs to predict therapeutic outcome, and as such these data need to be confirmed. But it is an exciting time and an exciting opportunity, being engaged at the early stages of translating a fundamental discovery.”

## A New View of Liver Cancer

The question of predicting survival and response is not limited to colon and lung cancers. One cancer for which there is a significant need for new prognostic tools is liver cancer. Currently, surgical resection or transplantation are the best options available for liver cancer patients; however, based on assessments of liver function, tumor size, and stage, only 10 to 20 percent of patients are eligible for these surgical options. Even those who are able to undergo surgery face an uncertain future; the frequency of metastasis and/or recurrence is very high.

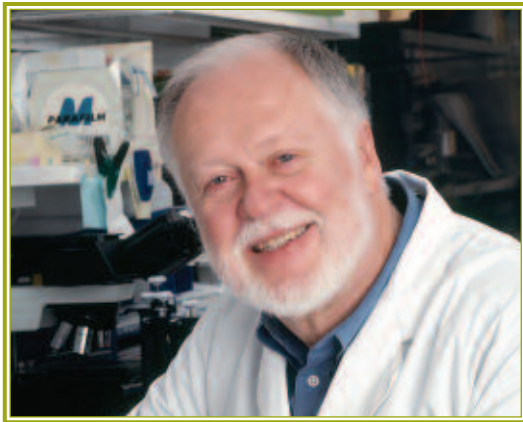
Knowing that microRNAs were starting to show promise for prognosis in other tumors, Xin Wei Wang, Ph.D., Head of the Liver Carcinogenesis Section in CCR's Laboratory of Human Carcinogenesis, began to look at whether such patterns could be applied to liver cancer as well. By comparing cancerous and noncancerous liver tissues, Wang and his collaborators identified 20 microRNAs whose expression correlated with risk of metastasis and did so with greater accuracy than classical pathology staging. They also found that this pattern itself could be used as an independent

measure for predicting a patient's clinical outcome.

Wang looks at these results in terms of clinical benefit. Methods for microRNA isolation and analysis are advancing rapidly, which creates conditions for turning microRNA profiling into a standard procedure for liver cancer patients. Having a profile in hand that can distinguish high- and low-risk patients would allow clinicians to decide early after a diagnosis how aggressive a treatment approach to take and give insights into how to personalize treatment for an individual patient.

# A Diverse Team

(Photo: R. Baer)



**Curtis C. Harris, M.D.**  
**Chief, Laboratory of Human Carcinogenesis**

"It all started in a small farming community in Kansas and at state science fairs," Curt Harris quipped when asked about his background. But he came to CCR with an inborn instinct for translational medicine. "One of my medical school mentors, with whom I had done some collaborative research, suggested that I should continue my work at NCI. I started out with a small laboratory while finishing my clinical training and haven't ever thought about leaving."

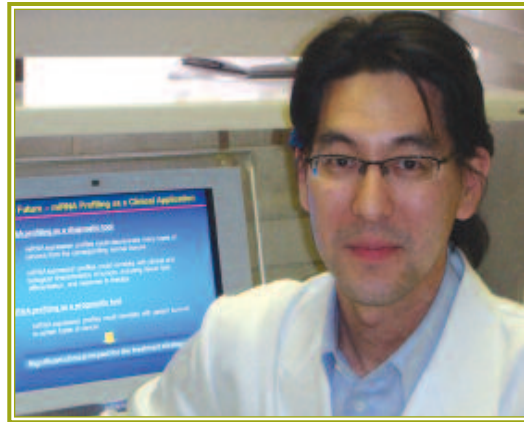
His research interests, at first glance, cover a range of topics. "My lab's research is diverse, which reflects being a physician-scientist. We have a strong motivation for understanding basic research and translating that knowledge into the clinic." What truly excites him, though, are moments of unexpected convergence. "I love it when there are two parallel lines of research in the laboratory, and there is a connection that we never would have predicted, leading us into something much more interesting."

This eye for convergence has fueled his work on biomarkers and prediction. "We have done a lot of work on molecular pathogenesis of cancer and how normal cells become cancer cells," Harris said. "We try to look at cancer from a scientific standpoint, a clinical standpoint, and a public health standpoint."

Harris finds working with his growing web of laboratory alumni a fruitful and enjoyable aspect of his job. "We maintain what we like to call the 'LHC Family.' I find it very satisfying to collaborate with former fellows who have cultivated their own independent careers and with whom I can have a collegial relationship."

Harris earned his medical degree from the University of Kansas School of Medicine and did his clinical training at the University of California at Los Angeles and at NIH.

(Photo: Courtesy of N. Yanaihara)



**Nozomu Yanaihara, M.D., Ph.D.**  
**Harris Laboratory Alumnus**

Nozomu Yanaihara worked with the Harris lab from 2004 to 2007 as a Research Resident and a Visiting Fellow from the Jikei University School of Medicine in Tokyo, Japan. "I am a gynecologist and an obstetrician by training, with a focus on gynecological oncology. When I decided to come to the National Cancer Institute for additional experience, one of my mentors, a former Fellow in Curt's lab himself, suggested I contact him."

Now an Assistant Professor of Obstetrics and Gynecology back at Jikei University, Yanaihara maintains an active relationship with his colleagues in the Harris laboratory. "One of the biggest things I learned at CCR," Yanaihara noted, "was that while researchers from different countries may struggle with language barriers, there are no barriers in research as long as we have shared goals."

His interests in microRNA and cancer, fueled by his work at CCR, have now crossed into his work in Japan. "I took part in several microRNA-related projects while at NCI, including the lung cancer prognosis prediction project. I am now applying the techniques and results that I brought back to prognostic research in gynecological cancers. My hope is to carry out this work collaboratively with Curt and others back in the United States."

Yanaihara received both his M.D. and his Ph.D. from Jikei University.



**Aaron Schetter, Ph.D., M.P.H.**  
**Cancer Prevention Postdoctoral Fellow**

Aaron Schetter's path to the Harris lab has followed a route that places him squarely at the intersection of public health and basic science. "I started off doing basic research studying cell biology in *C. elegans*," said Schetter. "After I finished my Ph.D., I wanted to switch to a field that was more relevant to human disease. After deciding to study cancer, I joined the NCI's Cancer Prevention Fellowship Program."

Launched in 1987, this program trains multidisciplinary experts in cancer prevention. Scientists, clinicians, and other health professionals are encouraged to earn an M.P.H., followed by mentored research with NCI investigators. "It's given me a completely different set of skills than what I developed as a Ph.D. student," Schetter noted.

After completing the academic portion of the program, Schetter looked for a laboratory where he could put those skills to work. "It's difficult to find labs where you can do both basic science and epidemiology well. Curt's lab is a large and diverse group, with people doing basic science and ones who exclusively do this kind of epidemiologic work, so to me it seemed a good fit."

The translational aspect of Harris' microRNA research also drew him in. "I saw how the microRNA work could rapidly turn into something that could affect human disease itself. MicroRNAs are pretty easy to work with in this regard. You can see which ones are altered in colon cancer and then move quickly into functional studies testing your hypotheses."

Schetter's experiences give him some pretty broad options for the future. "I haven't decided yet whether to go the academic route or the industry route, but regardless I hope to continue in the field of discovering and testing biomarkers and therapeutic targets in cancer."

Schetter earned his Ph.D. at Cornell University and his M.P.H. at the University of California at Berkeley.

**Jane Sohn, Ph.D.**  
**Postdoctoral Fellow**

With a research background in microbiology, Jane Sohn brings a different perspective to research on cancer. "I came to Curt's lab to work on colon cancer and a related disease called inflammatory bowel disease (IBD). It's thought that bacteria contribute to cancer risk by inducing inflammation. Though the etiology of IBD is not known, it is a disease of inflammation, and IBD patients have an increased risk for colon cancer."

Her current work stems from a realization she had while working on her Ph.D. "I was working in a laboratory that focused on bacterial pathogenesis and realized that I was interested in looking at the interface between inflammation and cancer. MicroRNAs may help us better understand this interface."

Sohn finds the environment within the Harris lab to be truly unique. "Curt's lab does three different things: basic research, translational research, and molecular epidemiology. NIH encourages basic scientists to collaborate with others doing translational science or epidemiology, and here it is already happening just within this lab.

"I came from a basic science setting," Sohn noted, "and now have an appreciation for human disease that I didn't have before."

Sohn did her doctoral work at the Massachusetts Institute of Technology.



(Photo: R. Baer)

**Aaron Schetter, Ph.D., M.P.H. (left), and Jane Sohn, Ph.D. (right)**

# A Better Immunotoxin

*Through inspired engineering, proteins that enter and kill cancer cells are finding their place among cancer treatments.*

(Photo: R. Baer)



Ira Pastan, M.D.

*Just as computer makers continue to devise smaller, faster, and more powerful laptops, the scientists in CCR's Laboratory of Molecular Biology (LMB) will not stop re-engineering their cancer-killing immunotoxins until they have created the optimum life-saving treatment.*

Immunotoxins are proteins designed to deliver a lethal blow directly to cancer cells. Each has two parts: a custom-designed antibody that can home in on a specific target on the surface of the cancer cells and a toxin, which delivers the fatal blow. When the antibody binds to its target, the whole complex is pulled inside of the cancer cell, where the toxin can do its work.

The team of CCR researchers, led by Ira Pastan, M.D., the Laboratory's Chief, shares a drive to do more for people with cancer. They have already begun to see their determination to devise better immunotoxins pay off, but they are continuing to dig deeply into the workings of the cell to learn why immunotoxins work in some patients and not in others. And their ability to quickly translate these insights from the labs at CCR to the infusion rooms at the NIH Clinical Center is helping them move deftly toward new anti-cancer therapies.

After spending most of his career studying cell surface receptors and their signaling pathways, Pastan shifted gears. "Since I was a physician trained to do research," he recalled, "I wanted to use what I knew to do something relevant to the treatment of cancer." He began working with *Pseudomonas* exotoxin A (PE), a bacterial protein with potent cell-killing activity, and became intent on finding a way to deliver the lethal toxin to tumor cells.

## Proof of Concept

When Pastan first started working with immunotoxins in the early 1980s, existing PE-based immunotoxins had several limitations. They were complex and costly to produce, and their large size prevented them from efficiently penetrating bulky tumors.

Pastan was able to make several important improvements by using recombinant DNA techniques to engineer smaller, more nimble immunotoxins that could be

**The LMB-2 trial clearly illustrated that powerful, targeted cell-killing agents could be designed and produced using genetic engineering.**

produced inexpensively in large quantities. "That conversion," said Pastan, "from coupling toxins to antibodies using chemistry to using genetic engineering to make these molecules, was really a big breakthrough."

His team's first target was a receptor, CD25, found on the surface of many B- and T-cell cancers as well as some normal B and T cells. Using an immunotoxin called LMB-2, Pastan and CCR colleague Robert J. Kreitman, M.D., Head of the LMB Clinical Immunotherapy Section, conducted a Phase

I clinical trial in patients with CD25-expressing hematological malignancies that had failed previous treatment.

LMB-2 was well tolerated by the study's patients. More importantly, several patients on the trial experienced a disease response within one week of treatment with LMB-2. In particular, four trial participants with a rare cancer called hairy cell leukemia (HCL) all demonstrated major responses to the immunotoxin. In one patient, the count of malignant cells dropped to an undetectable level. The LMB-2 trial clearly illustrated that powerful, targeted cell-killing agents could be designed and produced using genetic engineering.

Though the results were encouraging, Pastan and his colleagues wanted to develop immunotoxins to treat more common cancers (only 900 to 1,000 people are diagnosed with HCL in the United States each year). They decided to target CD22, a membrane protein commonly expressed on B-cell lymphomas and leukemias. While CD22 is also expressed on normal B cells, it is not present on the stem cells that generate B cells, allowing the body to readily regenerate its B cells and making CD22 a potentially good target for immunotoxin therapy.

Pastan teamed up with David J. FitzGerald, Ph.D., Head of the LMB Biotherapy Section, and Kreitman to design a new immunotoxin—dubbed BL22—to deliver PE to CD22-expressing cells and test it in a Phase I clinical trial in patients with chemotherapy-resistant non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), and HCL.

Even at low doses, many HCL patients responded to BL22. With increased doses, their responses grew more frequent and more dramatic. The cancer cells of many HCL patients completely disappeared from the blood, bone marrow, and spleen, while normal blood cells—which are commonly at dangerously low levels in patients with advanced blood malignancies—returned to normal levels.

More than half of the patients with advanced HCL were able to return to a normal life, and many remain in complete remission more than three years after the initiation of treatment. In a 2005 review article, Pastan noted that “This clinical experiment, in which more than half of the patients with an advanced malignancy were able to resume a normal life, was certainly the most rewarding experiment in my research career.”

### Beyond Hairy Cell

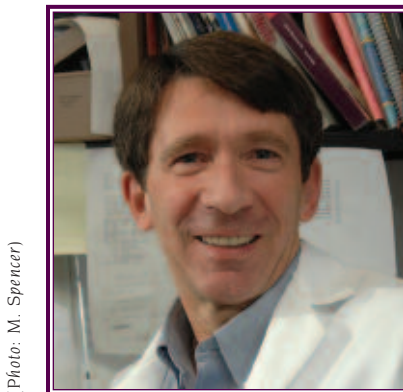
Although the responses in HCL patients to BL22 were profound, results in patients with other cancers were less encouraging. This information left the Pastan research team with a challenging decision. Should they continue to work on BL22 and try to get it approved for treatment of the relatively rare HCL? Or should they try to engineer an immunotoxin that could effectively treat more common CD22-expressing cancers like CLL, which afflicts more than 15,000 each year?

The group decided they could make an immunotoxin that did both. They knew that they had to find a way to deliver more immunotoxin to CLL cells. “CLL cells don’t have as much CD22 as HCL cells,” Kreitman explained. Indeed, a single HCL cell might have as many as 70,000 CD22 proteins on its surface, giving BL22 ample opportunity to latch onto the cell. CLL cells often have fewer than 1,000.

They needed an antibody that binds with higher affinity (more tightly) to CD22. This would make it less likely that the immunotoxin will fall off once it binds to the cell, increasing the probability that it will be ushered inside.

To optimize BL22’s affinity, the Pastan lab utilized a method called hot spot

mutagenesis, which mimics the way the immune system would do it. They altered specific regions of their anti-CD22 antibody and looked for mutants that bound more tightly to CD22. When they found what they were looking for, they built HA22—which is up to 20-fold more powerful than its predecessor. Pastan and his colleagues recently launched a Phase I/II trial to find out if HA22 can more effectively combat both cancers. Initial results have been promising, and the team hopes to know within the next few years whether the new immunotoxin will prove to be an improved treatment against CD22-expressing malignancies.



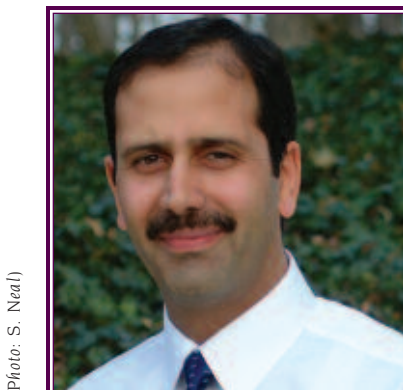
(Photo: M. Spencer)

**David J. FitzGerald, Ph.D.**



(Photo: M. Spencer)

**Robert J. Kreitman, M.D.**



(Photo: S. Neal)

**Raffit Hassan, M.D.**

### Tackling Solid Tumors

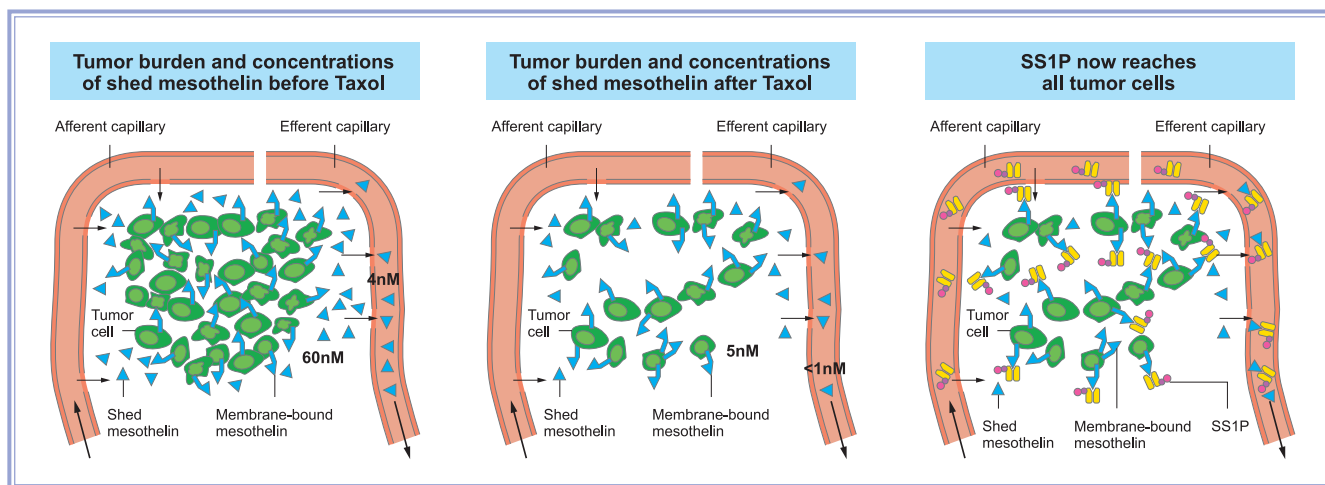
While hematological malignancies are well suited for immunotoxin treatment because they are easily accessible via the bloodstream, solid tumors are harder to reach and to penetrate. Pastan and his group, however, believe that these barriers are surmountable and have set out to identify a target to help them confirm their belief.

More than ten years ago, Pastan and his LMB colleague Mark Willingham, M.D., now at Wake Forest University School of Medicine, identified mesothelin when they were searching for proteins expressed in ovarian cancer cells. Mesothelin is highly expressed in more than 70 percent of ovarian cancers, as well as a high percentage of mesotheliomas (cancers of the membrane that encapsulates most of the major organs), lung adenocarcinomas, and gastric and pancreatic cancers.

Though its function in cancer cells has not been well characterized, there is some evidence that mesothelin’s interactions with another protein, CA-125, may promote the spread of ovarian cancer. Mesothelin is normally expressed on the epithelial cells that line body cavities but not in essential organs such as the heart, brain, liver, and kidneys. “Since there is high expression in tumors, but very little expression in normal tissue, it makes a good target for antibody-based therapy,” said Raffit Hassan, M.D., Chief of LMB’s Solid Tumor Immunotherapy Section.

While hematological malignancies are well suited for immunotoxin treatment because they are easily accessible via the bloodstream, solid tumors are harder to reach and to penetrate.

(Image: J. Kelly)



To be effective against solid tumors like those of ovarian cancer, immunotoxins like SS1P (which targets a protein called mesothelin) need to be able to penetrate deeply into the tumor. In mice, treating tumors with a chemotherapy agent like Taxol® before administering SS1P greatly increases the immunotoxin’s activity, possibly in part by disrupting the tumor’s structure.

To target mesothelin, the Pastan lab engineered an immunotoxin called SS1P. In laboratory tests, SS1P had impressive activity against cells from ovarian cancer patients. It also made tumors shrink and prevented lung metastases in mouse models. Based on these preclinical data, Pastan, Hassan, and Kreitman launched two separate Phase I clinical trials to test SS1P in patients with recurrent mesothelioma, ovarian cancer, and pancreatic cancer.

SS1P did not cause toxicities in any essential tissues, such as heart and liver, but it did cause an uncomfortable but not life-threatening inflammation of the body cavity lining. Patient responses to treatment were modest, but they were considered encouraging when compared with results seen with other immunotoxins and antibody-based therapies used against solid tumors.

There currently are limitations to SS1P’s effectiveness. Like other immunotoxins and antibody-based therapies, SS1P has trouble penetrating solid tumors. Such tumors have poor vascular and lymphatic systems, impeding the delivery of treatment agents. They also have high internal pressures, which favor an outward rather than an inward flow of material. In addition, the Pastan lab has found that solid tumors shed large amounts of mesothelin from their cell surfaces, which may absorb the immunotoxin and keep it from interacting with and killing target cells.

Interestingly, Pastan’s group has found that tumor-bearing mice treated first with a chemotherapy agent called Taxol® and then with SS1P show dramatic tumor regressions due to a synergistic interaction of the two drugs. “We think the synergy is caused by two things,” Pastan explained. “We think the Taxol kills some tumor cells and disrupts the organization of the tumor, allowing the immunotoxins to penetrate better. And the shed mesothelin, which is very high in the tumor, falls to very low levels and doesn’t soak up the immunotoxin. Taken together, this strategy gives us fantastic synergy.”

Pastan and Hassan are preparing to test their theory in a Phase II clinical trial

Even with cutting-edge high-throughput techniques, finding the ideal immunotoxin target is a difficult task.

combining chemotherapy with SS1P. The researchers hope that this combination approach will be a newly effective strategy for targeting solid tumors with immunotoxins.

The other protection solid tumors have that hematologic malignancies do not is a strong immune system on the part of the

patient. “So far we can only give patients with solid tumors one or two cycles before their immune systems mount a very good antibody response to the toxin and neutralize it,” Pastan lamented. This issue is rarely a problem in patients with hematological malignancies because their immunity is much weaker, having been suppressed by both the disease and previous chemotherapies. As a result, these patients can receive multiple cycles of therapy.

Pastan’s team is pursuing several strategies to enable the immunotoxins to evade the immune system. For example, they are trying to identify regions of the toxin recognized by patients’ immune systems and then modify or delete them using protein engineering.

### Immunotoxins of the Future

The CCR research team continues to learn how to make immunotoxins even more deadly to cancer cells and to identify new therapeutic targets. Scientists know that the PE toxin inhibits protein synthesis once inside the cell but not how this triggers cell death. FitzGerald is exploring where the toxin goes after gaining entry; perhaps, he said, “Different cell surface targets may shuttle the toxin along different intracellular paths. If one pathway offers greater benefit to patients, perhaps an immunotoxin could be engineered to follow that better path.”

Even with cutting-edge high-throughput techniques, finding the ideal immunotoxin target is a difficult task. Proteins must meet several stringent criteria to qualify as potential immunotoxin targets. They must appear on the cell surface, because immunotoxins are unable to cross cell membranes unassisted. And they must be expressed mainly on cancer cells and nonessential normal cells, not in essential tissues such as the kidneys, heart, and brain; any attack by an immunotoxin on these tissues could result in serious side effects.

Pastan and his colleagues found one new receptor by scouring the literature to identify proteins that are selectively expressed in certain types of cancers. FCRL1 is part of the Fc receptor-like (FCRL) family of proteins, which is expressed by different cells of the immune system. FCRL1 is selectively expressed on B cells and is present at high levels on the tumor cells of CLL patients. The scientists have generated two immunotoxins that recognize FCRL1, both of which have shown promise in the culture dish. These new immunotoxins are now ready for testing in animals.

The Pastan lab is also particularly interested in identifying new targets for breast and prostate cancers, since people can survive without the normal tissues associated with these organs. Searching through gene databases, Pastan lab Staff Scientist Tapan Bera, Ph.D., and colleagues in the lab have found a potential target for prostate cancer, a protein called NGEF, that is expressed in prostate cancer and normal prostate cells but not in essential tissues. The team is now developing antibodies to NGEF, which will be used as the basis for new immunotoxins that they hope will represent a new treatment option for prostate cancer patients.

Pastan's work has been over 20 years in the making, but his progress in turning his basic discoveries into effective tools has been accelerating quickly. Bera joined the Pastan lab in 1995, "to be involved in a research project where you can see the clinical benefit right away." He has not been disappointed.

## From Dangerous Fatigue to an Energetic Retirement

(Photo: Courtesy of D. Brenneman)



As he neared his fiftieth birthday, Dave Brenneman, an engineer for the State of Pennsylvania, began to notice that his energy was flagging. He tried to convince himself that it was all in his head or the inevitable result of getting older, but when his fatigue persisted, he decided to get a check-up. His family doctor told

him that his blood counts were lower than normal. A visit to a local oncologist and a series of additional tests revealed the "odd-looking" cells of hairy cell leukemia (HCL) in his blood.

Like many HCL patients, Brenneman was treated with the drug cladribine. His blood counts climbed back to normal over the next few months. "I was feeling great," Brenneman related, "but after approximately three years, my counts started to drop again." Two years later, he received another round of cladribine, but this time the cancer began to come back after only a year.

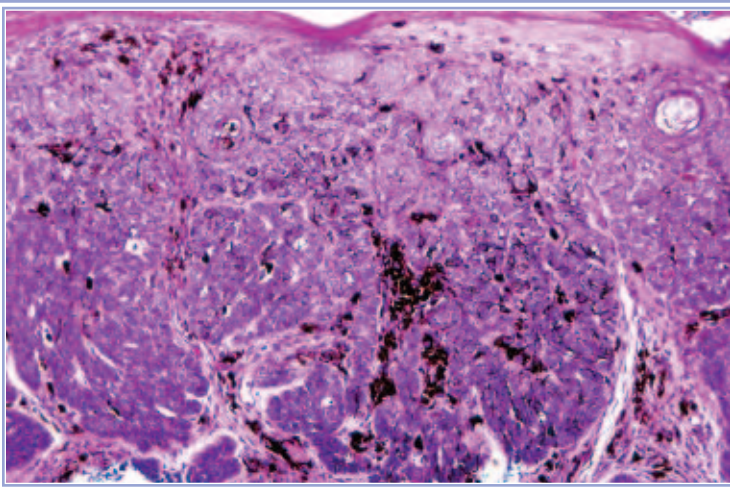
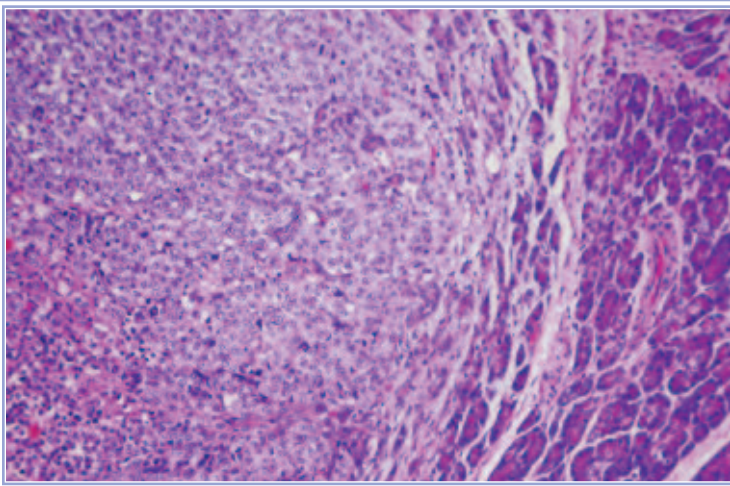
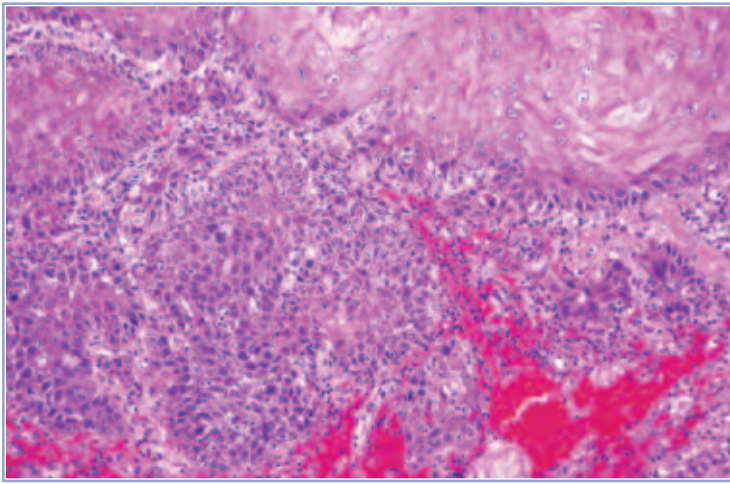
A proactive patient, Brenneman turned to the Internet to find other potential treatment options for HCL. He stumbled across information on a clinical trial being conducted on a new drug called BL22 and contacted Robert Kreitman, M.D., at CCR to find out more.

Kreitman and his colleagues looked carefully at Brenneman's cancer and decided that he was a good candidate to receive BL22. "His bone marrow was 70 to 80 percent HCL cells," Kreitman recalled, "and his neutrophils, which help fight infection, had decreased to a count of 508. Less than 500 is considered life-threatening."

Brenneman had reservations about joining a clinical trial. "I was very skeptical, but as I learned more and talked with Kreitman and his staff, I became more comfortable with the idea." He traveled to the NIH Clinical Center to receive his first cycle of the immunotoxin in September 2005. That one cycle was enough to trigger a complete remission. "His neutrophil count four weeks later was normal and the hairy cells were gone from his bloodstream," Kreitman explained. "Repeat tests showed no hairy cells left in his bone marrow either." The benefits of that single cycle of BL22 have lasted; Brenneman's bone marrow biopsies and blood tests have remained clear for over two years.

Thanks to BL22 and the dedicated team of CCR researchers, Dave Brenneman is making the most of his recent retirement. He has plenty of energy to maintain the dairy farm that has been in his family for three generations, work on his classic car, and spend time with his wife, children, and grandchildren. His strong response also illustrates the potential of BL22 and next-generation immunotoxins for treating HCL and—with continued improvements—other cancers as well.

# No Longer Skimming the Surface of Skin Cancer



When *Stuart H. Yuspa, M.D.*, began studying the skin in the late 1960s, scientists understood some of the basic biology of skin cancer. They knew what was required to produce and diagnose benign and malignant tumors—but not much more. “We’ve made remarkable leaps since then,” says the Co-Chief of CCR’s Laboratory of Cancer Biology and Genetics (LCBG).

Squamous cell carcinoma (top) is a relatively aggressive skin cancer, and it shares many of the features of lethal solid tumors of the internal organs. The Yuspa laboratory’s efforts to model the biology of skin have given great insight into the genesis and treatment of this and other major skin malignancies, such as basal cell carcinoma (middle), and malignant melanoma (bottom).



Stuart H. Yuspa, M.D.

(Photo: E. Branson)

(Photos: C.R. Lee, CCR)

“Now we know the genetic changes associated with each stage of cancer development and how those genetic changes translate to biochemistry in each stage,” Yuspa, a specialist in squamous cell skin cancers, explained. “And we have markers to recognize where we are in the progression from normal to malignant.”

Yuspa added, “We also know a lot more about normal skin. We know how skin homeostasis is controlled and the pathways that regulate it. That’s extremely important because you have to understand normal to understand abnormal.” To generate this understanding, over the last 36 years the Yuspa laboratory has developed *in vitro* models that recapitulate the normal growth and differentiation of skin epithelial cells called keratinocytes—precursors to the squamous cells that give squamous cell skin cancer its name. The models also reproduce each stage of carcinogenesis as it occurs in mouse skin cells.

These model systems have been “really important for understanding mechanisms of cancer,” said Adam Glick, Ph.D., a former Postdoctoral Fellow and Principal Investigator who still collaborates with Yuspa.

And the work is moving from discovery to application. Yuspa, Glick, and their colleagues recently identified genetic markers that distinguish low-risk benign skin tumors from high-risk tumors in mice and opened possibilities for targeted therapies that

block early tumors from progressing to invasive lesions.

Yuspa’s models are making inroads for other cancers as well, since similar epithelial cells are involved in cancers of many internal organs, such as the lungs, head and neck, esophagus, colon, and stomach.

### A Good Decision

Yuspa first came to NCI in 1967 to work in the lab of Richard R. Bates, Ph.D., who was working on skin carcinogenesis. “There were two of us and a few technicians,” Yuspa recalled. “We did some very nice work looking at carcinogen binding to DNA and how that caused mutations. I loved it! I stayed for the two years of my United States Public Health Service Commissioned Corps obligation, then asked to stay a third.” The young M.D. then left to pursue his clinical training to see what aspect of medicine he liked most—research or clinical work.

“Dick said he’d hold the spot for me, which would be impossible today, but it was the early days of the War on Cancer,” Yuspa continued. After finishing his medical residency training—and realizing that the lab was where he wanted to be—Yuspa returned to the Bates lab to work on the skin model. He became a Senior Investigator at NCI in 1972.

### The Place to Be

“Stu’s lab was a place that people came to from all over the world—and still do—to

learn how to culture the skin,” according to Molly Kulesz-Martin, Ph.D., who was a Postdoctoral Fellow in Yuspa’s lab from 1979 to 1981 and is now Director of Research and Professor in the Department of Dermatology at Oregon Health & Science University (OHSU).

When Kulesz-Martin joined the Yuspa lab, very few people were working on epithelial cells, even though most human cancers arise from them. “Everybody was working on cells that were easy to grow, but there was no way to assure they were like some particular organ in the body. And they weren’t,” she recalled.

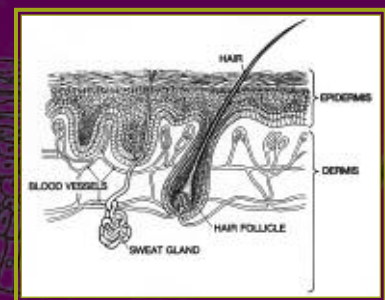
“Stu was working out ways to grow epithelial cells so they behaved as they do in the body. We had to find a way to make culture conditions good for growing epithelial cells,” said Kulesz-Martin. Her work built on earlier laboratory observations that reducing calcium levels enabled cells to grow for much longer than two weeks. From that, she created a transformation assay to quantify the strength of various carcinogens.

Kulesz-Martin’s experiences in Yuspa’s lab set the path for her future as a researcher. At OHSU, she continues to work with mouse and human cells to determine the initial changes and later insults that push a cell from normal to malignant to metastatic. “I didn’t fall far from the tree,” she admitted. “I still want to study cancer, still study skin as a model, and I’m still

## What Kind of Skin Cancer?

Each type of skin cancer—melanoma, basal cell carcinoma, and squamous cell carcinoma—arises in different cells within the skin. Melanoma forms in melanocytes (skin cells that make pigment) and is the most dangerous form of skin cancer. Basal cell carcinoma forms in cells found in the hair follicles. Basal cell cancers are the most frequent human tumor in Caucasians, but they grow slowly and rarely spread to other parts of the body.

Squamous cell carcinoma, the focus of the work of Stuart Yuspa, M.D., begins in squamous cells (flat cells that arise from keratinocytes and form the surface epidermis of the skin). They are not the most frequent skin cancers, but they share many characteristics—genetically and biochemically—with highly lethal cancers that arise in internal organs like the lung, head and neck, esophagus, and stomach.



The skin’s epidermis harbors the keratinocytes and melanocytes that give rise to skin cancer. The Yuspa laboratory’s efforts to model the biology of skin have given great insight into the genesis and treatment of skin cancers.

(Graphic: NCI)

learning new things about cancer genes that tell us about skin cancer and other kinds of epithelial cancers, such as prostate, liver, and kidney cancers.”

“Many of the events that Stu has identified in the mouse have been confirmed as also being causal genetic alterations that result in human tumors,” said Dennis R. Roop, Ph.D., who worked with Yuspa at NCI in the 1980s and is now Chair of Regenerative Medicine and Stem Cell Biology at the University of Colorado Health Sciences Center. “His early studies in the mouse provided the groundwork for understanding that after a stem cell is initiated, it can sit for a long time, then get an accumulation of other genetic events to lead that initial epidermal stem cell to result in a tumor.”

The epidermis, where the keratinocytes live, is a hotbed of cell turnover, Roop explained. This outer layer of our skin replaces itself every three to four weeks in humans. “Yet many of us develop skin cancer late in life. Only the stem cells that reside for a lifetime are around to accumulate enough genetic damage to lead to cancer.”

Roop added, “Stu was also really the first to identify mutations in the Harvey *ras* gene [a proto-oncogene known for its connections to skin and bladder cancers] as a complete initiating event by showing that just a single Harvey *ras* mutant gene in a keratinocyte [that is grafted onto an animal model] would result in formation of a tumor.”

“All of us have learned so much from the work Stu started and others who’ve further developed the model,” Kulesz-Martin added.

### A Physician’s Dream

“It’s everyone’s dream,” said Yuspa, “particularly if you’re a physician who’s done basic science for the last 30 years, to translate your discoveries into treatments for patients.” To do that, Yuspa’s team is focusing on two relatively new discoveries. Using a mouse model of induced skin inflammation, they are studying how inflammation influences the frequency with which skin cancers develop. In this model, skin cancer is much more frequent. The group identified a receptor that seems to be essential for tumors to develop. He hopes that it could be a novel target for preventing or treating skin cancer.

The second discovery builds on an insight made nearly 10 years ago. They have shown that inactivation of the tumor suppressor gene *p53* is important for malignant

# A Generous Spirit

(Photo: Courtesy of M. Kulesz-Martin)



**Molly Kulesz-Martin, Ph.D.**

(Photo: Courtesy of A. Glick)



**Adam Glick, Ph.D.**

Colleagues and former students of Stuart Yuspa, M.D., unanimously point out his generosity with his ideas and time—and his love of science.

He has built a dynamic community of researchers exploring skin cancer. He sees mentoring as a happy obligation. “The only legacy that will be remembered is the people you’ve trained,” Yuspa said. “Part of my job as a scientist, mentor, and member of the NIH is to help anybody I can to succeed.”

About 36 postdocs have moved through his lab. “I couldn’t be more proud of the people who’ve trained here. They’ve been uniformly successful and most [30] have stayed in the same field,” Yuspa beamed.

“The reason that many of us have continued to work in science is that his enthusiasm for science is infectious,” said Dennis Roop, Ph.D. “When we trained with him, we could see how much he enjoyed science. He hasn’t lost that. He’s still like a little kid when he starts talking about science.”

Adam Glick, Ph.D., called Yuspa the “grandfather of the field.” Go to any of the

big meetings, he noted, and everyone is somehow connected to Yuspa, either as collaborators, postdocs, or postdocs of his postdocs.

Replying wryly that he’d rather be called the “father of skin carcinogenesis,” Yuspa agreed that his proudest moments in science occur at meetings when he sees his protégés and theirs presenting important and interesting information. “It feels very good.”

“Once you go to Stu’s lab, you become part of a huge network of people from around the world who are doing top-rate science,” said Molly Kulesz-Martin, Ph.D. “We’re all excited about the skin and the science of the skin.”

And these “Yuspa graduates” open their arms to the new people who continue to come through the lab. “We share with anybody who asks. It’s a trait of Stu’s that we learned at his knee,” Kulesz-Martin continued. “I remember talking to Stu about how competitive science is and how people don’t want to share. He said, ‘I’ve always shared data. Maybe sometimes you get burned, but I always learn something new when I share.’”





(Photo: Courtesy of D. Roop)

Dennis Roop, Ph.D. (left), presents Stuart Yuspa, M.D. (right), with the Stephen Rothman Memorial Award for Investigative Dermatology in 2004. The Rothman Award is the highest award given by the Society to members who have made outstanding contributions to investigative cutaneous biology.

conversion, a late event in carcinogenesis in the skin. Using keratinocytes from *p53*-null mice, they discovered one of *p53*'s transcriptional targets, a gene called chloride intracellular channel 4 (*CLIC4*). *CLIC4*'s gene product, CLIC4, was originally described as a chloride channel protein but, as it turns out, is multifunctional, being involved in cellular responses to stressors such as DNA damage, metabolic inhibition, and senescence. Yuspa's team found that, in mouse skin cancer and most major human cancers, CLIC4 is lost from tumor cells. The protein is essential for cell death induced by *p53*. They are trying to reactivate the expression of *CLIC4* in tumor cells—not an easy feat. “We know the gene is there; it's not lost, just silenced in some way,” said Yuspa. They are hopeful they can unsilence the gene, reactivate *CLIC4* expression, and have some influence on tumor cell growth.

Other pathways are standing out as significant players as well. Yuspa's group has identified a pathway through protein kinase

C delta (PKCdelta)—a regulator of many different signaling pathways studied in numerous solid and hematologic cancers—that is important to the development of skin cancer in mice. In an animal model, Yuspa has been able to stimulate PKCdelta in tumor cells and cause the tumor cells to regress, activating a cell death (or apoptosis) pathway.

An Australian company named Peplin is developing a natural product drug that works through a similar mechanism. In clinical trials, this drug appears to do the same thing to PKCdelta in humans that the Yuspa lab has done in animals. NCI has a cooperative research agreement with the company to sort out the pathways involved in the death of the tumor cells.

### Looking More than Skin Deep

Yuspa's group continues to make exciting discoveries today. A recent study with Adam Glick, now an Associate Professor in the Center for Molecular Toxicology and Carcinogenesis at Pennsylvania State University, has ramifications for early detection and prevention of skin and other cancers.

In May 2007, Yuspa, Glick, and colleagues from the American University of Beirut reported that they had found an 87-gene expression pattern, or genetic signature, in mice that distinguishes benign tumors with a high or low risk of becoming malignant. In particular, the high-risk lesions had reduced expression of immune function genes compared with low-risk lesions, a finding Yuspa calls a “surprising, important component of the study. High-risk tumors may in some way be protected from surveillance by the immune system.”

Now they will have to determine how those findings can apply to human cancer—and moving from mouse to human is neither easy nor automatic. Human skin cancers are induced by ultraviolet light, while the tumors in Yuspa's animal models are induced by chemical carcinogens or oncogenes. As a result, the genetics may differ. “But,” Yuspa added, “we feel that the pathways

that are downstream from the inducing agents are very similar.”

Knowing that skin cancer takes different forms, Yuspa has also started using his models to investigate different cells. He has initiated a collaboration with Glenn Merlino, Ph.D., his Co-Chief at the LCBG, who studies melanoma. Since both melanomas and squamous cell cancers occur in the skin, and there is an intimate relationship between keratinocytes and melanocytes in the progression of melanoma, “bringing the two models together will give us great insight into melanoma's induction,” said Yuspa.

He has also pulled in partners to make a difference in internal organ cancers as well. “I've convinced [LCBG Adjunct Investigator] Jonathan Wiest, Ph.D., to look at skin as a model for lung cancer,” Yuspa said. With Adam Glick, he's hoping to look at head and neck cancers.

“These are all areas where we think we can go beyond boundaries,” he said.

“It's everyone's dream,” said Yuspa, “particularly if you're a physician who's done basic science for the last 30 years, to translate your discoveries into treatments for patients.”

# A Series of Fortunate Events

*In the world of scientific research, methodical approaches, driven by logic, reign. Some of the most influential and lasting contributions to science, however, are the result of unexpected twists in experiments, serendipitous “accidents.” Penicillin, for example, was discovered in 1928 by Alexander Fleming after he neglected to properly clean bacteria cultures and left them in his lab. The renowned 19th century chemist and biologist Louis Pasteur once said, “Chance favors the prepared mind.” Rather than relying solely on methods or on chance, however, truly visionary scientific discovery can only be achieved through a balance of both. Nancy E. Davidson, M.D., former Medical Staff Fellow at CCR, now President of the American Society of Clinical Oncology and Director of the Breast Cancer Research Program at Johns Hopkins University’s Sidney Kimmel Comprehensive Cancer Center, offers her thoughts on how that balance has affected her career as a breast cancer clinician and researcher.*

As a high school student growing up in Potomac, Md., near the National Institutes of Health, I attended a program at the National Naval Medical Center. While there, I was drawn to biology, an interest that shifted to molecular biology during my undergraduate and medical studies. Through a series of serendipitous events, I spent part of my undergraduate years working part time in a laboratory that was focused on liver cancer, an experience that sparked my interest in cancer.

As a student at Harvard Medical School, I again found myself working in a lab during the summer, this time in a breast cancer laboratory at NCI. It was here that serendipity yielded to passion and planning. That summer was life-defining for me, as it helped me realize that I wanted to focus on breast cancer. This realization not only gave me a clear path for my training, but it ultimately led me back to NCI as a Medical Staff Fellow from 1982–1985, and then to Johns Hopkins, where I have been a faculty member for over 20 years.

The true power of a team can only come from listening and interacting with other researchers, clinicians, and advocates, all committed to advancing research and helping patients.

Good science is generally a combination of reason and providence, and translational breast cancer research requires a mix of laboratory insight and clinical observation. Investigation at the interface between the bench and bedside drives my research, even today, and it was a key factor in my development as an independent physician-scientist. Early in my career, I was given the opportunity to lead a large NCI-sponsored clinical trial focused on premenopausal women with breast cancer. At this critical early time, I was—and still am—fortunate

to participate in the cooperative group process as a committee member and chair involved in the conduct of a number of clinical trials that examined optimal treatment for women with breast cancer. These collaborations have helped advance the standard of care in breast cancer by establishing appropriate kinds of chemotherapy and hormone treatments, and they instilled in me a lasting fascination with the concept of hormones in breast cancer and why some types of cancer do not respond to estrogen-related therapies while others do.

In addition to my early clinical research experiences, the collaborative environment of the laboratory also influenced my work. When I first joined the faculty at Johns Hopkins, my research centered on the roles of oncogenes in breast cancer growth. A laboratory colleague of mine at the time, Johns Hopkins scientist John Isaacs, Ph.D., pointed out a disconnect between my research focus and my clinical practice. "As a physician," he noted, "you work with patients to destroy the cancer invading their bodies. But as a researcher, you devote your time to discovering how to make breast cancer cells *grow*."

This chance conversation had a transformative effect on my research. I turned from studying the proliferation of breast cancer cells to studying their death, refocusing my work on apoptosis in breast cancer cells. Over time, my focus shifted again to bring my laboratory research back in line with my abiding clinical interest in the unique biological connections between hormones and breast cancer.

These connections have been a common theme in the field for many years. The estrogen receptor (ER) has long been the target of therapies aimed at reducing the growth-promoting influence of estrogen on breast cancer cells; the first hormone therapies for breast cancer were actually used over 100 years ago. While this is not a new arena for us, it is kept vital by the strides that researchers have made in recent years toward understanding the biology of hormones and breast cancer and its clinical exploitation.

But about 25 percent of human breast cancers lack ER expression, a trait that makes them resistant to hormonal therapies. Our current research focus on the epigenetics of ER expression is aimed at understanding one mechanism by which ER expression may be silenced in some

breast cancers. With this knowledge in hand, we can work to develop a way to reverse this process, perhaps making it possible to treat these endocrine-resistant forms of cancer with traditional hormonal treatments.

As a new breast cancer researcher in the 1980s and 1990s, I had the good fortune to enter the field in a time of great change and promise, a feeling that persists even today. The advent of modern molecular biology techniques, advances in our knowledge of the human genome, engagement of breast cancer patients, and a shift toward very scientifically-based clinical trials should put us even closer to achieving our goal of reducing the burden of cancer.

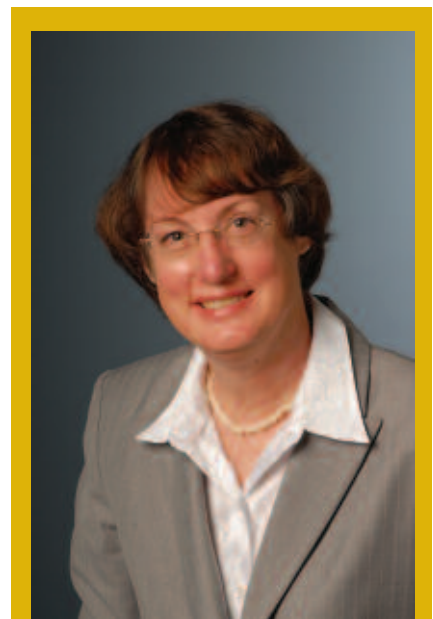
This optimism, however, is tempered by the reality of the research and healthcare environment that both researchers and clinicians face on a daily basis. Though we have made a number of inroads, we are still confronted by challenges that make it difficult to take advantage of these opportunities in the current research and clinical climates. Reduced research funding, increasing healthcare costs, and a population of approximately 47 million uninsured Americans<sup>1</sup> make it more difficult to use our growing knowledge about cancer to improve the well-being of all patients.

To overcome these research and clinical challenges, it is important that we not only remain open to those serendipitous moments that present themselves but also utilize them in the most efficient way possible. In addition, we must recognize the benefit of collaboration in our work. The true power of a team can only come from listening and interacting with other researchers, clinicians, and advocates, all committed to advancing research and helping patients.

As a researcher, I am ever more interested in working at the interface between

the lab and the clinic. My lab science grows more reflective of questions that I see in the clinic, and I like to think that my clinical research and clinical practice are ever more driven by good science and rigorous evidence. Of course all of the techniques and methods used in breast cancer research can be applied to different types of cancer as well. I believe that all cancer researchers can make focused and practical choices while staying receptive to the unexpected moments that can help drive truly beneficial science forward.

<sup>1</sup>National Coalition on Health Care. "Health Insurance Coverage." <http://www.nchc.org/facts/coverage.shtml>. Accessed February 27, 2008.



(Photo: Courtesy of N. Davidson)

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# Combating HIV, One Tumor at a Time

*Robert Yarchoan, M.D., vividly remembers the day in 1981 when, as a Clinical Fellow in the Metabolism Branch of NCI, he saw a young man who had developed a profound immunodeficiency. That patient, who had almost no T lymphocytes, represented a moment in medical history—the first NIH patient with what would come to be known as acquired immunodeficiency syndrome (AIDS). Since that time, Yarchoan, now Chief of the HIV and AIDS Malignancy Branch and Director of the newly formed NCI Office of HIV and AIDS Malignancy (OHAM), has seen firsthand how HIV/AIDS has risen as a global epidemic, how the development of highly active antiretroviral therapy (HAART) has revolutionized AIDS treatment, how malignancies associated with AIDS have tested both patients and doctors, and how the long-term effects of living with AIDS can bring with them a new host of challenges.*

**The discovery by Luc Montagnier and Robert C. Gallo of a novel human retrovirus, and Gallo's demonstration that this virus was the cause of AIDS, were the first major milestones in our efforts to understand, prevent, and treat the disease.**

An estimated 35 million people suffer from HIV/AIDS worldwide, about one million of whom live in the United States. However, when the first patient with AIDS crossed the threshold of the NIH Clinical Center in 1981, we had no way of foreseeing the shape that the future AIDS epidemic would take or the global, cultural, and societal impacts that it would have. All we knew was that we were seeing something new. It was soon evident that clusters of cases of rare tumors like Kaposi's sarcoma were part of this same condition. And over several months, it became apparent that this disease was widespread in the U.S., and that the more severe patients with AIDS we were seeing represented just the tip of the iceberg.

There was great confusion and distress in the medical community as AIDS first

surfaced. Though heroic measures were often utilized to try to treat these patients, they generally died within several months. It was not known how many people had this disease, how it was transmitted, or if anything could be done to treat it effectively.

The NCI had many tools in place to address this new disease, including expertise in immunology, retrovirology, tumor biology, and drug development. I believe that the rapid development of advanced treatments for HIV, in particular highly active anti-retroviral therapy (HAART), was the result of a keen and farsighted, though sometimes controversial, research focus at NCI on HIV itself.

## To Treat a Virus

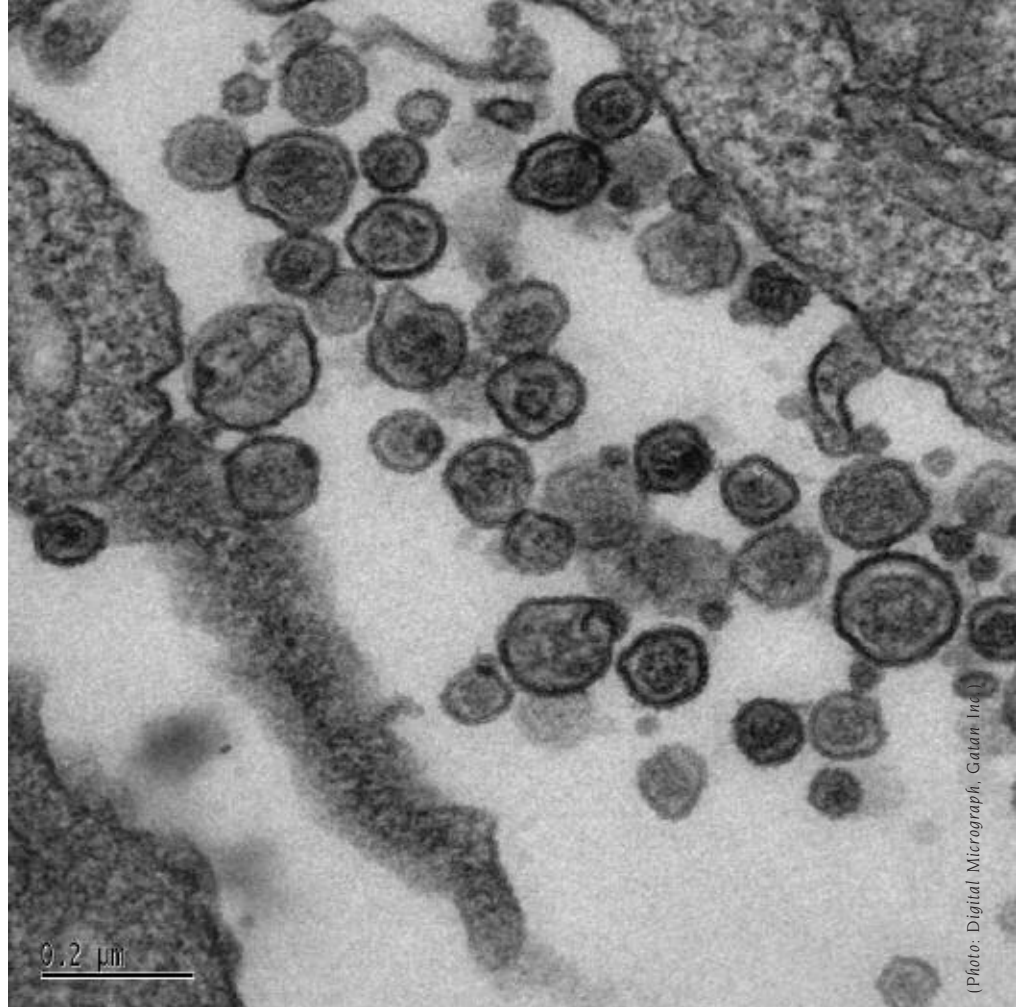
The discovery by Luc Montagnier and Robert C. Gallo of a novel human retrovirus, and Gallo's demonstration that this virus was the cause of AIDS, were the first major milestones in our efforts to understand, prevent, and treat the disease. Prior to these discoveries, most of the research on AIDS was descriptive.

Once the scientific community understood what was causing the immunodeficiency, it was possible to envision effective therapy directed at the root cause. Then-NCI Director Vincent DeVita, Jr., M.D., asked Samuel Broder, M.D., and his group, of which I was a member, to spearhead an effort to develop treatments for AIDS. Broder, Hiroaki Mitsuya, M.D., Ph.D., now Head of the Experimental Retrovirology Section of the HIV and AIDS Malignancy Branch, and I constructed the hypothesis that by blocking HIV replication, it might be possible to reverse the immunodeficiency caused by the virus. This idea was somewhat controversial at the time, as no such therapies existed for other progressive viral diseases, and it was

not clear that blocking HIV would lead to immunologic improvement. In a short time, our group in NCI developed the first therapies to effectively fight HIV infection, including zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC). AZT was developed in collaboration with Burroughs Wellcome Co., while ddI and ddC were developed within NCI and then licensed to pharmaceutical companies. These drugs all blocked an essential and unique enzyme of HIV, reverse transcriptase. The initial clinical trials of these drugs, both singly and in combination, gave rise to the first effective anti-HIV treatment regimens.

A limitation of these initial regimens was the frequent development of viral resistance. Thus, about a decade later, the first drugs were developed that attacked a different HIV target, the viral protease. The creation of these so-called protease inhibitors, which block the active site of HIV protease, was in part based on structural studies of this enzyme conducted in the NCI-Frederick program. When combined with two nucleoside reverse transcriptase inhibitors (like AZT and ddI), protease inhibitors were able to suppress HIV replication dramatically, often to undetectable levels. This combination drug therapy, the backbone of HAART, revolutionized the treatment of HIV. Patients improved clinically and had marked improvement in their immune function. There was a dramatic drop in the death rate from AIDS. In addition, there was a marked decrease in the number of AIDS-associated tumors, and survival of patients with these tumors improved. For our contributions to the development of HAART,

**This combination drug therapy, the backbone of HAART, revolutionized the treatment of HIV patients.**



**Figure 1: Tireless work on the part of researchers at NCI and other institutions has led to dramatic decreases in the mortality associated with HIV/AIDS and AIDS-related malignancies. But challenges still remain.**

Mitsuya and I were honored in 2006 with the first NIH World AIDS Day Award (Figure 2).

The first-generation protease inhibitors, while effective, had significant side effects and required high doses to be effective. Moreover, the development of resistance has been a vexing problem. Therefore, a number of scientists (including Mitsuya) and pharmaceutical companies have been looking into the development of improved, second-generation protease inhibitors. Darunavir, developed by Mitsuya and collaborators and approved by the U.S. Food and Drug Administration in 2006, is one such drug. Darunavir has the advantage of retaining its activity even in patients who have become resistant to other inhibitors.

For several years, my colleague David Davis, Ph.D., and I have been studying the possibility of inhibiting HIV protease by a new mechanism: by blocking the linkage of its two monomers into an active dimer.

Working with the two of us, Mitsuya recently found that in addition to blocking the enzyme's active site, darunavir does just this, making the drug quite different from other approved HIV protease inhibitors. This observation was highlighted as one of CCR's major scientific advances of 2007 (see "Multiple Strategies for Attacking HIV").

### The Tumors of AIDS

One of the hallmarks of HIV is a dramatic increase in the incidence of certain unusual cancers, especially Kaposi's sarcoma and certain aggressive B-cell lymphomas. Kaposi's sarcoma was frequently the cause of death for patients in the early days of the AIDS epidemic. This rare sarcoma—which, prior to the rise of HIV/AIDS, had primarily been known as a cancer of elderly men of Mediterranean or Eastern European Jewish descent—develops in the endothelial cell lining of blood vessels and often affects

(Graph: R. Yarchoan, CCR)

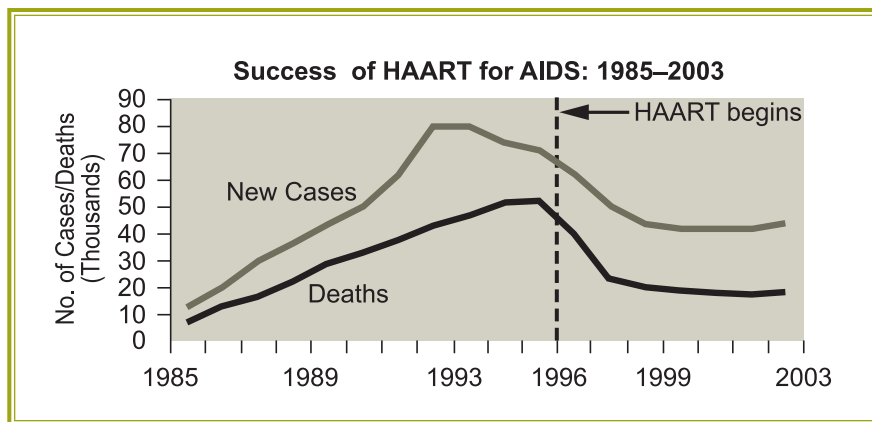


Figure 2: The development of highly active antiretroviral therapy (or HAART) has been the driving force behind a revolution in the treatment of HIV/AIDS.

the skin, though it may also involve the mouth, gastrointestinal tract, and lungs. The tumors appear as angry red and purple patches, often on the legs and feet. These lesions are a visible manifestation of HIV disease, often causing substantial psychological distress for patients in addition to severe pain and debilitation.

The cause of Kaposi's sarcoma remained a mystery until 1994, when Yuan Chang, M.D., and Patrick Moore, M.D., M.P.H. (now at the University of Pittsburgh), discovered a new gammaherpesvirus and showed it to be the cause of Kaposi's sarcoma. We now know that this virus, called Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus-8 (HHV-8), is also the etiologic agent of two other tumors seen in AIDS patients—primary effusion lymphoma (PEL) and some cases of multicentric Castlemans disease (MCD).

After focusing initially on treatments for HIV, my group started turning its collective attention to AIDS-associated malignancies. In the early 1990s, we showed that paclitaxel (Taxol®) was active against Kaposi's sarcoma. Then, since Kaposi's sarcoma is a cancer of blood vessels, we began exploring anti-angiogenic approaches that would interfere with blood vessel growth. We subsequently showed that thalidomide, a strongly anti-angiogenic agent, was also active against the tumor.

More recently, my colleagues James Pluda, M.D., Giovanna Tosato, M.D., and Richard Little, M.D., and I have turned our attention to the cytokine interleukin-12 (IL-

12), a powerful immune system regulator, as a possible treatment for Kaposi's sarcoma. We believed that there were at least three ways in which IL-12 could act against Kaposi's sarcoma:

- It could enhance the immune response against KSHV.
- It could act as an anti-angiogenic agent, shrinking the cancer's heavily vascularized tumors.
- It could work through another protein, a chemokine called inducible protein-10 (IP-10), which inhibits a virally encoded G-protein-coupled receptor that is important for the pathogenesis of Kaposi's sarcoma.

We carried out a clinical study, published in 2006, showing that IL-12 was active against Kaposi's sarcoma when used as a single agent. Subsequently, we decided to see if more rapid and complete responses could be attained by combining IL-12 with a cytotoxic agent, pegylated liposomal doxorubicin (or Doxil®), with known activity against Kaposi's sarcoma.

This research led to the launch of a Phase II clinical trial, spearheaded by Little, in 36 patients with advanced AIDS-associated Kaposi's sarcoma who were either on HAART or needed urgent therapy. All of the patients received IL-12 plus Doxil for 18 weeks, followed by IL-12 alone.

Thirty of the patients demonstrated significant responses, including nine complete remissions. Most of the patients maintained, or even improved, their condition while on the IL-12 only portion of the study, so we published the results in

December 2007. While this regimen has yet to be tested in a controlled trial, the uncontrolled results are quite promising.

### Coming to the Foot of the Matter

Working within CCR provides physician-scientists exceptional opportunities to conduct translational research—studies that can be viewed as a two-way street between the laboratory and the clinic. For some time, clinicians treating Kaposi's sarcoma had been struck by the tendency of this tumor to preferentially develop in certain parts of the body, particularly the feet. The feet are relatively poorly oxygenated, raising the possibility that hypoxia might have some connection to the growth and development of Kaposi's tumors. Later, in discussing this finding with colleagues, it dawned on us that PEL, another of the tumors caused by KSHV, also formed in a site with limited oxygenation (pleural effusions, accumulations of fluid within the chest cavity).

In thinking about these peculiarities with my colleagues, we considered the possibility that KSHV might somehow be responsive to hypoxia. Like other herpesviruses, KSHV can undergo either latent or lytic replication. My group, including Davis and Muzammel Haque, Ph.D., found that in PEL cell lines the virus was activated to lytic replication by hypoxia, and that certain specific genes of the virus could directly respond to hypoxia-inducible factors (HIF) produced by host cells. This was the first time that any virus had been shown to respond to hypoxia or HIF, and it gave great insight into the pathogenesis of KSHV-induced tumors (see "Why Do Kaposi's Sarcoma Lesions Most Often Develop on the Feet?").

This observation led us to consider a novel therapeutic approach for KSHV-induced associated tumors. When activated by hypoxia or other factors, KSHV expresses two lytic genes that encode enzymes capable of catalyzing AZT and another antiviral drug, ganciclovir, into forms that are toxic to human cells. We reasoned that this capability could be used to target tumors caused by KSHV, in particular PEL and MCD, a disease marked by serious, sepsis-like illness caused in part by the production of a form of the cytokine interleukin-6 encoded by KSHV



(Photo: B. Branson)

Robert Yarchoan, M.D.

itself. MCD is also unique among KSHV-related diseases because the virus's lytic genes are already activated.

Together with Little and Deirdre O'Mahony, M.D., we are investigating the use of these two drugs in patients with MCD in a unique clinical trial that is also helping us to further understand the disease's natural history. This trial plays to one of CCR's main strengths: its ability to recruit patients from around the nation and study very rare diseases. Thus far, about half of the MCD patients treated with high doses of AZT and valganciclovir (a prodrug of ganciclovir) have responded. The trial involves a collaboration of many groups within NCI and the NIH that we hope will lead to a better understanding of MCD's pathology and responses to therapy; in the future, we hope to explore this approach in patients with PEL as well. But this chain of events—from the clinical observation of Kaposi's anatomical distribution, to the discovery of KSHV's hypoxic elements, to a novel therapeutic option for MCD—is a prime example of the how clinical observation can lead to laboratory insights

that then lead back to the clinic to inform the design of a new treatment.

### Long-Term Survival, Long-Term Challenges, and Long-Term Opportunities

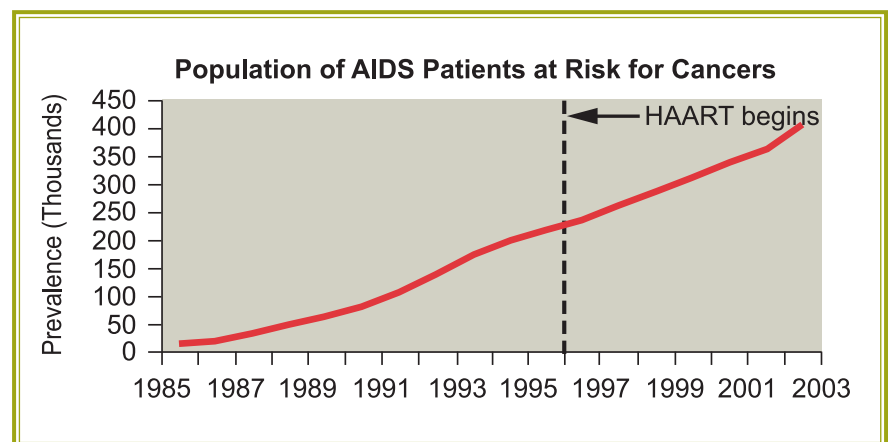
The development of HAART has arguably been the greatest advance in the prevention and therapy of AIDS-related tumors. One of the major victories of HAART has been a reduction in the incidence of certain AIDS-associated malignancies, especially those that develop in patients with markedly reduced CD4 counts. Patients are living for years on HAART, compared to the weeks or months that they might have hoped for 20 years ago.

However, as we enter the second decade of HAART therapy, the epidemiology of AIDS-related tumors and diseases is changing (Figure 3). In some patients, Kaposi's sarcoma tumors that had been under control for years are beginning to grow again. Also, tumors other than those "classically" defined as AIDS-related, such as Hodgkin's disease, lung cancer, and anal carcinoma, are on the rise in the HIV-infected population. The number of deaths from opportunistic infections and advanced AIDS is dropping, while cancer is fast becoming the most common cause of death in AIDS patients. In the meantime, in Africa and other parts of the world where HAART is not commonly available, HIV-associated tumors continue to be major causes of morbidity and mortality.

These new trends, as well as the new

As we enter the second decade of HAART therapy, the epidemiology of AIDS-related tumors and diseases is changing.

opportunities afforded by recent scientific advances, will require a renewed research effort by the National Cancer Institute. To this end, the NCI Director has recently created a new Office of HIV and AIDS Malignancy (OHAM) that will coordinate AIDS and AIDS malignancy research throughout the institute. At the same time, CCR has launched a new Center of Excellence in HIV/AIDS and Cancer Virology, led by Stuart Le Grice, Ph.D. With the renewed interest in this area and the creation of these two groups, the NCI is poised to have a substantial impact on AIDS malignancy and AIDS research on a global scale.



(Graph: R. Yarchoan, CCR)

Figure 3: The epidemiology of AIDS-related tumors and illness is changing as the number of AIDS patients in the United States grows. This represents an increasing population at risk of developing malignancies.

## Why Do Kaposi's Sarcoma Lesions Most Often Develop on the Feet?



(Photo: NCI)

**Because of hypoxia-responsive genes encoded by the virus that causes Kaposi's sarcoma, the lesions of this tumor have a tendency to show up in relatively poorly oxygenated tissues, like the feet.**

In his original description of Kaposi's sarcoma in 1872, Moritz Kaposi noted that the purple, brown, or black lesions of the condition tended to occur on his patients' feet. Over time, researchers and physicians alike have been struck by a predilection of Kaposi's to involve the feet and other areas of the body with a poor blood supply. It has been speculated that this tendency is because the legs and feet often have relatively low tissue-oxygen levels (hypoxia). Interestingly, another tumor caused by Kaposi's sarcoma-associated herpesvirus (KSHV), primary effusion lymphoma (PEL), arises in pleural effusions—accumulations of excess fluid in the chest with no direct blood supply and, consequently, poor oxygenation.

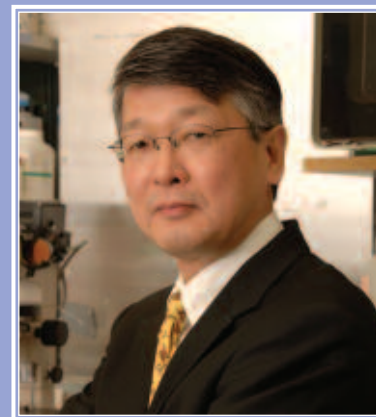
Generally, after initial infection, herpesviruses enter a dormant or resting phase, allowing them to remain within the host for years undetected only to arise when stimulated by various factors. This stimulation to lytic replication—which results in the rupture of the host cell—generally involves one or more switch genes, which in turn activate a cascade of viral genes.

In exploring KSHV-induced tumors' tendency to arise in hypoxic areas, Robert Yarchoan, M.D., and his colleagues found that lytic replication KSHV in PEL cell lines

is activated in hypoxic cells. In addition, the team found that certain specific KSHV genes could be directly activated by hypoxia, including a cluster of lytic genes stretching from ORF34 to ORF37. Cells exposed to hypoxia express increased levels of so-called hypoxia-induced factors (HIFs). Yarchoan's group found that the ORF34–37 gene cluster could be directly stimulated by the binding of HIFs to a single viral hypoxia response element (HRE) located in the cluster's promoter region. Because some of these KSHV genes play important roles in the pathogenesis of KS or other tumors, their hypoxic activation could thus help explain why these tumors arise where they do.

His group is now exploring the possibility that the activation of certain KSHV genes by hypoxia could be used as a means to treat tumors caused by KSHV. Two hypoxia-induced viral genes, ORF21 and ORF36, can chemically modify the drugs ganciclovir and zidovudine (AZT) into forms toxic to cells. Yarchoan is now testing whether these two drugs can be used to specifically target KSHV-induced tumors in which ORF21 and ORF36 are activated by hypoxia or other means.

## Multiple Strategies for Attacking HIV



(Photo: B. Branson)

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

While highly active antiretroviral therapy (HAART) is able to keep levels of HIV very low and deter the onset of AIDS, it does not provide a cure. Lingering viral reservoirs persist undetectable in the blood and other tissues, ultimately enabling HIV to develop resistance to antiretroviral drugs and begin propagating despite HAART. The identification of new types of drugs that attack HIV in different ways might help keep these drug-resistant viruses in check.

A group led by Hiroaki Mitsuya, M.D., Ph.D., recently developed a new antiretroviral called darunavir, which was specifically designed to tightly bind and inhibit the HIV protease. Darunavir is more potent than most available first-generation protease inhibitors (PIs), and it can block replication of HIV strains resistant to multiple other PIs.

Mitsuya recently teamed up with his long-standing collaborator Robert Yarchoan, M.D., and other researchers to learn more about how darunavir inhibits HIV protease. The HIV protease is made up of two protein monomer subunits that must come together, or dimerize, to create the mature, active form of the enzyme. It is the mature protease that plays a critical role in HIV replication.

Mitsuya, Yarchoan, and colleagues found that darunavir can work just like other PIs that have been used for years—by preventing the mature protease from processing other HIV proteins. However, they discovered that darunavir has another trick up its sleeve—it is also able to prevent the two parts of the protease from dimerizing. This study marked the first time that a small molecule was found to disrupt protease dimerization. Because it inhibits HIV protease using a unique mechanism (i.e., inhibition of protein dimerization), darunavir—and possibly other molecules like it—may be useful for treating individuals infected with HIV that have developed resistance to more traditional PIs and other antiretroviral therapies.