National Cancer Institute

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# CCR connections

CENTER FOR CANCER RESEARCH

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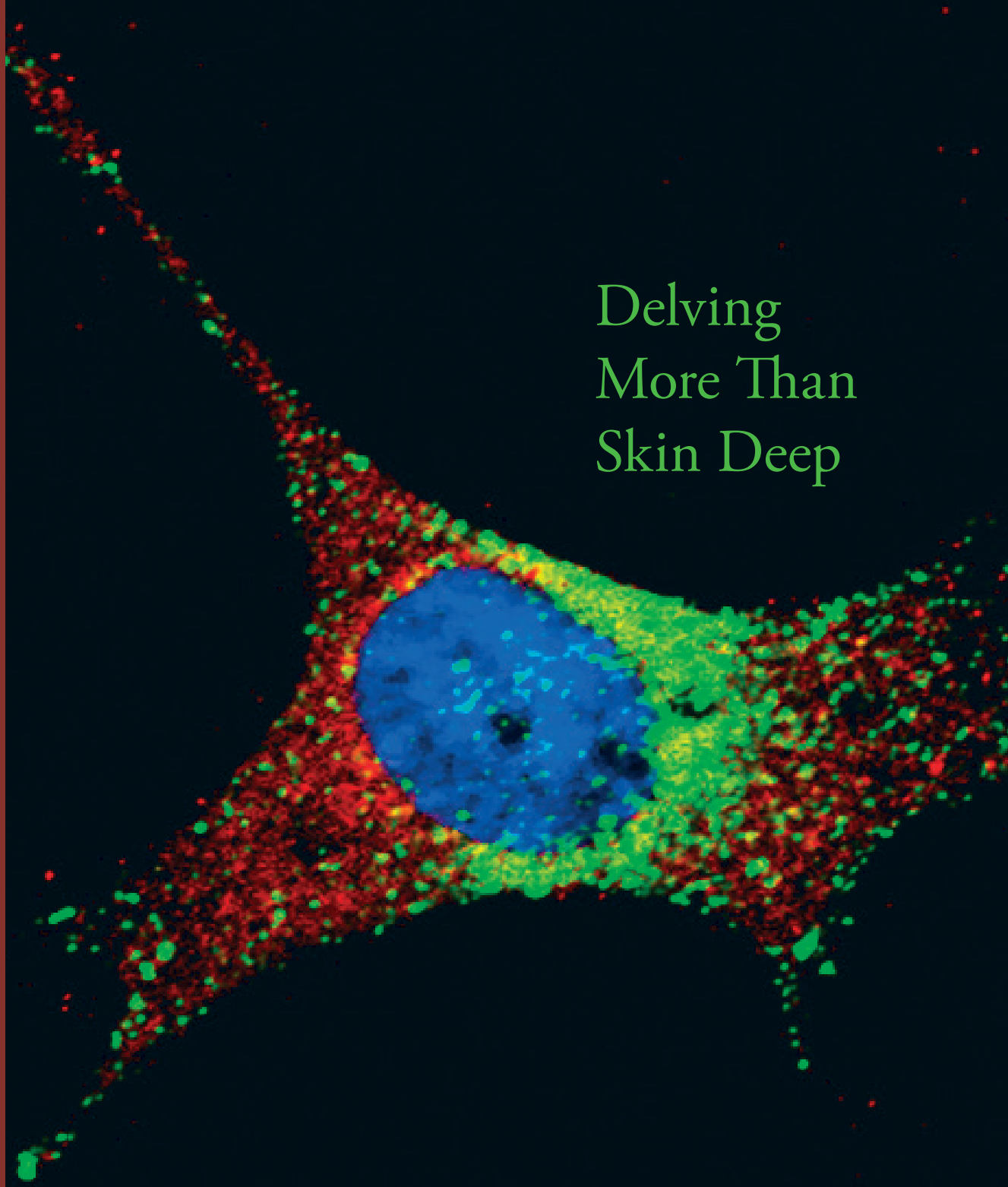
## Delving More Than Skin Deep



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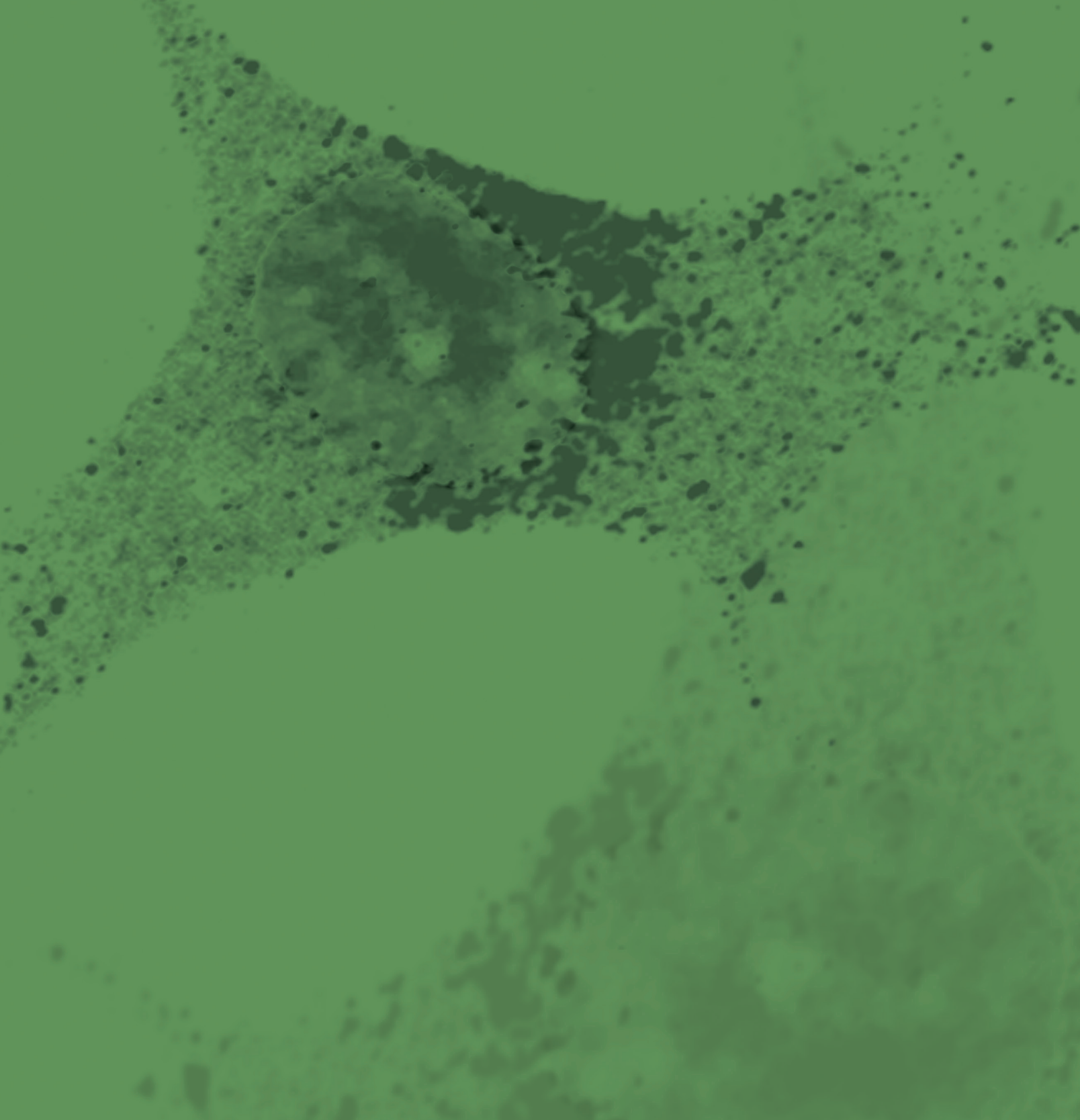

We invite your comments and suggestions about CCR connections.
Please email your feedback to tellccr@mail.nih.gov.

Center for Cancer Research

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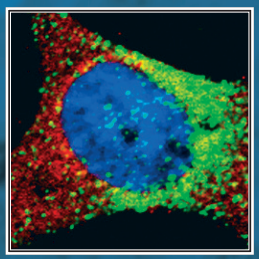

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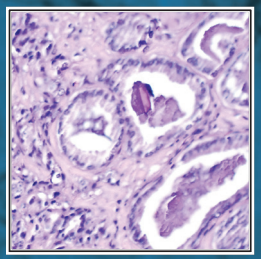

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CENTER FOR CANCER RESEARCH

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## The mission of CCR is:

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

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

# Planning for the UNKNOWN

*Recent, far-reaching increases in our understanding of cancer have positioned us for major strides in prevention and treatment in the decade ahead. The foundations for many recent transformational insights—as well as the prospects for their applications—rest upon sound planning and the often under-appreciated impact of scientific serendipity.*

It might seem contradictory to say that CCR, or any other research organization, can plan for unexpected experimental or clinical results. Yet the ability to plan sound hypothesis-driven science and anticipate several prospectively predictable outcomes, while still remaining flexible enough to recognize unanticipated findings of often greater value, is at the core of good science.

In CCR, we realize that meaningful advances in cancer research do not happen by random chance, but by building and sustaining an innovative environment with potential to scale up to big science when necessary, to foster team science when appropriate, and to empower individual researchers when they demonstrate exceptional creativity. The ability to recognize valuable outcomes, both predictable and unexpected, is also a necessity.

Therefore, in addition to supporting solo investigators, our culture of interaction and innovation encourages interdisciplinary research teams to bring together their highly specialized skills to address difficult problems, while recognizing the value of anticipated and unanticipated outcomes. Our approach is to promote collaborations between our labs and clinics and with others around the globe to move the most promising discoveries into improved diagnostic and treatment strategies as quickly as possible. Because serendipitous findings may fuel important and rapid advances in science, we often use our flexibility in funding mechanisms to follow unexpected, but promising leads wherever they appear. In that sense, CCR's scientific planning and culture of innovation provide a novel environment and philosophy for preparing researchers for tomorrow's unknowns.

(*Photo:* B. Branson)

Robert Wiltrout, Ph.D.

This issue of *CCR connections* showcases some of the fruits that we are already reaping from our innovative environment—namely, unexpected results. Whether it is Mary Carrington, Ph.D., discovering how seemingly unrelated research focus areas—HIV infections and cervical cancer—actually inform one another, Stefan Ambs, Ph.D., M.P.H., identifying a distinct interferon-related gene signature in the prostate tumors of African-Americans, or Ying Zhang, Ph.D., generating a mouse model to study the function of Smurf2 only to discover that this protein may play a role in preventing tumor formation, the theme is the same: Planning and innovation accelerate progress in cancer research, and the ability to recognize value in unexpected findings accelerates progress even more.

# New Web-Based Tools

## Make Systems Pharmacology More Accessible Using Data from the NCI-60

High-throughput biological techniques, like microarrays and drug screens, generate an enormous amount of data that may be critically important for cancer researchers and clinicians. Being able to manipulate the data to extract those pieces of interest, however, can require computational or bioinformatics skills beyond those of the average scientist. One rich source of information is the NCI-60 panel of tumor cell lines. Originally developed to screen anticancer compounds by NCI's Developmental Therapeutics Program, these 60 cell lines have also been analyzed for their gene and microRNA expression levels, DNA mutation status, and DNA copy number variations. Researchers from CCR and the Division of Cancer Treatment and Diagnosis, led by Yves Pommier, M.D., Ph.D., of CCR's Laboratory of Molecular Pharmacology, wanted to make this data more readily available so they generated a series of online tools, which are freely accessible through their CellMiner web-application.

The tools, described in a recent *Cancer Research* paper, allow users to access expression levels for 26,065 genes and 360 microRNAs as well as the activity of 20,503 compounds, including 98 that are already approved by the Food and Drug Administration. An additional tool that performs pattern comparisons can be used to identify connections between these three parameters or with a user-supplied pattern of interest. The tools perform the complex computational tasks required to normalize the data captured from five distinct microarray platforms and varying numbers of drug experiments and present it in a format that is easier for users to analyze.

To demonstrate the usefulness of their tools, the researchers tested a colon-specific pattern. Of the genes with a highest colon specificity that were returned, two are known to be over-expressed in colon cancer, and a third, which had the highest specificity, is a little-studied gene that may make an excellent colon cancer biomarker. Several drugs were also highly active in colon cancer cell lines. Three of these are currently being tested in clinical trials, while the fourth drug with the strongest colon-specific activity pattern, could be considered for testing. Looking at the activity pattern across all 60 cell lines for one of the drugs under clinical investigation, the researchers also noted strong activity in melanoma cell lines suggesting the drug could be a therapy for that cancer type as well.

The researchers plan to add more tools in the future including two that provide access to DNA copy number variations and whole genome sequences across the NCI-60. Together, these freely available tools can help researchers with little bioinformatics training find novel hypothesis-generating associations from data previously buried in complex databases.

(*Photo: Christopher Spielmann*)


Gene Chip is one of five microarray platforms used to determine gene expression.

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

*For more information about CellMiner and to access its tools, please visit http://discover.nci.nih.gov/cellminer.*

## New Targeted Therapies Available for Licensing:

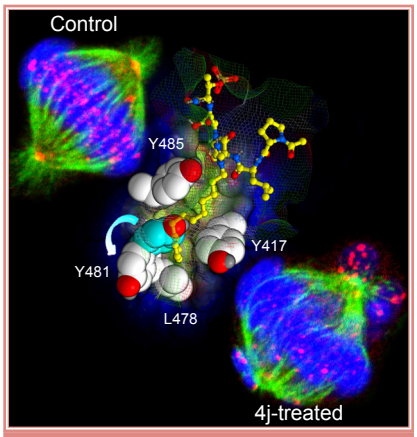
# Inhibitors of Polo-like Kinase 1

While a Postdoctoral Fellow at Harvard University studying cellular proliferation and mitotic controls, Kyung Lee, Ph.D., made a seminal discovery of the key role played by the noncatalytic polo-box domain (PBD) of mammalian polo-like kinase 1 (Plk1). He demonstrated that the PBD plays an essential role for the function of Plk1 by targeting its catalytic domain to specific subcellular structures. Plk1 is of interest to cancer research because it is over-expressed in a broad spectrum of human cancers, and also because blockade of Plk1 function efficiently kills cancer cells, but much less efficiently kills normal cells, due to the addiction of the former to a high level of Plk1 activity.

After coming to CCR in 1998 as a tenure-track investigator in the Laboratory of Metabolism, Lee discovered a 5-residue long phospho-peptide, PLHSpT, which specifically binds to the Plk1 PBD with a high affinity. He sought the expertise of CCR investigators, Alexander Wlodawer, Ph.D., Chief of the Macromolecular Crystallography Laboratory, and James McMahon, Ph.D., Chief of the Molecular Targets Laboratory, to determine the exact crystal structure and binding mode of the PBD-PLHSpT complex. This collaboration provided Lee's research team with the molecular look they needed to better understand the nature of Plk1 PBD-dependent interactions. Lee and his colleagues further found that disrupting the function of Plk1 PBD by PLHSpT derivatives induces mitotic arrest and apoptotic cell death in cultured cancer cells.

Given that catalytic domain inhibitors frequently exhibit a high level of cross-reactivities, Lee suspected that Plk1 PBD inhibitors might make excellent targeted therapies for cancer because of their unparalleled affinity and specificity.

(*Figure: T. Burke and K. Lee, CCR*)




A high affinity PBD inhibitor, 4j (yellow ball and stick) disrupts Plk1 (red fluorescent signals in the immunostained cells; green, alpha-tubulin; blue, chromosome) localization and induces cancer cell killing. Binding by 4j to the PBD is achieved by the rotation (curved arrow) the Y481 side chain (semi-transparent cyan), which reveals a newly discovered hydrophobic channel on the surface of the Plk1 PBD.

Consequently, in the spring of 2008, Lee approached Terrence Burke, Ph.D., in CCR's Chemical Biology Laboratory, to explore the possibility of further developing the PLHSpT-based inhibitors. Since that meeting, Burke's expertise in the generation of peptide derivatives coupled with the biochemical and cell-based analyses of Plk1-dependent events performed by Lee's lab, has yielded several highly potent Plk1 PBD-binding compounds.

As Lee explains, "While systematically analyzing various PBD-binding proteins, my research team and I identified a PLHSpT-containing motif from a kinetochore protein. Next, synthetic work in Burke's laboratory led to the serendipitous discovery that certain modifications to the natural amino acid histidine within the PLHSpT peptide imparts up to a 1000-fold better binding affinity to PBD."

"This," according to Burke, "makes CCR's Plk1 inhibitors currently the most potent ones yet reported, because we managed to maintain good Plk1 target selectivity along with exceptional binding affinity."

The Plk1 PBD-binding inhibitors are currently in preclinical development and are available for licensing through the NIH Office of Technology Transfer.

*To inquire about licensing Plk1 PBD-binding inhibitors, please contact Patrick McCue, Ph.D., at McCuepat@mail.nih.gov.*

*For a description of the new agents, please visit http://www.ott.nih.gov/Technologies/abstractDetails.aspx?RefNo=1971.*

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

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

# Smurf2 Regulates DNA Repair and Packaging to Prevent Tumors

The blueprint for a cell's functions is written in the genetic code of DNA sequences as well as in the landscape of DNA and in histone modifications. DNA is wrapped around histones to package it into chromatin, which is stored in the nucleus. It is important to maintain the integrity of the chromatin structure to ensure that the cell continues to behave appropriately. Recently, Ying Zhang, Ph.D., in CCR's Laboratory of Cellular and Molecular Biology, and her colleagues showed that alterations in the organization of DNA can lead to tumor growth in a variety of tissues. This study appeared in the February 2012 issue of *Nature Medicine*.

To understand how cancer cells might acquire changes in the chromatin landscape, Michael Blank, Ph.D., a Postdoctoral Fellow in Zhang's lab, investigated the role of the protein Smurf2. Previous studies have demonstrated that Smurf2 functions as an enzyme that adds a tag, called ubiquitin, to proteins to signal their destruction, but there is only scant information about whether Smurf2 has any role in cancer.

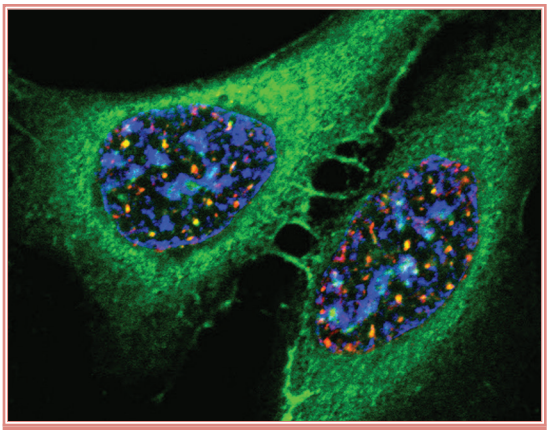
The researchers generated a mouse model in which they prevented Smurf2 expression (Smurf2$^{-/-}$). Smurf2$^{-/-}$ mice develop normally and have no obvious physical problems early on. However, tumors begin to grow in a variety of tissues as the mice age, suggesting that Smurf2 may play a role in preventing tumor formation.

Therefore, the researchers studied cells from normal mice and from Smurf2$^{-/-}$ mice. Over time, the Smurf2$^{-/-}$ cells began to grow faster and expressed genes distinct from the normal cells. Smurf2$^{-/-}$ cells also formed tumors when injected into mice. Re-expressing Smurf2 in the Smurf2$^{-/-}$ cells did not correct their altered growth and indicated that changes in the cells' DNA may have already occurred. Other studies indicated that Smurf2 plays a role in regulating the DNA damage response and the packaging of DNA.

The addition of certain molecules to histones allows DNA to wrap more or less tightly around histone proteins. The researchers found that certain histone modifications were increased in Smurf2$^{-/-}$ cells and that Smurf2 can directly target the protein RNF20 for destruction by adding ubiquitin. Decreasing the levels of RNF20 in Smurf2$^{-/-}$ cells decreased the histone modification, increased DNA packaging, and decreased cell growth. Expressing RNF20 in normal cells increased their growth rate. The researchers also found that Smurf2 and RNF20 move to sites of DNA damage where Smurf2 decreases the level of RNF20. These results show that Smurf2 plays an important role in tumor formation in the mouse by regulating RNF20, which controls the DNA damage response and DNA packaging. But is the same pathway important in human tumors?

To address this question, the researchers examined a number of human tumor cell lines. Similar to mouse cells, they found that removing Smurf2 resulted in increased RNF20 levels and its associated histone modification, while loss of RNF20 increased DNA packaging. In a panel of 40 breast tumors, the investigators found that 32 tumors expressed high levels of RNF20 protein. A set of 55 lymphomas showed similar elevated levels of RNF20.

This research has shown that human DNA is sensitive to the levels of Smurf2 and RNF20 and that loss of Smurf2 function may contribute to human tumor formation via changes in the DNA damage response and chromosomal organization. Future studies will need to investigate whether inhibiting RNF20 activity or reactivating Smurf2 can prevent tumor formation in human cells.



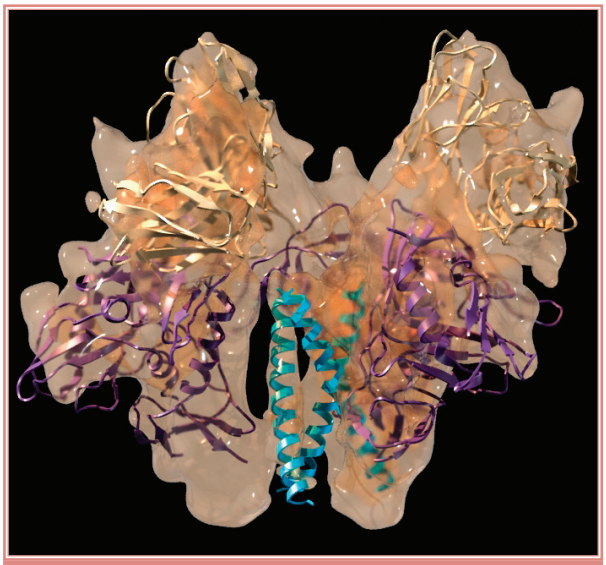
(*Image:* M. Blank, CCR)

Following DNA damage, Smurf2, labeled in green, moves to sites of double-stranded DNA breaks, shown in red. Because Smurf2 regulates a cell's response to DNA damage at these sites as well as the organization of the DNA in the nucleus, loss of Smurf2 may promote tumor formation.

*To learn more about Dr. Zhang's research, please visit her* CCR Web *site at http://ccr.cancer.gov/staff/staff.asp?name=yzhang.*

# Finding a Chink in the Armor:
## Investigating the Structure of HIV

HIV infection depends on two proteins expressed on the virus surface: gp41, which sits in the virus membrane, and gp120, which sits on top of gp41. Three copies, or trimers, of each gp41/gp120 pair make up the protein, Env. Env coats the virus surface and interacts with its receptor, CD4, and a co-receptor on the T cell. Binding to the receptors is thought to cause a structural reorganization of Env, which exposes a fusion peptide that inserts into the T cell membrane and actually forces the virus and host membranes together, initiating an infection. However, the structural details of this process are lacking.



(*Figure: S. Subramaniam, CCR*)

A three-dimensional rendering of the structure of trimeric Env bound to 17b is shown. The map was fitted with three copies of the X-ray structures with gp120 shown in purple and 17b in gold. One copy of the gp41 N-terminal helix, shown in cyan was fitted individually into each of the three densities and occupies the central region of the spike, which is essentially a cavity in the unbound state.

Antibodies directed against different parts of Env may be able to prevent HIV infection, but only if essential structural elements are targeted. Erin Tran, Ph.D., a Postdoctoral Fellow, and Mario Borgnia, Ph.D., a Staff Scientist, working with Sriram Subramaniam, Ph.D., in CCR's Laboratory of Cell Biology, and their colleagues investigated how the structure of Env changes as it binds to its receptor. The conformations of Env in different situations may suggest new regions for antibody targeting that are likely to block HIV infection. Their findings were published July 12, 2012, in *PLoS Pathogens*.

The researchers first examined the structure of Env on intact viruses incubated with a soluble version of CD4 to mimic normal receptor binding or with an antibody called 17b, which simulates co-receptor binding. They rapidly froze the samples to preserve the structures and to prevent ice crystals from forming. The frozen samples were then imaged using electron microscopy and three-dimensional structures were generated. Binding to either soluble CD4 or 17b caused the gp120 molecules to rotate outward, forming an opening in the center of the Env structure. This more open conformation is similar to the structure of Env bound to both CD4 and 17b, indicating that 17b binding alone is capable of inducing a conformational change in Env similar to the change induced by the natural CD4 receptor.

To get an even more detailed picture of Env in the open conformation, the researchers used single particle electron microscopy techniques, which provide higher structural resolution. Incubating soluble Env trimers with 17b again produced the open Env conformation, but this time the researchers were able to detect three helical structures in the center of the Env complex. The researchers attributed these helices to one end of gp41 and suggest that the helices are a part of the HIV fusion peptide, but at a step prior to insertion into a target cell membrane. The researchers also noted that the portion of gp41 that forms each helix is one of the most conserved regions across different HIV strains. This high-resolution structure of Env may provide a new blueprint for building vaccines. Vaccines directed against this structure could stimulate the production of antibodies that will recognize this highly conserved region of Env at a stage where the virus is still poised to infect target cells.

*To learn more about Dr. Subramaniam's research, please visit his CCR Web site at http://electron.nci.nih.gov.*

# Recent CCR Awards

### Arthur S. Flemming Award for Exceptional Service

For his work in cancer cell biology

**Thomas Misteli, Ph.D.**
*Laboratory of Receptor Biology and Gene Expression*

### 2012 Outstanding Clinical Care Award in Psychosocial Oncology

*American Psychosocial Oncology Society*

For outstanding clinical contributions to the field of psychosocial oncology

**Lori Wiener, Ph.D.**
*Pediatric Oncology Branch*

### 2012 Honorary Member Society of Toxicology

For outstanding and sustained achievements in advancing health through the science of toxicology

**Frank Gonzalez, Ph.D.**
*Laboratory of Metabolism*

### NIH Asian and Pacific Islander American Organization Award

For outstanding accomplishments in biomedical research

**Ying Zhang, Ph.D.**
*Laboratory of Cellular and Molecular Biology*

### 2011 American Association for the Advancement of Science Fellows

For distinguished contributions to translation and selenium biology fields, especially expanding the genetic code and determining the biosynthetic pathway of selenocysteine in eukaryotes

**Dolph Lee Hatfield, Ph.D.**
*Laboratory of Cancer Prevention*

For HIV/AIDS vaccine development, replicating and non-replicating adenovectors, mucosal/systemic vaccination strategies, neutralizing and non-neutralizing mechanisms of antibody protection

**Marjorie Robert-Guroff, Ph.D.**
*Vaccine Branch*

### 2012 SER-CAT Young Investigator Award

*Southeast Regional Collaborative Access Team*

**Jason Stagno, Ph.D.**
*Macromolecular Crystallography Laboratory*

### 2012 Brigid Leventhal Merit Award

*Conquer Cancer Foundation of the American Society of Clinical Oncology*

**Fernanda I. Arnaldez, M.D.**
*Pediatric Oncology Branch*

### The Scientist Magazine: Best Places to Work Postdocs 2012

Ranked 13

**National Cancer Institute, Bethesda/Frederick**

TheScientist BEST PLACES TO WORK POSTDOCS 2012

## CCR Researcher Receives Presidential Award

"The impressive accomplishments of today's awardees so early in their careers promise even greater advances in the years ahead."
**President Barack Obama**


(Photo: E. Branson)

Daniel Larson, Ph.D., Head of Systems Biology of Gene Expression in CCR's Laboratory of Receptor Biology and Gene Expression, is among the 96 researchers named by the White House as recipients of the 2011 Presidential Early Career Awards for Scientists and Engineers (PECASE). The awards are the highest honor bestowed by the U.S. government on science and engineering professionals in the early stages of their independent research careers. Larson received the award for his studies on transcription dynamics of single human genes.

The awards, established by President Bill Clinton in 1996, are coordinated by the Office of Science and Technology Policy within the Executive Office of the President. Eleven federal agencies, including the Department of Health and Human Services, join together annually to nominate the most meritorious scientists and engineers. Winning scientists and engineers are selected for their pursuit of innovative research at the frontiers of science and technology and their commitment to community service as demonstrated through scientific leadership, public education, or community outreach.

# Staff News at CCR

## Announcements

(Photo: B.Branson)

### William Dahut, M.D.

Dahut has been named a CCR Deputy Director. He received his M.D. from Georgetown University and completed clinical training in internal medicine at the National Naval Medical Center. He completed a fellowship in hematology/oncology at the former NCI-Navy Medical Oncology Branch. He returned to NCI in 1998 as Head of the Prostate Cancer Clinic, and in 2009, he was appointed CCR Clinical Director, a role which he will retain as deputy director. Dahut is a leader in the development of novel therapeutic strategies for the treatment of adenocarcinoma of the prostate.

(Photo: R. Baer)

### Tom Misteli, Ph.D.

Misteli has been named CCR Associate Director for Scientific Development. He is a Senior Investigator in the Laboratory of Receptor Biology and Gene Expression. He trained at the University of London, U.K., and the Cold Spring Harbor Laboratory, N.Y. He is internationally recognized for his studies in cell biology of genomes and gene expression in living cells. In his new role, Misteli will guide CCR in enhancing its strategic vision and in identifying high-priority, transformative, and novel scientific opportunities.

## Newly Tenured CCR Scientist

**Philip Tofilon, Ph.D.**
*Radiation Oncology Branch*

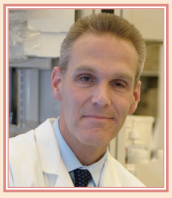
## New Tenure-Track Scientists

(Photo: B.Branson)

### Piyush Agarwal, M.D.

Agarwal joins CCR's Urologic Oncology Branch as a tenure-track investigator and Head of the Bladder Cancer Section. Although he researches all aspects of bladder cancer, his work focuses on, BCG-refractory disease and molecularly targeted therapy. As a surgeon, he focuses on minimally invasive surgical techniques.

(Photo: B.Branson)



### James Hodge, Ph.D.

Hodge is now a tenure-track investigator in CCR's Laboratory of Tumor Immunology and Biology, where he is Head of the Recombinant Vaccine Group. He has made significant contributions to the design and development of novel recombinant vaccines and vaccine strategies for cancer immunotherapy, including recombinant vectors to deliver tumor antigens, the use of costimulation to enhance antitumor T cell responses, the use of whole tumor cell vaccines, and the use of diversified prime and boost strategies to enhance antitumor immunity.

(Photo: B.Branson)

### Damien Kovalovsky, Ph.D.

Kovalovsky joins CCR's Experimental Immunology Branch. Kovalovsky's current research focuses on understanding the genetic control of effector and regulatory differentiation in lymphocytes.

(Photo: B.Branson)

### Vanja Lazarevic, Ph.D.

Lazarevic joins CCR's Experimental Immunology Branch. Lazarevic's laboratory focuses on understanding at a fundamental level the transcription factors responsible for maintaining self-tolerance.

# CTCF, a Novel Regulator of Alternative Splicing

In a study published in the November 3, 2011, issue of *Nature*, Shalini Oberdoerffer, Ph.D., of CCR's Mouse Cancer Genetics Program, and Sanjeev Shukla, Ph.D., a Postdoctoral Fellow in her lab, investigated how exons with weak splicing signals are included using the *CD45* gene as a model system. At different stages of lymphocyte development, exons 4, 5, and 6 are specifically incorporated or excluded from the CD45 mRNA. Oberdoerffer previously found a protein, hnRNPLL, regulates exons 4 and 6, but the mechanism for regulating exon 5 was unclear.

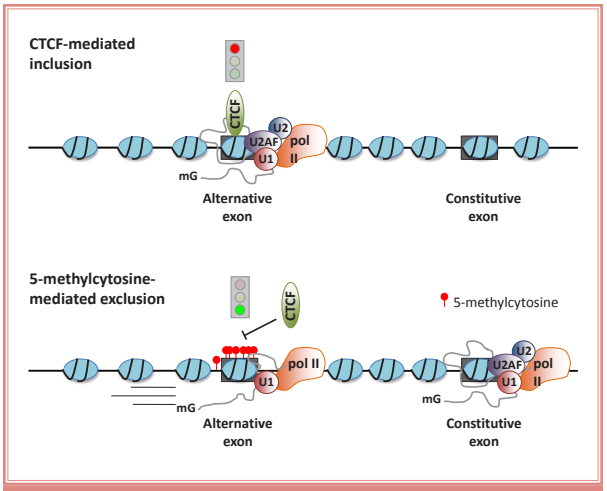
Analyzing previously published data, the researchers found that the DNA-binding protein CTCF, which is thought to shield inactive regions, had a strong interaction with *CD45* exon 5, even in cells expressing high levels of CD45 protein. This interaction was also observed in mouse immune cells. Contrary to previous studies, this data suggested that CTCF binding may play an important role in exon 5's inclusion in the CD45 mRNA transcript.

Next, by examining Burkitt lymphoma cell lines with variable exon 5 exclusion the researchers found that exon 5 was more likely to be included when CTCF was bound and that in cells where CTCF failed to bind CD45-5 expression was reduced. One way CTCF binding might help incorporate exon 5 is by affecting the activity of RNA polymerase II (pol II), the enzyme that produces mRNA transcripts. More active pol II was associated with exon 5 in cells expressing high levels of CD45 that incorporates exon 5 (CD45-5), which suggests that pol II spends more time, or pauses, at exon 5 in these cells. Depleting CTCF protein reduced pol II binding at exon 5. By slowing down pol II, CTCF could provide time for the splicing machinery to recognize the exon 5 splice site and incorporate exon 5 into the CD45 mRNA transcript.

The researchers then investigated how CTCF binding to exon 5 is regulated since CTCF is always expressed but exon 5 is only included in the CD45 transcript at certain stages of lymphocyte development. The addition of a methyl group to DNA nucleotides is known to interfere with CTCF binding, and in cell lines with methylated DNA at exon 5, CTCF failed to bind. To see whether this was also the case in normal lymphocytes, the investigators studied T cells that expressed higher or lower levels of CD45-5 and observed increased methylation and reduced CTCF binding in the T cells expressing less CD45-5. Inhibiting DNA methylation in the cells expressing lower CD45-5 increased CTCF binding and pol II pausing at exon 5. Importantly, these results are the first to link the processes of DNA methylation and alternative mRNA splicing.

Since CTCF binding sites are located in the exons of genes other than *CD45*, the researchers reduced CTCF levels in cell lines and then looked for RNA sequences that differed with the loss of CTCF. The researchers determined that exons with a CTCF binding site downstream were preferentially excluded when CTCF was depleted. Similar to CD45 exon 5, CTCF binding downstream of these exons induced pol II pausing.

These studies have revealed the importance of CTCF in the inclusion of alternative exons. Changes in CTCF function may play a critical role in diseases such as cancer where altered splicing and DNA methylation patterns have been observed.




(*Figure*: S. Oberdoerffer, CCR)

By binding downstream of some alternative exons, CTCF causes RNA polymerase II (pol II) to pause giving components of the splicing machinery time to incorporate the alternative exon. When the CTCF binding site is methylated, however, CTCF cannot bind, pol II does not pause, and the alternative exon is not incorporated into the transcript.

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

# In Conversation:
## Research Fellow Sanjeev Shukla, Ph.D.

(*Photo:* J. Summers, SPGM, FNL)

Sanjeev Shukla, Ph.D.

Among the multiple alterations present in cancer cells are an abundance of aberrant mRNA transcripts. Whether this abnormal gene transcription is a by-product of cellular transformation or whether it represents aberrant splicing that contributes altered proteins to cancer cells is not yet clear.

**CCR:** Sanjeev, your research path is a bit unusual. What prompted you to switch your earlier research focus on prognostic biomarkers for oral cancers to the world of alternative splicing and its regulation?

**Sanjeev:** Actually, from my perspective, I have not changed my focus. My ultimate goal is to contribute to cancer research by unraveling the basic mechanisms that enable cancer cells to churn out aberrant splice products, and biomarkers are among these products. I realized that in order to understand cancer's aberrant proteins, I need to thoroughly understand the mechanism of alternative splicing, and I need to know its regulators.

**CCR:** Did you come to CCR as a Visiting Fellow to help you achieve those goals?

**Sanjeev:** Yes, it offered me the opportunity of a mutually beneficial collaboration. I knew what my long-range goal was, and I discovered that Shalini Oberdoerffer's lab could provide me with the training and technical tools to ask the right preliminary questions about the mechanism of alternative splicing. Shalini's interests are more focused upon alternative pre-mRNA splicing in maturing immune cells, but we share an interest in deconstructing all the regulators and steps involved in alternative splicing, and she has the perfect CD45 pre-mRNA model system to study the regulators.

**CCR:** Have you mastered the basic mechanism of alternative splicing or gathered any preliminary data toward your ultimate goal of deconstructing alternative splicing's link to cancer?

**Sanjeev:** Yes. I am pleased with our progress so far. Shalini and I knew that alternative splicing decisions are determined by the ability of weak splice sites to compete with strong splice sites for detection by the spliceosome, but we did not know how epigenetic factors could alter this process. We have discovered a key regulatory role for DNA methylation. And it regulates splicing in a most unusual manner.

**CCR:** What exactly does methylation do to alter the basic splicing mechanism?

**Sanjeev:** We discovered a novel mechanism for regulation of alternative splicing via DNA methylation. In lymphocytes, the mechanism of CD45 exon 5 splicing was not clear. We found that a protein called CTCF usually binds to exon 5 DNA. Now CTCF's usual role is to form DNA loops and give chromatin a spatial conformation. So this might mean that epigenetic modifications may be maintained on DNA to aid the spliceosome in the process of exon selection. DNA methylation regulates CTCF binding, and that, in turn, regulates alternative splicing. So by slowing down pol II, CTCF might be providing time for the splicing machinery to both recognize the exon 5 splice site and incorporate exon 5 into the CD45 mRNA transcript. Isn't that fascinating?

**CCR:** Have you given any thought to how you will mentor your own Postdoctoral Fellow in the future when you run your own lab?

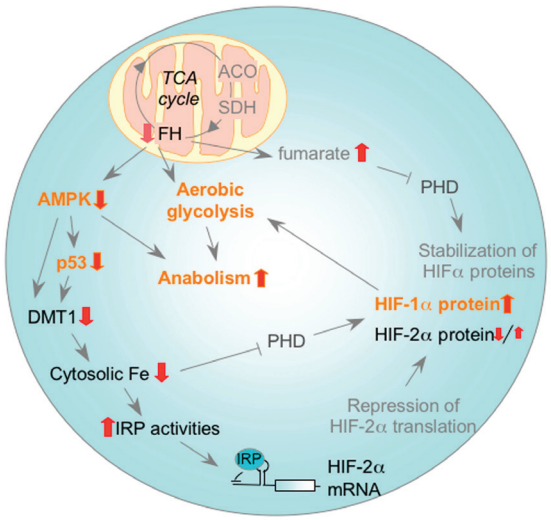
**Sanjeev:** I plan to repeat the same approach that worked for me at CCR. I think the key is for the Postdoctoral Fellow to think clearly about his overarching career goal. Then I would suggest that he or she shop for the lab that can offer the technology and training that can best help him or her to approach the questions that need to be answered to reach that goal.

As the head of the lab, I plan to expect that type of focus from my prospective Postdoctoral Fellows, and I expect the candidates to articulate their "big picture" goals to me. Then I will explain what expertise and technology training my lab can bring to bear upon their goals.

## Collaborating to

# Outwit HLRCC's Pathways

Marston Linehan, M.D., Chief of CCR's Urologic Oncology Branch, has spent his entire career at CCR crafting better approaches to treating kidney cancer, and he has done so by establishing effective collaborations and collecting properly annotated tissue samples. Almost 30 years ago, when he partnered with Berton Zbar, M.D., then Chief of the Laboratory of Immunobiology, to identify the first mutation that established a genetic basis for kidney cancer, he had no way of knowing the amount of genetic diversity he was facing. What Linehan did recognize was the importance of stored tissue and samples from kidney cancer families. This foresight, along with his ability to establish strategic collaborations, has enabled Linehan and his colleagues to identify and study more underlying mutations, not just in clear cell renal cell carcinoma, but also in papillary kidney cancer, TFE3 kidney cancer, hereditary chromophobe kidney cancer, and most recently in hereditary leiomyomatosis renal cell carcinoma (HLRCC).




In this working model of cells deficient in the fumarate hydratase (FH) gene, impairment of the Kreb's cycle results in a shift of energy production from respiration to glycolysis that induces an AMP-activated protein kinase dependent decrease in p53 and increased expression of hypoxia inducible factor-1.

(*Figure: Marston Linehan, CCR*)

When studying HLRCC, Linehan and his research team collaborated with Tracey Rouault, M.D., of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, to determine how kidney cells missing the fumarate hydratase (FH) gene—an essential enzyme of the Krebs citric acid cycle—manage to survive and thrive. Knowing HLRCC patients lacked the FH gene, Linehan once again turned to his collection of kidney cancer tissue samples. Linehan worked with Rouault to demonstrate that inactivation of the Krebs cycle in FH-negative cells and tissues from HLRCC patients results in a glycolytic shift that decreases levels of AMP-activated protein kinase (AMPK). The team showed that when AMPK signaling is reduced, the expression of tumor suppressor gene p53 also is reduced, and hypoxia inducible factor is increased.

Linehan and his collaborators wanted to know how HLRCC cells manage to supply themselves with energy under such restricted conditions, so again they established an appropriate collaboration. This time they turned to colleagues Ralph DeBerardinis, M.D., Ph.D., at The University of Texas Southwestern Medical Center, and Navdeep Chandel, Ph.D., at Northwestern University, for their expertise in techniques such as C13 glucose and glutamine metabolite tracking. Working together, they demonstrated that these tumor cells have defective mitochondria that use a glutamine-dependent reductive carboxylation pathway rather than the typical oxidative path to compensate for the metabolic shift in these rapidly growing fumarate-hydratase-deficient kidney cancer cells.

This work, and subsequent, in-depth radiolabeled tracer metabolite analysis performed with colleagues Teresa Fan, Ph.D., and Andrew Lane, Ph.D., at the University of Louisville, is revealing some potential targets and novel approaches to therapy for HLRCC and related malignancies.

"These results are very encouraging; these tumor cells are completely dependent on this alternate energy pathway for rapid growth," says Linehan. "So, hypothetically, we can block any of the pathway components to stop HLRCC cells' growth."

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

# Delving More Than Skin Deep

*There are several schools of thought on cancer. One claims it's a basic knowledge problem. A lot of things can be done, but we still don't have a complete understanding of the process.* Vincent Hearing, Ph.D., *Deputy Chief of* CCR's *Laboratory of Cell Biology, belongs to this school. He has spent* 42 *years at the bench characterizing a single cell type called a melanocyte. For him, this groundwork is necessary in order to target the abnormal melanocytes that often result in the deadly skin cancer—melanoma.*

Melanocytes are specialized skin cells that are embedded between fibroblasts in the dermis and keratinocytes in the epidermis. They are the only type of cell in the body that can produce light-absorbing, high-molecular-weight biopolymers called melanins. Two major types of melanin molecules give skin its color: brown-black eumelanins and yellow-red pheomelanins. The capacity of melanocytes to produce mixtures of these light-absorbing pigments is genetically determined, but the amounts of pigments made and their ratios to one another can change over time in response to physiological requirements and/or to environmental influences.

Hearing began his research career studying pigmentary disorders, and his interest has never waned. His sustained attention to the molecular mechanisms that underlie pigmentary changes in the skin has produced a much clearer picture of the biochemistry and regulation of melanins, their role in the photoprotection of human skin, and their usefulness in targeting pigmentary diseases and melanoma.

After much study, Hearing and his research team have helped to paint the molecular picture of what normal epidermal melanocytes do: They produce, package, and move light-absorbing pigments called melanins to keratinocytes which then distribute them towards the skin's surface. Melanocytes package melanins in membrane-bound organelles called melanosomes. The maturation of melanosomes depends upon the proper trafficking of structural and enzymatic components, the synthesis of melanin, followed by their transport to the melanocyte's dendritic extensions. Once there, melanosomes are transferred to adjacent skin cells called keratinocytes. When all goes well, keratinocytes form vesicles to engulf and internalize the donated, pigment-rich melanosomes, and transport machineries, including a special protein called dynein, then move them to the vicinity of the keratinocyte's nucleus.

"This magnificent biochemistry shields the underlying skin cell's DNA from damage caused by ultraviolet radiation (UV). And all this action is usually accelerated automatically in skin cells in response to UV exposure," explains Hearing.

## Meet the Proteins: Builders and Modulators

Over the years, Hearing and his colleagues have drilled down to the molecular level to identify just how melanins—which are actually synthesized from the amino acid tyrosine via the action of a critical enzyme tyrosinase (TYR)—are produced and distributed to protect human skin from UV radiation. One by one, the team has identified key players in the process. Hidenori Watabe, Ph.D., who worked in Hearing's lab and is now at Haruhino Dermatological Clinic in Kawasaki, Japan, and Julio Valencia, M.D., currently a Staff Scientist in Hearing's lab, followed the movement of two major melanosomal proteins, TYR and PMEL17, showing that they first interact with additional proteins named TYRP1, DCT, and MART1 to regulate the structure of melanosomes and to modify the types of melanin produced, while PMEL17 acts later in melanogenesis, building the structural fibrils essential to the

maturation and movement of mature melanosomes.

Other members of the Hearing lab characterized the proteins that move these organelles out to keratinocytes in an orderly manner. Still others identified several signaling proteins that respond to environmental factors by modulating pigment production. They showed how agents like melanocyte stimulating hormone (MSH), agouti signal protein (ASP), dickkopf1 (DKK1), and neuregulin 1 (NRG1) regulate the expression and function of pigmentation genes and modulate their responses to UV radiation.

## Melanin Modulators of Interest

One interesting finding from Yuji Yamaguchi, Ph.D., formerly of the Hearing lab and now at Abbott Pharmaceuticals in Japan, explains why the skin on a person's palms and soles are always thicker and lighter in color than on the rest of the body. He showed how a modulator protein called DKK1 is expressed at high levels in dermal fibroblasts derived from the lightly pigmented skin of an individual's palms and soles, but is expressed at much lower levels in fibroblasts derived from skin on the body's trunk. The high levels of DKK1 produced by fibroblasts in the palms and soles both reduce pigment there and thicken the skin in those areas.

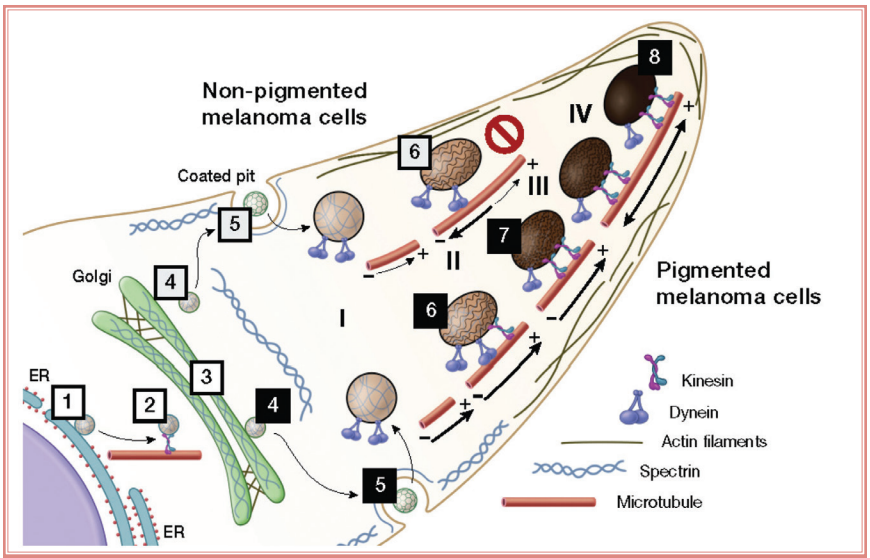
Wonseon Choi, Ph.D., a Research Fellow in Hearing's lab, produced another interesting finding. She identified the important role of NRG1 to modulate skin pigmentation. She demonstrated how this protein, when it is secreted by fibroblasts, regulates ethnic differences in constitutive skin color. NRG1 does this by regulating melanocyte differentiation and by altering the amount of melanins produced.

"Constitutive skin pigmentation is strongly associated with the incidence of skin cancers among ethnic groups. The rates of basal and squamous cell carcinomas and melanomas in Caucasians are more than 70- and 30-fold greater, respectively, than in African-Americans, and the rates of these cancers in Asians, Hispanics, and other ethnic groups with intermediate levels of skin pigmentation fall within these two extremes," said Hearing, who believes that the mechanisms underlying the different cancer risks of these ethnic populations are due at least in part to the UV-absorbing and chemical properties of the different types of melanins.

Realizing that manipulating melanin modulators—either DKK1 and/or NRG1—may have clinical utility, the Hearing team and NCI have filed patents for bioactive peptides to target them as possible therapies to correct hypo- or hyper-pigmented conditions or to modulate the production of melanin. Manipulating pigmentation and studying its effects is of even greater importance to cancer research because hypo-pigmentation is a common characteristic of very aggressive melanomas.

## Beware of Traffic Jams

Realizing that their analysis of melanogenesis at the molecular level would not be complete without addressing the trafficking of melanosomal components—the Hearing team took a close look at pigmented and unpigmented melanocytic cells. (See Melanosomal Trafficking figure below.) They learned that in pigmented cells, the early movement of melanosomes involves the endoplasmic reticulum, while later transport to the dendritic extensions depends upon a protein called dyenin, and final movement to the periphery requires the interaction of well-positioned spectrin and actin filaments. When all these components are in the right place at the right time, working together, pigmentation occurs. Interestingly, they found that this orderly process does not occur efficiently in unpigmented melanocytic cells. Spectrin is




(*Image:* V. Hearing, CCR)

**Movement of melanosomes in unpigmented (top) and in pigmented (bottom) melanoma cells.**

Vesicles containing melanosomal proteins bud from endoplasmic reticulum (1), and are then moved forward by dynein (2) and spectrin (3). At the transGolgi network (4), delivery to the plasma membrane region begins. New vesicles are internalized (5) and directed to melanosomes (6). Kinesins promote their transport to the cell periphery via microtubules (7) and actin filaments enable their secretion (8). Adapted from H. Watabe et al., J. Invest. Dermatol. 2008, 128:162-174.

missing in the plasma membrane, so interactions with actin do not occur, and pigment trafficking breaks down.

## A Breakthrough Discovery

Because melanosomal proteins are complex, transient, and always in motion—even when all is functioning correctly—identifying and correcting malfunctioning components is like trying to hit a moving target. Fortunately, Thierry Passeron, M.D., Ph.D., a former Postdoctoral Fellow in Hearing's lab and now a researcher in the Department of Dermatology at the University Hospital in Nice, France, succeeded in doing just that. In a series of salient experiments performed while he was in the Hearing lab, he discovered a gene coding for a transcription factor called *SOX9* that functions upstream in regulating the melanogenic pathway, so it regulates both DCT and TYR and other key melanosome-linked genes.

Gene expression of *SOX9*, which occurs in both neonatal and in adult human skin, increases pigmentation in response to UVB exposure and acts counter to a pigmentation inhibitor called ASP. Passeron noticed that the loss of *SOX9* expression was a consistent marker of skin cancer malignancy. As melanoma progressed in aggressiveness, *SOX9* expression decreased; and as the disease turned metastatic, *SOX9* expression vanished completely.

So Passeron wondered if *SOX9* expression could be reversed. Could expression of *SOX9* be restored in melanoma cell lines? And if so, would tumorigenicity be inhibited? The answer was a resounding, "yes." Passeron restored *SOX9* function in melanoma cells by treating them with a substance found naturally in the body called prostaglandin D2 (PGD2).

While confirming this result in human ex vivo models, Passeron and his collaborators uncovered a possible explanation for its mechanism of action. When *SOX9* expression ceased, over-expression of a melanoma antigen called PRAME occurs, which inhibits the retinoic acid receptor, making the melanoma cells insensitive to retinoic acid therapy (RA). This helped explain why RA, an effective therapy for many other cancer types, often proves ineffective for melanoma. They found that RA sensitivity returned when *SOX9* expression was restored and that treatment with either PGD2 or PGD2 plus retinoic acid inhibited the proliferation of melanoma cells by 50 percent and 75 percent, respectively.

To translate this discovery into clinical benefit as quickly as possible, Passeron, Hearing, and NCI have patented this noncytotoxic PGD2 approach to targeting and restoring *SOX9* activity and thereby restoring melanoma's sensitivity to RA treatment. Passerson is testing this and other *SOX9* gene re-activators in his laboratory. This is an important contribution, because treatment options for aggressive melanoma are limited.

## Finding Protein Biomarkers

In an attempt to find better melanoma therapies, the Hearing team is also tackling another very sinister behavior of aggressive melanomas. As melanocytes turn cancerous and become increasingly malignant, they tend to stop differentiating, and so they express fewer differentiation antigens. This allows them to evade detection by the body's immune system. So Hearing and his team are searching for preserved melanocyte-specific biomarkers, ones that are expressed at an earlier-stage and continue to be expressed by less-differentiated melanocytes and melanoma cells. These markers will be useful for detecting melanoma earlier and may even serve as targets for antibody-based therapies. Toward this end, Hearing's group is developing techniques needed to purify earlier stages of melanosomes and to characterize them in terms of new markers within the melanosome proteome.

The search for new biomarkers is important because the markers currently used to detect melanoma lack either specificity in the case of

(*Photo: R. Baer*)

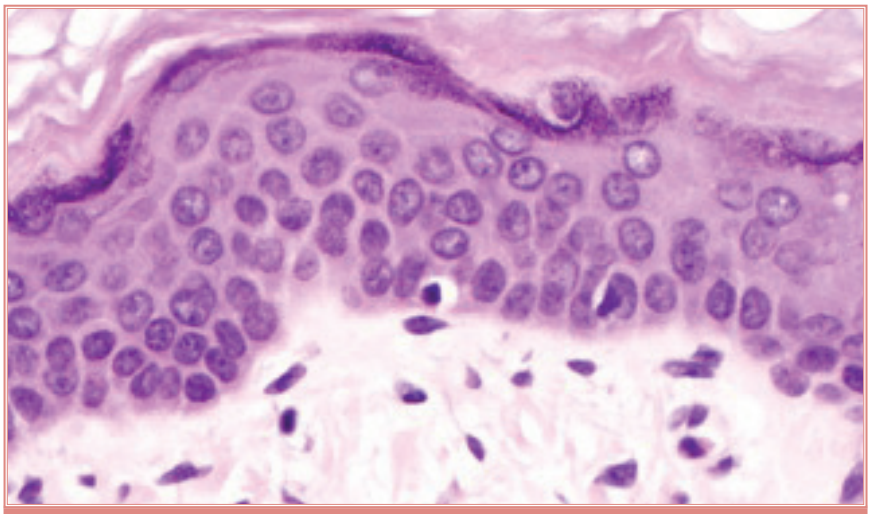
Vincent Hearing, Ph.D., and Sergio Coelho

the S100 protein, or sensitivity in the case of the PMEL17 protein. One possible biomarker being investigated by the Hearing lab is a melanosome-specific proteolytically released protein called GPNMB that appears in the melanosomes of nonmetastatic melanoma and is enriched and secreted from mature melanosomes.

## Understanding UVA and UVB Exposures

To determine the effects of UVA and UVB exposures on human skin, the Hearing team participated in collaborative clinical studies with the U.S. Food and Drug Administration (FDA). They discovered that the two component wavelengths of UV, UVA and UVB, produce different effects on the skin. Yoshinori Miyamura, Ph.D., now at the University California-Davis, and Sergio Coelho, M.S., a Research Biologist in Hearing's lab, noted that, after both types of UV exposure, melanins are distributed in all epidermal layers, but the pigment granules are larger after UVB exposure, and are only intermediate in size after UVA exposure. Also, while both wavelengths induced visible tans, the UVA-induced tans allowed more DNA damage to occur, while the UVB-induced tans provide a modest photoprotection against a subsequent UV exposure.

"We found that the areas that had been irradiated with UVB only or with UVA+UVB actually synthesized new melanin, which provided some protection against the subsequent UV challenge, but the UVA-only tans did not synthesize new melanin and provided no photoprotective benefit. This is an important consideration given that UVA-rich lamps are now frequently used in the indoor tanning industry to promote tanning with the implied potential benefit of reducing DNA damage and increasing protection against subsequent UV exposure," said Hearing.



(Image: Duke University, Department of Pathology)

Melanocytes comprise from 1 to 2 percent of the cells in the basal layer of the epidermis. They are pigment producing factories that provide a surface tan to protect the human body from UV-induced DNA damage to deeper layers of the skin.

Another important factor identified in this study was the highly variable rate of DNA damage removal and/or repair among individuals even within the same ethnic group. Some individuals were highly efficient at repairing DNA lesions and no damage was evident one week after UV exposure; other individuals were inefficient in this process and repaired less than 50 percent of the initial UV damage within one week.

This research will help cancer researchers to better understand the factors responsible for photocarcinogenesis, and the data also will inform the FDA as it regulates UV lamps.

## Armed with Antibodies and 3-D Skin Models

Future research will include studies by Coelho, who is optimizing antibodies to eumelanins and pheomelanins. These antibodies will enable Hearing and his colleagues to assess the types and distributions of melanins in future projects. In addition, Hearing is working with 3-dimensional artificial, but physiologically relevant, skin models. These models will permit the Hearing team to regulate precisely the type, amounts, and distribution of melanins in the skin and then challenge the system with different wavelengths of UV to more critically assess the roles of different types of melanins in photoprotection.

Of course, Hearing and his colleagues will then have to validate all of the activities seen in these model systems. They will have to demonstrate that the same changes occur in human skin and see whether there are the same consequences. With validation complete, Hearing will yet again contribute valuable insights to skin cancer research.

"While we already have drilled down more than skin deep, much remains unknown about relationships between DNA damage repair and the different types, quantities, and distribution of melanins in the skin. Ongoing studies within our laboratory will continue to clarify these and new questions as they arise," said Hearing.

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

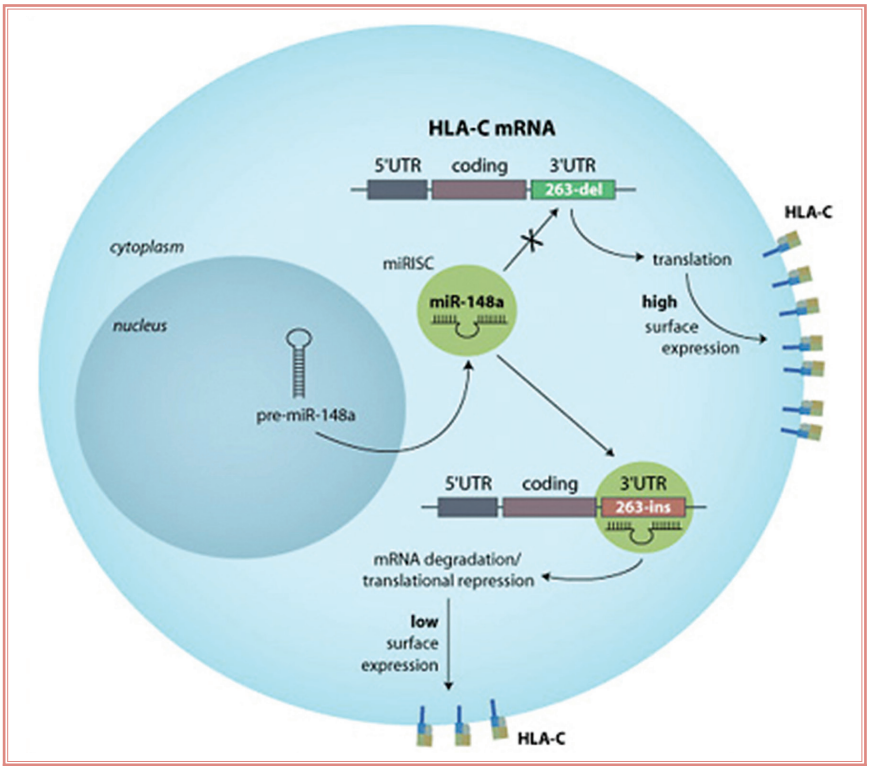
# Seeing the Unexpected

*Mary Carrington, Ph.D., Senior Investigator in CCR's Laboratory of Experimental Immunology and Director of the SAIC Basic Science Program at the Frederick National Laboratory for Cancer Research, has a talent for seeing unexpected molecular interactions, and for interpreting their implications. While studying the genes that code for human leukocyte antigens (HLAs)—the molecules that distinguish "self" vs. "nonself" on human cells, tissues, and organs—and the role they play in a person's susceptibility to HIV infection, she and her colleagues made a novel discovery. They found that tiny variants called single nucleotide polymorphisms, located within untranslated regions of the HLA-C gene—where microRNAs like to bind—can actually change the amount of "self" molecules produced and displayed.*

She and two Postdoctoral Fellows, Smita Kulkarni, Ph.D., who works in her lab, and Ram Savan, Ph.D., who worked with Howard Young, Ph.D., a colleague in the Laboratory of Experimental Immunology, realized immediately that this unexpected post-transcriptional interaction between microRNA and a particular *HLA* variant could be a powerful modulator that regulates *HLA* expression and, in turn, influences host immune responses in human disease, whether triggered by HIV infections, autoimmune diseases, or cancer.

## Another Unexpected Interaction

The immune system is a complex network. In many cases, the actions of various components are intertwined, and the effects of one component may be greatly affected by its relationship with a different component. In their study of the host immune responses and diseases, Carrington and her collaborators have made another important and unexpected contribution. They have carefully documented how *HLA* and a completely separate gene cluster, the killer cell immunoglobulin-like receptor (*KIR*) genes, conspire to operate in functionally relevant combinations for good or ill. Working




(*Figure:* M. Carrington, CCR)

Carrington and colleagues have found evidence that a single nucleotide polymorphism (-35 SNP) marks the presence of a variant in the HLA-C microRNA 148a binding site that directly determines whether or not there is an increase in expression of HLA-C.


(*Photo: R. Baer*)

Xiao-jiang Gao, Ph.D., and Mary Carrington, Ph.D.

together, these gene clusters determine how "non-self" invading pathogens or "self" tumors are either tolerated or destroyed by the body's immune system.

KIR molecules, the product of *KIR* genes, are the largest category of receptors expressed on the surface of natural killer (NK) cells. These cells are a key component of the innate immune system, the body's immediate response against foreign invaders and tumor cells. When a foreign cell enters the body, its own HLA class I "self" molecules present a piece of its protein on its surface. The NK cell's KIR receptor binds to it, and sends a signal to the NK cell that activates or inhibits its activity. So by using the invading cell's own HLA "self" markers, KIR molecules help to regulate the NK cell's ability to kill other cells.

Although the *HLA* and *KIR* gene clusters work together, they do not reside together. The *HLA* and *KIR* clusters are located on chromosomes 6p and 19q, respectively, so they are inherited independently. This increases genetic variation, which in turn, creates extensive diversity among HLA class I and KIR molecules. However, to be effective KIR molecules and corresponding specific HLA molecules must be present together to regulate NK cell activity. Therefore, it is not surprising that variation of these gene groups likely influences the risk of a variety of diseases.

> "Over the past several years, we have discovered the breadth and depth of influence exerted by *HLA/KIR* genetic-variant combinations," said Carrington, "but much remains to be discovered, understood, and applied before we can improve the lives of cancer patients and of patients who suffer from infectious diseases."

## *HLA/KIR* Combinations

*HLA/KIR* variant combinations may have a protective effect. Very strong evidence exists showing that the *HLA/KIR* immune response genes are under natural selection in some regions. "Very different HLA/KIR variant frequencies have been found in particular geographic locations. We believe these frequency differences may be related to the types of diseases endemic to particular regions," said Carrington. "For example, certain HLA/KIR gene forms may be protective against infection with the parasitic protozoan *Plasmodium malariae* in a region where malaria is prevalent, so a higher frequency of

these malaria-protective variants may be found in the population located there. This would probably occur because individuals who inherited the protective variants would live longer and reproduce. Conversely, in other regions of the world, where malaria is not a big problem, there might not be selection pressure for the malaria-protective *HLA/KIR* gene variants."

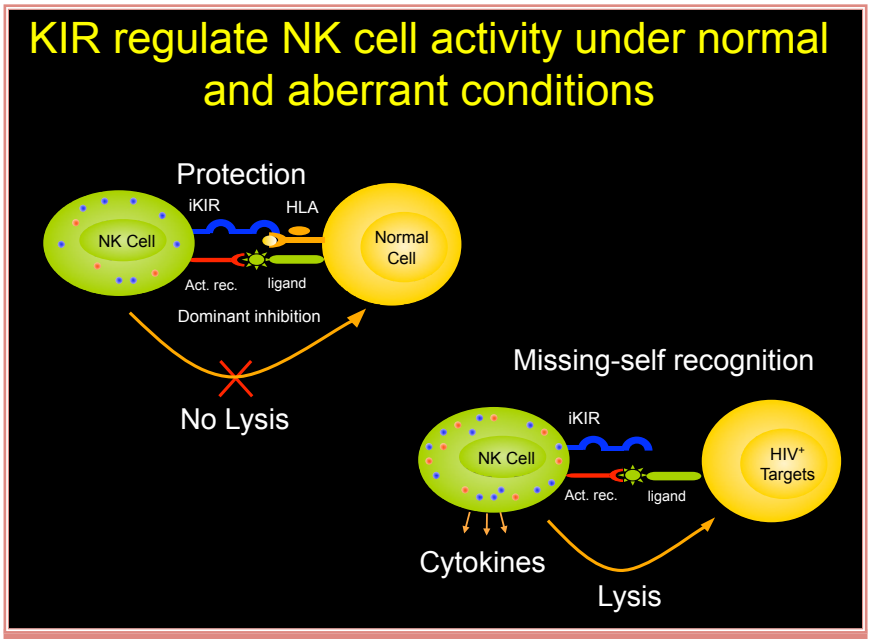
*HLA/KIR* combinations also play a role in immune surveillance. *HLA* gene variants manufacture products with extensive diversity that serve the immune response well by interacting with an unlimited number of "non-self" fragments. Whether encountering an external pathogen or an enemy from within, such as a cancer cell, the molecules produced by *HLA* variants notify the body's immune system that a response is needed. "*HLA* diversity ensures the survival of our species by providing resistance to a wide breadth of infectious organisms and even the ability to eliminate cancer cells," said Carrington.

The diversity generated when many different types of *HLA* markers on target cells can coexist in concert with many different KIR molecules on NK cells, increases the effectiveness of the immune surveillance system. As the combinations *HLA/KIR* genetic variants change, their products change, and NK cell activity changes, ranging from strong inhibition to strong activation.

Genetic variation plays another important role, too. KIR molecular diversity also produces inhibitory KIR molecules that allow NK cells to identify normal body cells as "self." This prevents autoimmune attacks on healthy autologous ("self") cells.

## *HLA-C/KIR* Combinations and Cancer

Aware that expression levels of *HLA/KIR* variants can influence host immune responses in both human infection and disease, the Carrington team applied their discoveries to better understand a woman's risk for cervical cancer, which is now known to be caused by infections from one of about 15 strains of HPV, most often HPV16.




(*Figure*: M. Carrington, CCR)

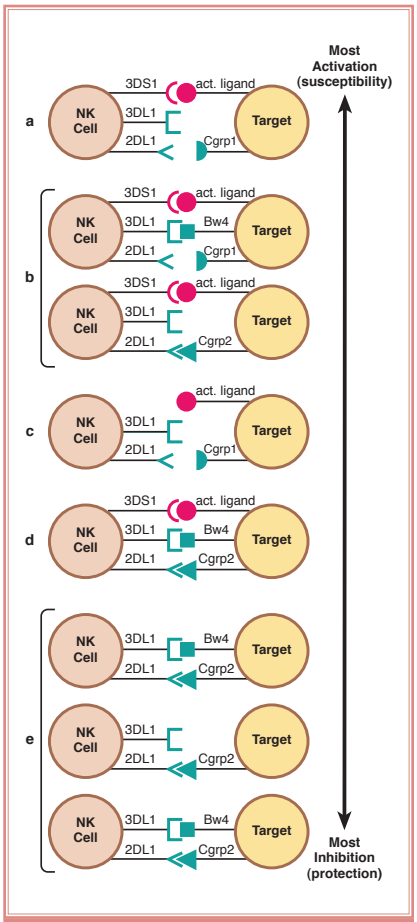
NK cells express KIR on their cell surface that can send either activating or inhibitory signals to NK cells. Inhibitory KIR recognize specific HLA class I molecules on target cells. If these molecules are present, KIR will send a signal to the NK cell not to kill the cell. Some virally infected cells and some tumor cells downregulate HLA class I to escape NK cell recognition. In this instance, the inhibitory KIR will not see its HLA class I counterpart in the normal context and an activating KIR will send a signal to kill the cell because its HLA class I expression is abnormal.

HPV infection is the most common sexually transmitted disease in the nation. While most HPV infections exhibit no symptoms and clear up without treatment, some can persist and lead to cancer. The Carrington team was curious about what constitutes this persistence at the molecular level. Specifically, are certain *HLA/KIR* genotype variants, or genetic variations, involved in a woman's risk of developing cervical cancer?

Carrington and her team have a longstanding collaboration to investigate cervical cancer with Allan Hildesheim, Ph.D., an Investigator in NCI's Division of Cancer Epidemiology and Genetics. In one study, the researchers investigated whether *KIR* genes are involved in the risk of developing cervical neoplasia, a cancer caused by HPV. The two research teams grouped *HLA-B (HLA-B Bw4)* and *HLA-C (HLA-Cw* groups 1 and 2) molecules according to their specificity for certain *KIR* receptors. Having obtained samples from patients in three large cervical cancer studies, the teams compared the presence of the HLA groups in normal and abnormal cervical tissue samples that were classified as intraepithelial neoplasia 3 (CIN3)–also known as stage 0 cervical carcinoma. When they merged data from all studies, Carrington and Hildesheim found that *HLA-Cw* group 2 and *HLA-B Bw*4 groups were significantly associated with a decreased risk of cervical cancer. The presence of both of these alleles, forms of the KIR gene, had a stronger protective effect than the presence of either alone.

"This suggests that these *HLA* class I variants may be exerting influence on cervical cancer based on

(*Image:* M. Carrington, CCR)




**Model shows KIR-mediated NK cell types associated with risk of developing cervical neoplasia.**

Resistance to cervical neoplasia increases when genotypes are ordered by their ability to confer the most activation (susceptibility) to the most inhibition (protection). The red shapes represent activating receptors and HLA class I molecules; the green shapes represent inhibitory receptors and HLA class I molecules.

their capacity to bind inhibitory KIR molecules, which regulates NK cell activity," said Carrington. By contrast, the presence of an activating KIR receptor, KIR3DS1, was found more frequently in CIN3 tissue samples than occurred in noncancerous cells. So, activating and inhibitory KIR molecules appear to be involved in a woman's risk of developing cervical cancer.

Allelic groups of *HLA* whose gene products bind KIR3DS1 molecules could trigger an immune response, while those produced by *HLA-Cw* group 2 and *HLA-B Bw4* allelic groups could bind to KIR2DL1 and KIR3DL1 molecules, respectively, and avert immune activation. "Therefore, it appears that *KIR*-associated immune activity is linked to cervical pathogenesis," said Carrington.

Aware that only a few HPV infections can persist and lead to cancer, the Carrington team developed a model to explain this observation in molecular terms. They suspected that there is a gradation of influences, ranging from *HLA/KIR* genotype combinations that are most activating to ones that are most inhibitory. Then they discovered examples of just that—inhibitory HLA/KIR molecule pairs that decrease the risk of developing cervical cancer and the presence of the activating receptor KIR3DS1—particularly when not accompanied by *HLA-Cw* group 2 and *HLA-B Bw4* groups—that results in susceptibility to cervical cancer.

## Variants Are Complicated

Genetic epidemiological studies show that compound genotypes expected to result in immune-activating phenotypes may be associated with protection against a particular infectious disease, and may also be associated with susceptibility to autoimmune disease. Genotype is the internal inheritable allelic information within a person. Phenotype is the external physical manifestation of all the inheritable allelic information within a person. Overall, Carrington and her collaborators have found a general trend for the association of an overactive KIR component with a favorable outcome in infectious diseases, but with an unfavorable result in autoimmune disease and cancer.

## *HLA-C/KIR* and Cervical Cancer Risk

Continuing their collaboration with Hildesheim, Carrington and her team are studying a large collection of CIN3/cervical cancer samples from the Guanacaste Natural History Study (NHS). NHS is a population-based cohort of 10,000 women from Guanacaste, a rural province of Costa Rica with a high incidence of invasive cervical cancer. Cervical and blood samples were obtained and HPV tests were conducted annually for over seven years.

Having these cervical samples available now permits the Carrington team to expand on their earlier discovery of the interaction between untranslated regions of the *HLA-C* gene and microRNAs in HIV infection. Having discovered that microRNA post-transcriptional activity with *HLA-C* can actually change the amount of "self" molecules, the team can now study *HLA-C* variants and their interactions with KIR genotypes in relation to cervical cancer risk.

"In particular, my colleagues and I are now interested in identifying all the factors that can influence levels of *HLA-C* expression in cervical tissue because we have evidence that there is a decreased risk of cervical neoplasia when strongly inhibitory *HLA-C/KIR* combination genotypes are present," explains Carrington. "Over the past several years, we have discovered the breadth and depth of influence exerted by *HLA/KIR* genetic-variant combinations," said Carrington, "but much remains to be discovered, understood, and applied before we can improve the lives of cancer patients and of patients who suffer from infectious diseases."

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

# Deconstructing Health Disparities

*Survival rates for African-Americans with cancer are simply not as encouraging as those for other racial groups. Many factors have been examined—differences in socioeconomic status and access to health care, PSA screening, age at diagnosis, and disease stage and grade—to identify reproducible causes for these substantial racial disparities, but so far, no convincing explanation has emerged. Stefan Ambs, Ph.D., M.P.H., a Senior Investigator in CCR's Laboratory of Human Carcinogenesis, who heads the Breast and Prostate Unit, is determined to change this situation. He has set out to unravel some of the causes for cancer's unequal burden within the African-American population by taking a broad biological view of the disease.*

## Seeing with New Eyes

Ambs is well suited for the task. He has a track record of seeing old facts with new eyes. Earlier in his career, long before he set out to tackle health disparities, he began approaching existing data and seeing patterns that were not visible to his colleagues. While many scientists were trying to understand differences in breast cancer risk and response to therapy, he looked beyond the usual suspects—the highly penetrant single-gene mutations like *BRCA1* and *BRCA2*—and realized that risk and treatment response could be influenced, instead, by interactions among a group of low-penetrant genetic variations. (When a mutation is penetrant, the internal change in DNA is linked to an external, visible trait.) His new way of seeing evidence has been successful. He and his colleagues discovered that one low-penetrant variant in the manganese superoxide dismutase gene, called *Val16Ala*, is commonly associated with resistance to cyclophosphamide-based therapies, and they produced a patented test to help patients find out if they carried this variant. This test can potentially save a patient from ineffective therapy because those carrying the *Ala* variant will not benefit from cyclophosphamide, a drug commonly added to combination chemotherapy for breast cancer.

## Novel Approach to Health Disparities

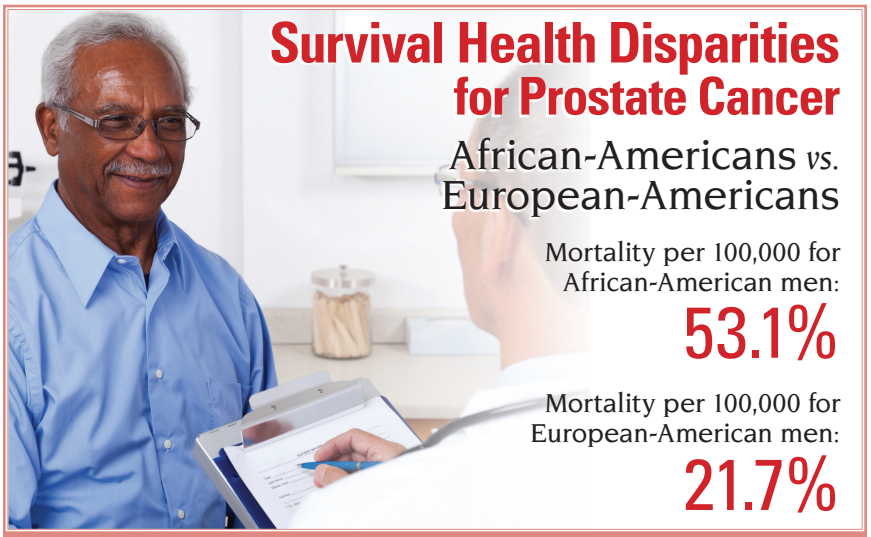
Turning his attention to the survival health disparity in breast cancer, Ambs suspected that unique epigenetic alterations may affect the tumor biology of African-American breast cancer patients. In a pilot study, he and his lab colleagues, Tiffany Dorsey and Atsushi Terunuma, M.D., Ph.D., collaborated with Songping Wang and Bernard Kwabi-Addo, Ph.D., from Howard University's Department of Biochemistry and Molecular Biology, to investigate the differences in CpG island methylation between African-American and European-American breast cancer patients focusing on several candidate gene loci. The team found that DNA methylation in breast tumors was different by race/ethnicity for a few key tumor suppressor genes. For example, the study revealed significant differences in the CDH13 methylation status. Several of the

(*Photo:* R. Baer)

Prachi Mishra, Ph.D., Adrienne Starks, Ph.D., Stefan Ambs, Ph.D., and Tiffany Dorsey

observed differences were even more pronounced in women with the estrogen receptor-negative disease and among young patients (women less than age 50). And, most important, CDH13 methylation seemed to be a marker for reduced overall disease survival. These initial observations are now being followed using state-of-the-art techniques for whole genome DNA methylation analysis. First results indicate that DNA methylation patterns in breast tumors indeed differ by race/ethnicity.

Since his main research focus is now prostate cancer, Ambs and his team are deconstructing the unequal burden of cancer borne by African-American prostate cancer patients by looking at the role gene expression profiles play in tumor biology. The Ambs team is not content with just finding differences in genomic expression profiles between different male ethnic populations and stopping there. They want to know how a collective set of gene activities contributes to the biology of the tumor, so they are searching for gene signatures that define tumor biology. After analyzing differences in gene expression in prostate tumors from African-American and European-American patients, overall, they recognized three broad functional areas that seem to differently affect the two patient groups: gene activities involved in making an immune response, those involved in helping cancer to spread, and those involved in fighting viral infections through the interferon pathway. Ambs and his colleagues also are analyzing microRNA differences for additional clues to changes in the biological mechanisms that are enabling more aggressive tumors to thrive in African-American patients. "Tumor biology is widely assumed to be the same among all ethnic groups, but evidence is emerging that population differences may be linked to tumor biology differences. Change in scientific concepts comes slowly, because change in itself is hard to accept. But if there really are tumor biology differences between ethnic groups, we all must change our thinking. It would mean that therapies already proven efficacious for white men may not work as well in other racial or ethnic groups," explained Ambs.




*SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations)*, National Cancer Institute. Bethesda, Md.

## Calling All Prostate Cancer Specimens

Ambs is interrogating prostate tissue directly to discover and dissect out the changes that occur in tumors of African-American men. He is grateful to his lab chief and collaborator, Curtis Harris, M.D., who heads the Laboratory of Human Carcinogenesis, because Harris had the foresight, 30 years ago, to collect annotated tumor specimens, blood samples, and epidemiological profiles from cancer patients among minority populations. But the work ahead will need many more tumor specimens—likely hundreds more prostate cancer samples—for Ambs to attempt to demonstrate a biological basis for the prostate cancer health disparities observed in the African-American community. The Ambs team is actively recruiting up to 1,000 prostate cancer patients and 1,000 healthy volunteers of African-American or European-American descent from two Baltimore-area hospitals: Veterans Affairs Medical Center and the University of Maryland Medical Center. Healthy volunteers are matched by age and race to men with prostate cancer. They take a survey that identifies possible risk factors for cancer development and progression, and donate samples of blood and urine; prostate cancer patients take a survey and donate their tumor specimens after prostatectomy.

Ambs and his research team are analyzing these samples in collaboration with Andy Hurwitz, Ph.D., an Investigator in CCR's Laboratory of Molecular Immunoregulation, and Ludmila Prokunina-Olsson, Ph.D., an investigator in NCI's Division of Cancer Epidemiology and Genetics, and with extramural collaborators, including Arun Sreekumar, Ph.D., a faculty member at the Baylor College of Medicine, and Carlo Croce, M.D., Director of The Ohio State University Medical Center's Institute of Genetics. "Our goal is to identify differences both in risk factor exposures and in tumor biology that are present among African-American and European-

American men. We want to know if environmental and genetic factors both contribute to the prostate cancer health disparity between these two groups by affecting tumor biology in specific ways," said Ambs.

For Ambs, the genome has to be used ultimately to determine tumor biology. Without discovering the function of expressed gene clusters, there will be no progress. And there's not much a scientist can do with a lot of mutations unless he or she can distinguish between what researchers describe as a "driver" mutation and a "passenger" mutation. To do that, the researcher needs to know the gene's raison d'etre, its reason for existence.

## Difference in Tumor Biology

The Ambs research team started looking at several known metastasis-promoting genes, including autocrine mobility factor receptor, chemokine CXCR4, and matrix metalloproteinase 9, and found the genes are more highly expressed in tumors of African-American men. Next using their cohort, they identified a two-gene tumor signature that accurately differentiates between African-American and European-American prostate cancer patients. In prostate tumors, the expression pattern of these two genes alone—phosphoserine phosphatase pseudogene 1 (*PSPHL*) and crystallin, beta B2 (*CRYBB2*)—could successfully classify 91 percent of African-American samples and 94 percent of European-American ones.

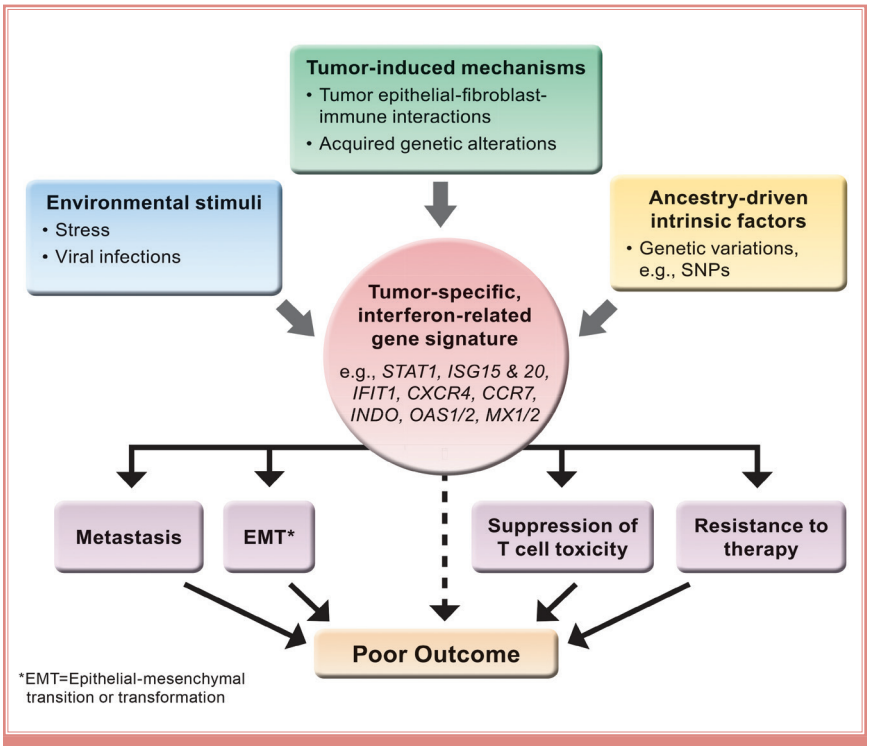
*PSPHL* is the most highly up-regulated gene in prostate tumors from African-American patients, displaying a 160-fold difference in gene expression levels when compared to prostate tumors from European-American men. *PSPHL* is located on chromosome 7q11.2, a chromosomal region related to advanced tumor stage in prostate cancer, yet there are no studies linking this gene's over-expression to cancer progression. Ambs, in collaboration with Jun Luo, Ph.D., and William Isaacs, Ph.D., at The Johns Hopkins University, may have discovered why this gene activity was not studied further, at least in European-American men with prostate cancer. The team found that the *PSPHL* locus is frequently germline deleted (permanently removed from the DNA in a germ cell, which is destined to become a sperm) in European-American men while remaining present in men of African descent.

Not content to stop at classification alone, the Ambs team is now seeking the function of *PSPHL*. "Since we now know that the *PSPHL* gene encodes for two transcripts that are both expressed in prostate cancer cells, we are currently investigating the function of these transcripts," said Ambs.

## Seeing Microenvironment as a "Driver"

Using microarray technology, Ambs and his colleagues took the broadest biological view possible in search of causative activities linked to health disparities. They performed genome-wide gene expression profiling of primary prostate tumors and normal prostate tissue—matched on clinical variables—donated from African-American and European-American patients and healthy volunteers.

"We analyzed the resulting datasets on disease-association-levels and pathway levels and found that each patient group has a distinct tumor microenvironment," said Ambs. "Many of the differentially expressed genes pointed to significant differences in tumor immunobiology and tissue inflammation pathways between the two patient cohorts. These




(*Figure: Adapted from S. Ambs, CCR*)

The distinct interferon-related gene signature found to be more prominent in African-American prostate tumors may be induced by tumor-signaling mechanisms, environmental stimuli, and inherited intrinsic factors. The signature has been associated with poor disease outcome, which may be caused by suppression of immune cells, metastasis, and resistance to therapy.

microenviromental differences could be among the "drivers" that are producing the survival health disparities among different ethnic groups."

Differing immunologic profiles could have many causes: environmental factors, genetic variations, or the interactions of both. Interestingly, chronic inflammation, which is believed to be a contributing factor in prostate carcinogenesis, was found to be more prevalent in nontumor prostate biopsy specimens from African-American men when compared with European-American men. This discovery continues to prompt active investigation.

## A Singular Interferon Signature

A distinct prostate-linked interferon signature also has been identified as more prominent in African-American tumors. It appears in about half of tumors of African-American origin and in about 20 percent of tumors of European-American origin for multiple datasets. This interferon signature is almost identical with an interferon-related, DNA-damage-resistance signature that serves as a breast cancer biomarker that predicts tumor resistance to chemotherapy and radiation.

In the literature, the breast-cancer interferon signature has been linked with metastasis and poor disease outcomes, suggesting that the similar—albeit not identical—prostate-cancer interferon signature may be driving the heightened aggressiveness of this cancer in African-American men. This particular interferon signature has also been linked to the foreboding prometastatic epithelial-to-mesenchymal transition of cancer cells. And the signature is induced by interactions between fibroblasts and tumor cells, suggesting that this expression pattern promotes cancer metastasis. Thus, the interferon signature in prostate tumors may alter the immune environment and also weaken the cytotoxicity directed against cancer cells. "The high prevalence of a distinct interferon signature in cancer cells from African-American patients could be clinically very significant and warrants further investigation into the origin of this signature, and how this signature can be targeted," said Ambs. "The presence of this signature in African-American tumors may not only affect their response to immune-based therapies, but it may also make them more resistant to standard therapies. It is possible that tumor-induced signaling, environmental stimuli, and inherited intrinsic factors collectively induce the distinct interferon-related gene signature in prostate tumors," added Ambs.

"The presence of this signature in African-American tumors may not only affect their response to immune-based therapies, but it may also make them more resistant to standard therapies. It is possible that tumor-induced signaling, environmental stimuli, and inherited intrinsic factors collectively induce the distinct interferon-related gene signature in prostate tumors."

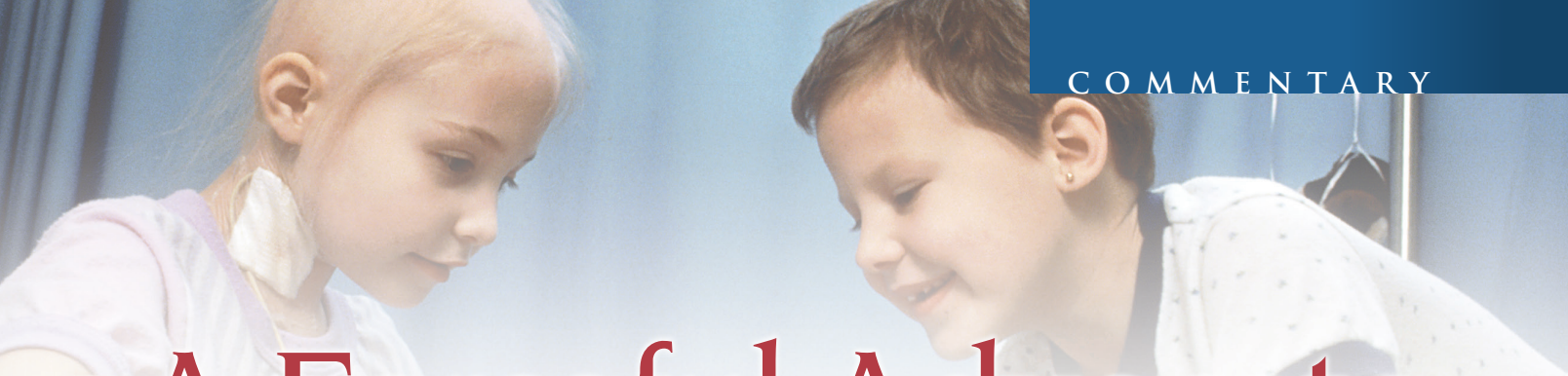
## Seeing Pathogens as a "Driver"

Elevated expression of an interferon gene signature in prostate tumors could also occur because it signals that an infection is under way. Such up-regulation of an interferon signature could be triggered by an invading external pathogen, such as a bacteria or virus, or it could be caused by reactivation of an endogenous retrovirus, like human endogenous retrovirus type K (HERV-K), often found in the tumor microenvironment.

This latter explanation is supported by Ambs' finding that the distinct interferon signature in prostate tumors coincides with a gene signature of retroviral activation. So he and his team are exploring the presence of endogenous retrovirus reactivation in tumors from African-American and European-American patients, in collaboration with Feng Wang-Johanning, Ph.D., at The University of Texas M.D. Anderson Cancer Center.

"Cancer health disparity is a very controversial issue, but we need to look at the old facts in new ways. It is imperative at a time when we have the technology to answer our queries that we begin to ask the right questions. Understanding the biology behind the observational studies of cancer health disparities will advance this field of research and make significant contributions toward countering the unequal burden of cancer. Whether the health disparities are occurring in breast or prostate cancer patients, we are familiar with the problem. I know I can do something about it, and I have a great resource at NCI and a great team of colleagues to help me do it," said Ambs.

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



# A Forceful Advocate for Young Cancer Patients

*"I consider our ability to cure a child with cancer the most important job in the world." With these words, Peter C. Adamson, M.D., Chair of the Children's Oncology Group—an NCI-supported consortium of more than 200 centers across North America, Australia, New Zealand, and parts of Europe—recently commented at a U.S. Food and Drug Administration briefing about the shortage of cancer drugs.*

(Photo: Peter Adamson)

Peter C. Adamson, M.D.

At the FDA briefing, I urged the agency to take the necessary steps to enforce early reporting of impending drug shortages. Moreover, I suggested that the government explore novel approaches, including incentives, which would result in the establishment of a strategic reserve of life-saving drugs for children with cancer.

The roots of my advocacy efforts can likely be traced back to my medical school training at Cornell University Medical College. It was during my first clinical rotations that I learned the importance of every patient having an advocate to help navigate the health care maze. I carry the importance of advocacy with me these many years later, which in part led me to speak with the FDA on the cancer drug shortage crisis. Being able to effectively advocate for children is an important skill for both pediatricians and pediatric subspecialists. Whether it is for an individual patient or a group of patients, advocacy for the children in our care has been stressed at various times throughout my pediatric training, both as a resident at The Children's Hospital of Philadelphia (CHOP), and during my 12 years of subspecialty training and work in NCI's Pediatric Oncology Branch.

During my time at NCI, in addition to the leadership and guidance of the then Chief of the Pediatric Oncology Branch, Philip Pizzo, M.D., I had the distinct privilege of having two of our fields' most outstanding mentors, David Poplack, M.D., and Frank Balis, M.D. I was afforded tremendous research opportunities

> Emerging research shows that even the more common childhood cancers are a mixture of diseases, with each subset potentially requiring a unique and specific type of targeted therapy. It is clear that we must develop a better and more efficient way to move novel treatments from our labs into the clinic.

early in my training. One of my first projects focused on understanding key elements of the clinical pharmacology of a unique type of targeted therapy for children and adults with acute promyelocytic leukemia (APL). All-trans retinoic acid (ATRA, Tretinoin) was emerging as a major therapeutic advance for patients with APL, but the ability to maintain necessary drug exposures in patients appeared to limit its efficacy. Our work, first in pre-clinical models and then in adult and pediatric clinical trials, helped define the important clinical pharmacology of ATRA, which when administered on a continuous basis quickly induces its own metabolism. We went on to demonstrate that an intermittent schedule of drug administration could in part overcome this effect, and since then, an intermittent schedule of drug administration has become the standard way of administering ATRA to patients.

After my years at NCI, I returned to CHOP to start a new program of pediatric drug development that would extend beyond pediatric oncology. We established the Division of Clinical Pharmacology and Therapeutics, and our group led or supported a broad range of clinical trials that were being performed under the Best Pharmaceuticals for Children Act (BPCA). At CHOP, I more recently had the opportunity to support a broad range of clinical-translational research efforts, serving for a number of years as CHOP Research Institute's Director for Clinical-Translational Research.

About 18 months ago I was elected to lead the Children's Oncology Group (COG; www.childrensoncologygroup.org), the world's largest organization devoted exclusively to childhood and adolescent cancer research. The COG unites more than 8,000 experts in childhood cancer at more than 200 leading children's hospitals, universities, and cancer centers across North America, Australia, New Zealand, and parts of Europe in the fight against childhood cancer. Today, more than 90 percent of the 13,500 children and adolescents diagnosed with cancer each year in the United States are cared for at COG member institutions. Research performed by COG institutions over the past 50 years has helped transform childhood cancer from a virtually incurable disease to one with a combined five-year survival rate of 80 percent.

Whether it is in the clinic, or in the development and execution of COG clinical trials, a key focus of our work will be to shorten the time it takes to get results.

My role as Chair of the COG has enabled me to rethink how the oncology research community can best move its discoveries into pediatric clinics. Emerging research shows that even the more common childhood cancers are a mixture of diseases, with each subset potentially requiring a unique and specific type of targeted therapy. It is clear that we must develop a better and more efficient way to move novel treatments from our labs into the clinic. Through COG, we can forge collaborations worldwide to improve efficiencies in all phases of clinical research.

There are a number of lessons from both the research realm and the clinical realm that influence how I now train pediatric residents and fellows. One common theme is that of the importance of time—whether it is the time to move an important idea from the laboratory into the clinic, or the time to deliver clinical test results to a patient and family. For families with children with cancer, there is perhaps no greater anxiety-provoking period than the time between the performance of a diagnostic test and the receipt of test results. Whether the news is good or bad, awaiting results in a remarkably stressful time for patients and families. So the delivery of results in a clear, compassionate, and straightforward manner is essential. Whether it is in the clinic, or in the development and execution of COG clinical trials, a key focus of our work will be to shorten the time it takes to get results.

Research examining the timeline between the creation of a new clinical trial concept and the actual start of the clinical trial shed important light on the inefficiencies of our national cancer clinical research enterprise, be it research at NCI-designated cancer centers or throughout the cooperative group system. The COG has taken a number of steps to shorten this timeline. One approach was to incorporate a new trial design for the conduct of pediatric phase 1 trials, the first-in-children studies we perform for new anticancer agents.

Our focus is now on target identification and evaluation of novel therapies. Not only is the goal to improve the cure rate, but also to decrease long-range deleterious effects of current-day treatment that too many of childhood cancer survivors face in young adulthood.

The pediatric phase 1 trial design we developed is called the rolling-six-method, and it allows us to reach a study endpoint, the recommended dose for children, in a significantly shorter period of time than was possible with prior designs. We used computer-based simulations to determine how the design would perform in the clinic, and have now moved to using the rolling-six as the standard approach to our phase 1 trials.

Since the introduction of chemotherapy for the treatment of childhood leukemia more than 60 years ago, the prognosis for children with cancer has indeed improved dramatically. The five-year survival rate for childhood cancers, many of which were uniformly fatal in the pre-chemotherapy era, is now approaching 80 percent. Despite these advances, several childhood cancers still have unacceptably low cure rates, and even when treatment is successful, the acute and long-term side effects can be substantial.

Our past success has come mainly through the more intense use of decades-old drugs, many of which were developed originally for adult cancers. But this approach to improving the outcome is, not surprisingly, paying diminishing returns. Thus our focus is now on target identification and evaluation of novel therapies. Not only is the goal to improve the cure rate, but also to decrease long-range deleterious effects of current-day treatment that too many of childhood cancer survivors face in young adulthood.

Relative to medical oncology, pediatric oncology faces some unique challenges in the development of novel approaches to the treatment of cancer. Despite a wealth of tantalizing leads from basic science, there is a near-complete void in commercial research and development for drugs specifically targeting pediatric cancer. As devastating as cancer is in children, the numbers affected are too small to drive innovation in the private sector. To potentially address this gap, we have envisioned a public-private partnership that could establish a virtual drug development company. Our ideas emerged from an Institute of Medicine committee I participated on in 2005. The report, *Making Better Drugs for Children with Cancer*, serves as a blueprint of how this could emerge, and is modeled in part on efforts undertaken for other diseases or illnesses including cystic fibrosis, tuberculosis, and malaria. A clear advantage that the pediatric oncology community has is that the clinical trial infrastructure afforded by the COG could conduct the full spectrum of pediatric clinical trials for drugs that result from such a venture, mitigating the major costs incurred by the private sector in drug development.

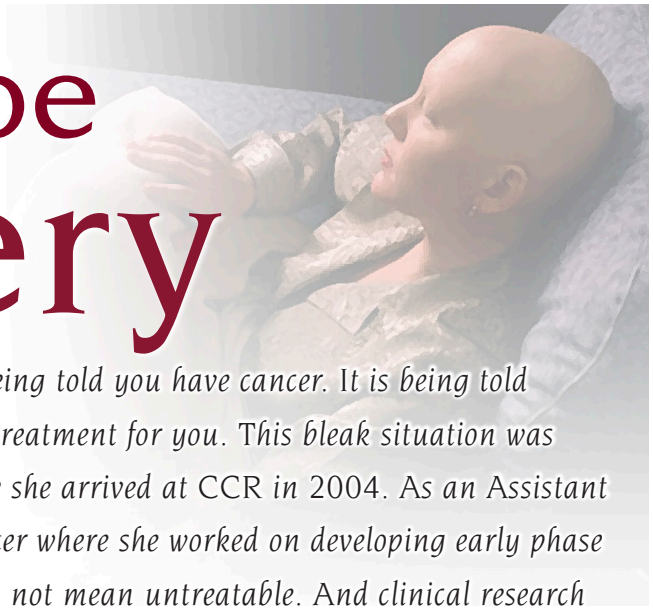
Cancer research is clearly entering into an age of discovery. We are using powerful analytical genomic tools—whose costs have fallen dramatically—to understand childhood cancer in terms of fundamental biology. Our ability to stratify patients for appropriate treatment continues to improve, and we foresee our lab-based efforts including genomics helping guide future research and therapy. The COG has a remarkable ability to partner with families in research, and this ability will provide a growing platform for discovery as we further link biology to outcome in the years ahead.

The COG has a remarkable ability to partner with families in research, and this ability will provide a growing platform for discovery as we further link biology to outcome in the years ahead.

Our discovery efforts are clearly focused on understanding the biology of all the childhood cancers, finding the Achilles' heels for every type of pediatric tumor, no matter how rare, and developing and delivering treatments that maximize the likelihood of cure while minimizing the near- and long-term effects of therapy. As one of the pioneers in pediatric oncology Giulio (Dan) D'Angio, M.D., taught me many years ago, "for children with cancer, cure is not enough."

Our discovery efforts are clearly focused on understanding the biology of all the childhood cancers, finding the Achilles' heels for every type of pediatric tumor, no matter how rare, and developing and delivering treatments that maximize the likelihood of cure while minimizing the near- and long-term effects of therapy.

# Bringing Hope Through Discovery

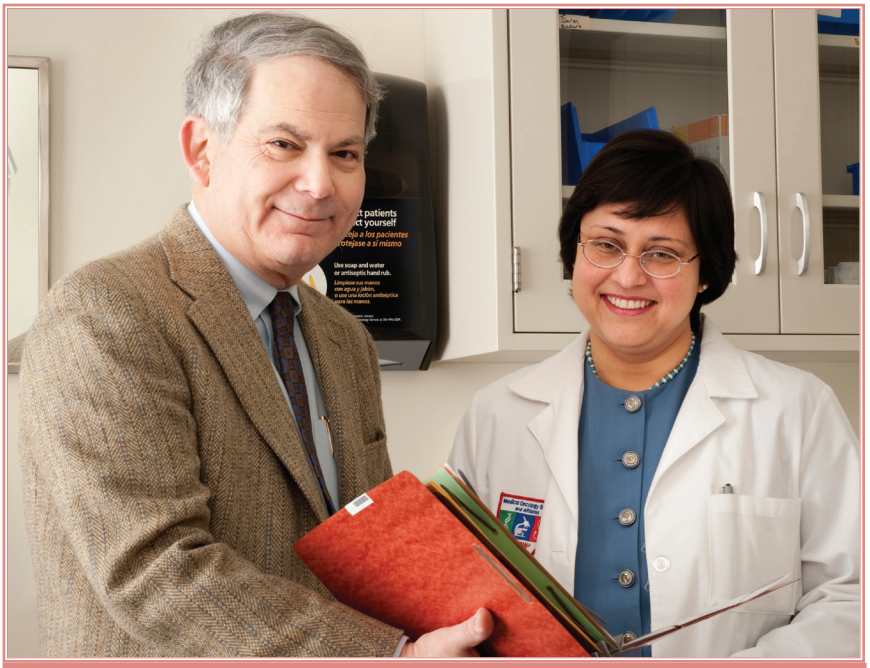


*CCR clinicians know there is something worse than being told you have cancer. It is being told that your cancer is incurable, and there is no known treatment for you. This bleak situation was unacceptable to Shivaani Kummar, M.D., even before she arrived at CCR in 2004. As an Assistant Professor in medical oncology at the Yale Cancer Center where she worked on developing early phase drugs, her view was, and remains, that incurable does not mean untreatable. And clinical research provides her with the opportunity to increase treatment options for all cancer patients.*

Since coming to CCR, Kummar has again focused her labor on early drug development as a Staff Clinician in CCR's Medical Oncology Branch. She is also Head of Early Clinical Trials Development, within NCI's Division of Cancer Treatment and Diagnosis (DCTD). Working closely together, the Kummar clinical team—clinical research coordinators, research nurses, nurse practitioners, and clinical fellows—is determined to see more discoveries translated into new therapy options for patients facing incurable cancers.

Setting out to establish new treatments, or better combinations of existing therapies, is a broad goal, so Kummar and colleagues take a comprehensive approach to drug discovery by offering more than a dozen new clinical trials at various stages of development. A few first-in-human phase 0 studies, which are early phase evaluations, investigate how the body responds to a drug and how the drug acts in the body. Several others look at drug safety and tolerance in phase 1 studies, and many more evaluate side effects and optimize a drug's effectiveness in pilot and phase 2 trials. All of the trials run by the Kummar team are kept small in size intentionally, so they can accumulate more in-depth data from a few representative patients which informs the further development of promising new agents.

(*Photo: R. Baer*)



James Doroshow, M.D., and Shivaani Kummar, M.D.

Phase 0 trials were conceptualized by James H. Doroshow, M.D., Director of DCTD and NCI Deputy Director for Clinical and Translational Research, as part of a national collaboration to improve the way clinical trials are conducted in cancer. The first step in shortening the drug development timeline was the addition of a new small-scale "proof-of-concept" trial called a phase 0 trial, prior to the formal phase 1 trials where they examine dose escalations of a drug. An ideal phase 0 trial comes armed

(*Photo: R. Baer*)

**The Kummar Team:** Front row: Yvonne Horneffer and Shivaani Kummar, M.D.; Second row: Lamin Juwara, Khanh Do, M.D., Abhilasha Nair, M.D., Giovanna Speranza, M.D., Deborah Allen, James Doroshow, M.D., Michelle Eugeni, Jennifer Zlott, and Ramya Parthasarathy, Not pictured: Janelle Bingham, Lauren Powell, and Woondong Jeong, M.D.

with a reliable assay that can monitor the changes that happen in the tumor with drug administration. It validates that a suspected target has been hit in human patients—as predicted from earlier non-human cancer models.

Kummar and her clinical team moved phase 0 trials from proposed concept to reality when they ran the first successful trial at NCI in 2006. They tested ABT888, also known as veliparib. This new drug inhibits an enzyme called poly (ADP-ribose) polymerase that is essential for repairing damage to DNA. The team used a rigorous assay to measure poly (ADP-ribose) in tumor tissue. Armed with the assay, they showed that ABT88 inhibited its target enzyme in tumor cells and in white blood cells. With data and a reliable assay from the phase 0 trial as a guide, veliparib has progressed on to more than 50 phase 1 and phase 2 trials nationwide that are now testing the new agent in various combinations with chemotherapy drugs. Cancers being treated in these trials include many types of solid tumors as well as lymphoid cancers, at all stages of disease.

In addition to running phase 0 trials, Kummar and her clinical team have made significant progress in several phase 1 and phase 2 studies. She reported to the American Society of Clinical Oncology in June 2011 the promising results from a phase 2 trial that evaluated cediranib for a rare cancer (makes up less than 1 percent of soft tissue sarcomas) called alveolar soft part sarcoma (ASPS). ASPS gets the "alveolar" part of its name from the arrangement of cells seen by the pathologist. Alveoli are small air sacks deep within the lung where oxygen is absorbed into the body, and this cancer, when examined under the microscope, appears similar to lung air sacks. ASPS results from a rare translocation between the ASPL locus on chromosome 17 and the TFE3 locus on the X chromosome (der(17) t(X;17)(p11q25)). This cancer strikes the young and frequently spreads, establishing small metastatic colonies throughout the body, especially in the lungs and even the brain of young patients. Until cediranib came along, there was no systemic drug known to be efficacious for metastatic ASPS.

Because pathologists consistently

## Kummar Clinical Team

***Research Nurses:***

Deborah Allen, R.N., O.C.N.

Ramya Parthasarathy, B.S.N., R.N., O.C.N.

Jennifer Zlott, B.S.N., R.N., O.C.N.

Michelle Eugeni, B.S.N., R.N., O.C.N.

***Nurse Practitioners:***

Yvonne Horneffer, C.R.N.P.

Lamin Juwara, C.R.N.P.

***Clinical Research Coordinators:***

Janelle Bingham, R.N.

Lauren Powell

***Clinical Fellows:***

Khanh Do, M.D.

Abhilasha Nair, M.D.

Woondong Jeong, M.D.

report ASPS as highly vascularized, this rare cancer appeared to be a good candidate for an anti-angiogenic strategy. So the Kummar team tested cediranib, a potent oral inhibitor of all three vascular endothelial growth factor receptor (VEGFR-1,-2,-3) tyrosine kinases that are needed for tumor vascularization.

The Kummar clinical trial included 36 ASPS patients, and cediranib showed substantial single-agent activity against their cancers, with a greater than 40 percent response rate (partial responses) and a disease control rate (DCR) of 78 percent. They also analyzed the gene expression profile of tumors over the course of treatment and showed that cediranib had an effect on genes in the angiogenesis pathway, and it also affected genes in the inflammatory response pathways.

Cediranib has yielded several long-lasting responses, but, in anticipation of the possibility that drug resistance or disease recurrence might eventually develop, Kummar and her colleagues are identifying and testing alternative anti-angiogenesis drugs. The Kummar team, along with several other major cancer centers, is now testing sunitinib, another anti-angiogenesis inhibitor. Toward that goal, they are conducting a multicenter, randomized phase 2 study with Dana-Farber Cancer Institute in Boston, Mass., and with The University of Texas M.D. Anderson Cancer Center in Houston to compare cediranib with sunitinib in ASPS patients. Patients will receive either cediranib or sunitinib alone, and then they will switch to the other drug if their disease progresses.

In their quest for bringing hope through treatment options, the Kummar clinical team is also looking at creative combinations of existing drugs that can disrupt several cancer signaling pathways at once.

One example of a new combination being studied in colon cancer builds upon knowledge that cetuximab targets the epidermal growth factor receptor, which is expressed in the majority of these solid tumors. Recognizing that one possible mechanism of drug resistance to cetuximab is through the Ras-Raf pathway, which controls cell growth, division, and differentiation,

**Representative Phase 1 and Phase 2 Trials of the Kummar Clinical Team**

| New Agent/Combo | Desired Effect |
|---|---|
| Pazopanib and Tivantinib | Disrupts blood vessel formation and the MET pathway |
| MK-2206 and AZD6244 | Disrupts both AKT and MEK pathways (Merck and AstraZeneca drugs) |
| Cyclophosphamide and Veliparib | Damages tumor DNA and PARP inhibitor prevents its repair |
| EZN-2208 and Bevacizumab | Disrupts cancer's DNA replication with longer acting version of the active form of camptothecin 11 that inhibits topoisomerase 1 and blocks new blood vessel formation (Enzon drug) |
| Belinostat | Turns silenced genes back on to limit cancer's growth. Being evaluated in patients with liver abnormalities |
| Z-Endoxifen | Disrupts estrogen-receptor-driven growth in patients with hormone positive cancers |
| Vorinostat | Turns silenced genes back on to limit cancer's growth; being tested as a treatment for adenoid cystic cancer, a rare disease |
| Phase 0 trial of IPdR Absorption, Metabolism, and Safety | Tests whether IPdR is absorbed in humans to eventually develop it as an oral radiosensitizer |
| EZN-2968 | Blocks the hypoxia inducible factor 1 alpha (HIF) production (Enzon drug) |
| Indenoisoquinolines LMP400 and LMP776 | Blocks topoisomerase 1 needed to cut DNA during cancer cell's replication (non-camptothecin inhibitors) |

and another common cancer ally is the vascular endothelial growth factor receptor (VEGFR2) pathway, the team is combining cetuximab therapy with sorafenib. Because sorafenib disrupts both Raf kinase and VEGFR2 tyrosine kinase activity, this strategy could enhance the clinical potency of cetuximab in metastatic colon cancer, especially in patients with KRAS mutation positive colorectal cancer.

The chart on the previous page provides some additional examples of new strategies being tested. These treatments range from drugs that inhibit the action of histone deacetylases and change the shape of chromatin to agents that directly disrupt cancer's replication activities.

An advantage of discovering effective combination treatments in addition to new agents is that, once they are identified, they may prolong or even eliminate the drug resistance that often develops during seemingly successful therapies. Another is that lower doses of each drug may be used when they act in unison. This should reduce the collateral damage to normal cells and tissues.

Kummar speaks for her entire team in summing up their focus this way, "Cancer is innovative and resourceful in its attempt to proliferate and metastasize. However, we will continue to work with researchers in various cancer-related disciplines to bring forth novel agents for the treatment of cancer. We will not rest until there is a treatment option for every incurable cancer."

*To learn more about Dr. Kummar's research and clinical trials, please visit her Web site at http://bethesdatrials.cancer.gov/investigator-profiles/default.aspx?investigatorid=127.*

*Patients or doctors interested in finding out what clinical trials are under way at the NIH Clinical Center need only visit bethesdatrials.cancer.gov.*

## Disease Control Rate (DCR): A Surrogate Marker

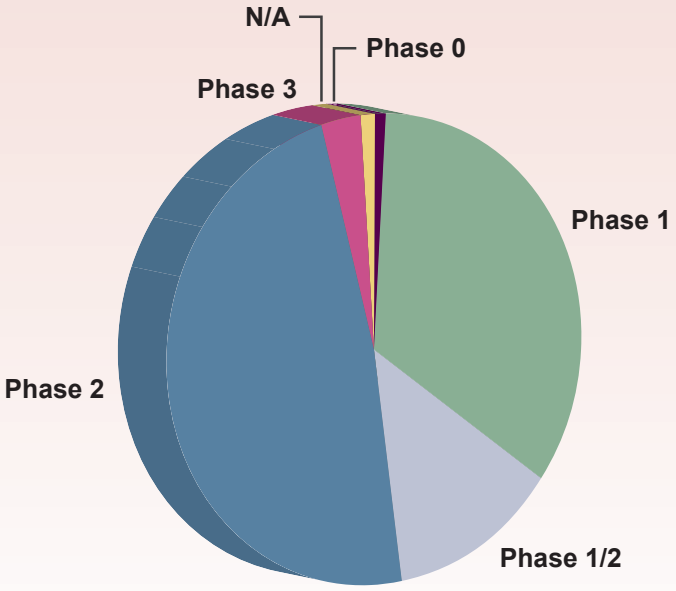
In clinical trials, a surrogate marker for the effect of a certain treatment looks like it correlates with a real clinical event—like cure or death—but the link is not guaranteed. That is why the NIH defines them as "a biomarker intended to substitute for a clinical endpoint."

Surrogate markers are used when the primary endpoint is undesired (for example, death), or when the number of events is too small to be statistically significant. During new drug development, the U.S. Food and Drug Administration (FDA) will often accept evidence from clinical trials that show a direct clinical benefit to surrogate markers.

Disease control rate (DCR) is one such surrogate marker. For most clinical trial treatments, patients will exhibit stable disease (SD) or progressive disease (PD) more often than a complete response (CR) or partial response (PR). DCR is a hopeful surrogate marker that opposes PD. It includes CRs, PRs, and SDs.

**CCR's Treatment Trials by Phase**

The majority of CCR's early-phase studies, from pre-Phase 1 (preIND) through Phases 1 and 2, are proof-of-principle trials that answer some of the basic questions about optimizing a new drug's dose, safety, and mode of delivery.



# Thyroid Cancer, **Meet Mr. Mac**

*Edgar MacIntosh (he goes by Ed or Mr. Mac) survived a life-threatening car accident as a teenager and endured over 150 X-rays to his neck and head during his recovery. Much later in life he was diagnosed with a rare thyroid cancer, and his oncologists explained to him that his cancer might be linked to those earlier X-rays, which helped save his life.*

Ed's cancer experience began in 2001 with a pathology report describing his thyroid tumor as a Hürthle cell type, that in some cases can become malignant and metastasize to other sites. Ed was one of the rare cases. He sought conventional treatment with surgery and radioactive iodine and completed several different drug protocols offered at two different hospital centers, until one fateful day when his oncologist told him that there was nothing more the doctors could offer him.

Ed did not consider that an acceptable response, so he took action. Armed with his computer and email, he described his diagnosis and the treatments he received, and he broadcasted a call for help across the Internet to several cancer centers nationwide and to the NIH. A CCR nurse answered his plea, and in July 2007, Ed paid a visit to the NIH Clinical Center to meet with Shivaani Kummar, M.D., and her research team. From that day forward, they have teamed together to fight his cancer.

Ed tried several clinical trials. As he says, "some worked and some didn't." A new study using a novel agent in combination with topotecan, a cancer drug approved for use in lung and ovarian cancer, stabilized his tumor but did not shrink it. He is now trying a combo therapy of pazopinib and tivantinib. Pazopinib blocks new blood vessel formation but, in doing so, it creates a state of low oxygen which can result in cancer cells becoming resistant to treatment. By adding tivantinib, one of the growth pathways of cancer cells is blocked and it may prevent the development of resistance in cancer cells. Ed hopes that the combo will kill the cancer cells faster than either single agent alone.

Ed sees his struggle this way, "My tumor knows me very well, but I know my tumor very well, too. I have been fighting it for years. Next year, it will be a teenager, but I can handle it. The treatment I am now taking did not even exist 5 years ago, so I will keep fighting, and new treatments will come along."

As new growths appear in Ed's lungs, he goes to the hospital for bronchoscopy and excision, and he donates biopsy samples regularly. According to Ed, "They are not painful, and the information they can provide to my clinical care team can save lives, so I'm glad to do it."

Ed does not get discouraged or depressed about his cancer. He values the CCR clinical staff who search for new treatment options for him. Meanwhile, he enjoys his family and grandchildren and soldiers on. As he explains, "If Dr. Kummar and her team are willing to keep on fighting my cancer, so am I."

(*Photo: R. Baer*)

**Clockwise from left to right:** Deborah Allen, James Doroshow, M.D., Michelle Eugeni, Ramya Parthasarathy, Edgar MacIntosh, Shivaani Kummar, M.D.

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

## Web Sites with More Information about CCR

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

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

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

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

## Patient Information on Cancer and Clinical Trials

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

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

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

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

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

## Additional Links

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

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

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



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