National Cancer Institute

Volume 5, No. 2 | 2011

CCR connections

CENTER FOR CANCER RESEARCH

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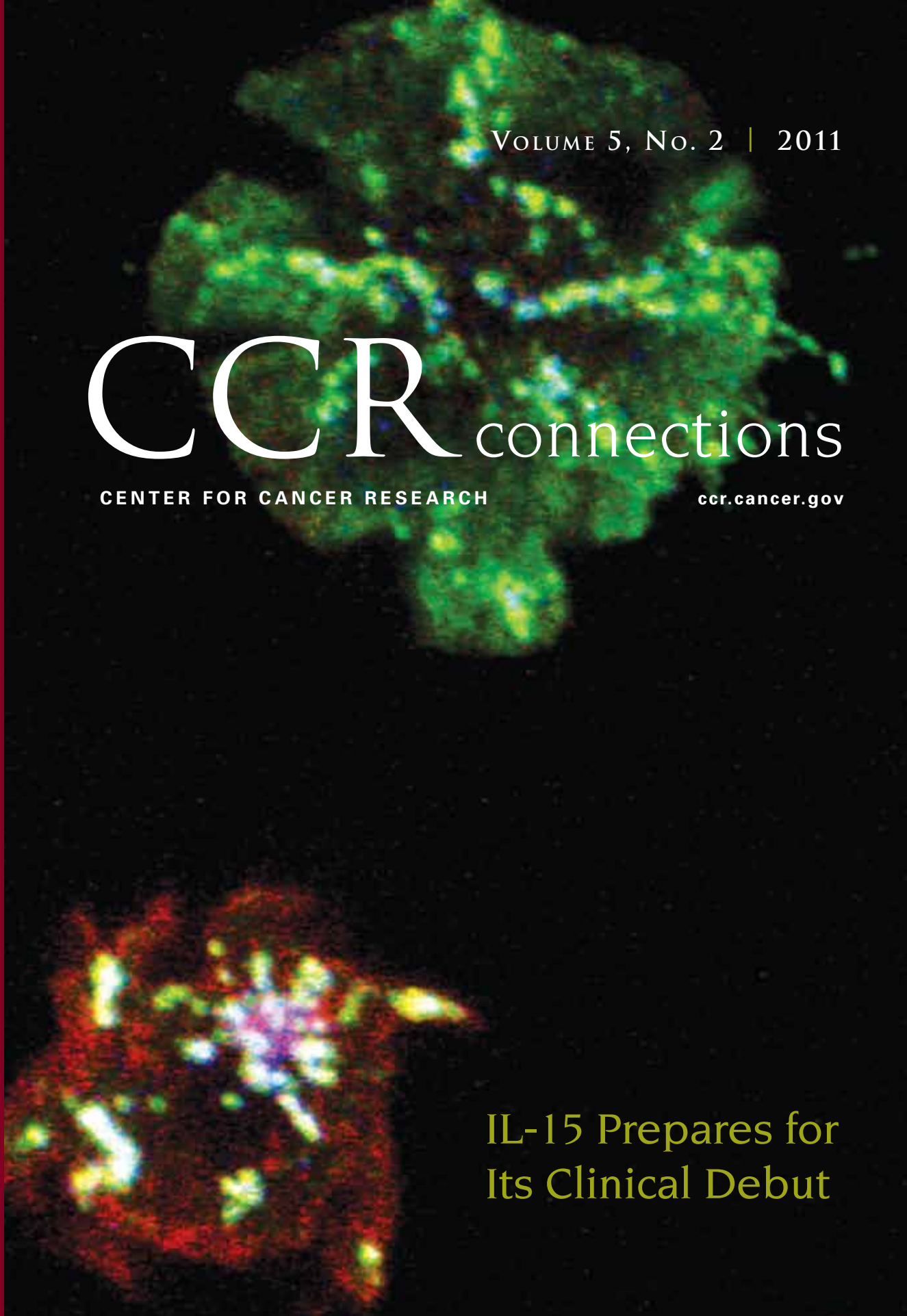

IL-15 Prepares for Its Clinical Debut

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

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

Center for Cancer Research

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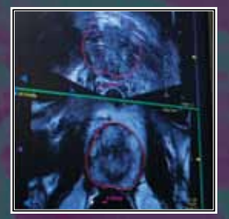

Imaging Minimally Invasive Therapy

CENTER FOR CANCER RESEARCH

The mission of CCR is:

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

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

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Our Translational Research Teams Include Our Patients

Translational research is routinely described as "from bench to bedside," evoking a picture of scientists moving novel findings from basic labs into hypotheses that are evaluated in clinical trials. While this is true in part, at the Center for Cancer Research (CCR), the picture is much broader.

CCR's story is one of translational successes: converting lab concepts into clinical advances, and taking clinical observations back to the laboratory to make more scientific breakthroughs. Our translational research is a sophisticated, multidisciplinary network that includes our patients, nurses, clinicians, and scientists, as well as database and technology experts—all offering their analytic, clinical, and communicative expertise, and all moving in unity to develop improved outcomes for our patients. We have built our network upon an enabling, patient-centered, and comprehensive infrastructure backed by a sustained commitment to stay the course in support of potential high-reward opportunities for breakthroughs.

In this issue of *CCR connections*, we showcase our translational research teams. The story told in "IL-15 Prepares for Its Clinical Debut," shares how our teams assemble *ad hoc* to bring IL-15 into patient trials.

We also offer insights into the key role our nurses play in seeing experimental approaches and agents advance from patient trials into oncology practice. "CCR Nurses: Collaborative, Committed, and Caring Amidst Complexity" captures their ability to remain the face of compassion and the hands of care, while cancer's complexity requires them to work behind the scenes, juggling data entry, modality scheduling, adverse reporting, and industry collaborations.

(*Photo: R. Baer*)

Lee J. Helman, M.D.

And taking a calculated risk based on sound proof-of-concept science is the *modus operandi* for our translational teams. Whether we are harnessing imaging to improve prostate cancer detection, by better guiding biopsy sampling and training oncologists to perform nerve-sparing robotic surgery as described in "Imaging Minimally Invasive Therapy," or leading the discovery and development of new molecules from the natural world to the clinic as described in "Faculty Successes: 'NExT' Opportunities for CCR Investigators in Drug Discovery and Development," CCR's translational researchers work hand in hand with our most valued members of the multidisciplinary network, our patients. So when we do make progress, our successes are their successes, too.

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

Giving His All for ALL

A personal visit to Guatemala evolves into a sustained global outreach program in pediatric oncology.

In 2006, when Michael Dean, Ph.D., Head of CCR's Human Genetics Section of the Laboratory of Experimental Immunology, visited Guatemala as a volunteer member of his church, he never expected that the trip would be the first of many to the region, or that it would become the start of a global outreach program in pediatric oncology.

Dean's story began when he met a teacher on that first trip to the country, who told him about Edgar, a seven-year-old orphan who had what sounded like retinoblastoma, a rare type of eye cancer that usually develops in early childhood. Dean connected Edgar with the Unidad Nacional de Oncología Pediátrica in Guatemala City where they diagnosed a form of ocular herpes rather than the cancer feared by Dean.

That experience brought Dean in contact with both the pediatric oncology hospital in Guatemala and St. Jude Children's Research Hospital in Memphis, Tenn., two institutions that have been international partners to improve the lives of children since 1997.

The diverse genetic makeup of the Guatemalan people forms a complex backdrop for clinical oncology studies, and Dean wanted to investigate the role that this diversity plays in childhood cancers, especially the genetic risk factors for acute lymphoblastic leukemia (ALL), the leading cause of cancer-related deaths among children. Previous research had identified genetic variants (called polymorphisms) in the *ARID5B* gene, a gene implicated in early B-cell development, as possible culprits, showing also that certain variants are more prevalent in Hispanic children than in Caucasian children.

Conducting clinical research in a busy pediatric hospital in Central America, where staff handle ten times the number of cases seen in a typical U.S. pediatric center, was a huge challenge, and Dean faced it head on, personally taking each family through the consent process in Spanish. After Dean's team had collected more than 1,000 DNA samples, they confirmed that certain genetic polymorphisms at the ARID5B allele within the Guatemalan population confer much higher risks for ALL. In fact, when Dean's research team compared the frequency of the risk variants in three major populations, they showed that individuals of European descent had a 30 percent frequency, Hispanics had 50 to 60 percent, and the indigenous Mayan population had an even higher frequency of 70 percent.

Why is this particular genetic polymorphism so powerfully linked to ALL? One possible hypothesis is that many of today's indigenous Guatemalan people descended from individuals who survived infectious diseases brought to the Americas by Europeans. The surviving individuals may have had a stronger immune system due to the presence of the ARID5B allele, which resulted in a greater production of B cells to fight infections. That increased production of B cells could now be conferring a higher risk of ALL on the young descendants of these populations.

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

Michael Dean, Ph.D.

Knowing that certain variants of the *ARID5B* gene confer a high risk for ALL, particularly in Guatemalan children, may one day enable clinicians to customize cancer care by specifically targeting the protein products of these particular polymorphisms. But before that can happen, Dean and his team will continue this international collaboration to unravel the molecular role that these variants play in cancer.

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

Faculty Successes

"NExT" Opportunities for CCR Investigators in Drug Discovery and Development

The development of new therapeutics to prevent and treat cancer is one of the most important goals of CCR. The Molecular Targets Faculty (MTF) was established 10 years ago to provide the infrastructure to accomplish this goal.

"In the late 1990s, we realized that to take promising molecules out of the lab and into the clinic, we needed a more comprehensive development process—we needed to screen more thoroughly, understand pharmacokinetics more deeply, and be able to preclinically test molecules more clearly," said Patricia Steeg, Ph.D., one of the main initiators of the MTF and currently co-chair of CCR's Molecular Targets Faculty Steering Committee (MTFSC) along with James Doroshow, M.D., NCI Deputy Director for Clinical and Translational Research.

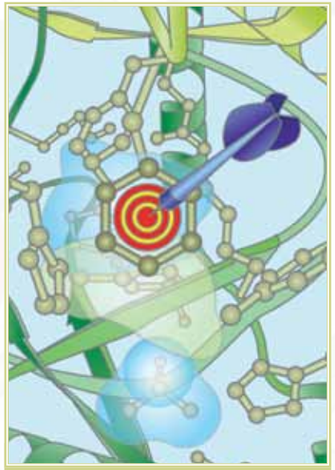
One of the first steps was to bring James McMahon, Ph.D., into the process with the establishment of the Molecular Targets Laboratory (MTL). McMahon's group provides intramural investigators with an essential first step—the development of screening assays to identify inhibitors and biologic agents that interact with their molecular targets of interest.

"We create high-throughput screening assays for use with both NCI's Natural Products Repository, the most chemically diverse repository in the world, and NCI's Chemotherapeutic Agents Repository, housing more than 200,000 pure chemical compounds," said McMahon. The end result of the screen, which is often a three- to six-month process, is a list of "hits" or likely molecular candidates, which are ultimately sent to the collaborating principal investigator's laboratory for further testing and validation.

The ultimate goal is to bring promising molecules to the NCI Experimental Therapeutics (NExT) Program, a partnership between the Division of Cancer Treatment and Diagnosis (DCTD) and CCR. The mandate of NExT is to advance clinical practice and bring improved therapies to patients with cancer by supporting the most promising new drug discovery and development projects.

One recent success of the MTF is that of Yves Pommier, M.D., Chief of the Laboratory of Molecular Pharmacology, and his efforts to bring a new class of cancer therapeutics called indenoisoquinolines from the bench to the bedside. Pommier and Mark Cushman, Ph.D., of Purdue University, initiated a high-throughput screen to discover compounds that inhibited the function of the DNA processing enzyme, topoisomerase 1, and spent years revising the resulting compounds to create better derivatives. Now, several years later, after productive collaborations with several additional researchers, with the MTF, and with NExT, two derivatives are currently in clinical trials at the NIH.

As Pommier noted, "It's all about commitment, and within the MTF, there are a lot of individuals who are committed to making drug development work." And work it does, with at least seven other molecules in the immediate pipeline, all hopefully headed to the clinic in the very near future.



(Image: I. Kelly)

The Molecular Targets Faculty works to identify and validate important molecular targets in cancer and AIDS.

To learn more about MTF, please visit https://ccrod.cancer.gov/confluence/display/CCRMTF/Home.

To learn more about A Phase I Study of Indenoisoquinolines LMP400 and LMP776 in Adults With Relapsed Solid Tumors and Lymphomas, please visit http://clinicaltrials.gov/ct2/show/NCT01245192.

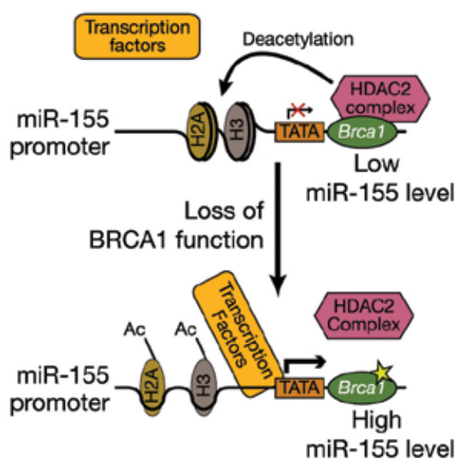
BRCA1 Variants Co-Conspire with MicroRNA-155

Recently discovered microRNAs (miRNAs) play an important biological role by switching "on" and "off" at different times during cell growth, death, development, and differentiation. They regulate gene expression by blocking messenger RNA's instructions for protein production.

Shyam K. Sharan, Ph.D., working with Suhwan Chang, Ph.D., a research fellow, in CCR's Mouse Cancer Genetics Program, recently reported in *Nature Medicine* how one of these miRNAs can also be harmful when the "on" and "off" switching occurs at the wrong time in the wrong place. This was discovered by researching the functional consequences of a BRCA1 variant called R1699Q that does not alter its DNA repair function. Instead, the Sharan team found that this variant co-conspires with microRNA-155 over-expression to help cancer thrive.

Sharan and colleagues uncovered a new function for *BRCA1*, a gene most commonly associated with hereditary breast and ovarian cancer when it is mutated. Working on mouse cells, they discovered that normal *BRCA1* suppresses the expression of another gene that codes for a microRNA called miR-155, which is known to be cancer-causing. These findings suggest that *BRCA1* functions as a tumor suppressor not only by playing a role in DNA repair, as known previously, but also by silencing oncogenic miR-155.

Using a mouse embryonic stem-cell-based assay, Sharan and colleagues also investigated precisely how normal *BRCA1* silences miR-155 in cells. They discovered that *BRCA1*, through its interaction with another protein called histone deacetylase2 (HDAC2), modifies proteins called histones that wrap around DNA and help maintain its structure. As a result of these modifications, DNA is prevented from expressing miR-155. When BRCA1 is absent or a mutant BRCA1 that cannot bind to HDAC2 is present, these deacetylation modifications of DNA cannot occur, and consequently miR-155 is over-expressed.



(*Image: S. Sharan, CCR*)

If BRCA1 is absent, or if a variant form—such as the R1699Q—is present in the cell, the interaction of the BRCA1-HDAC2 complex is disrupted. This increases the level of acetylated H2A and H3, which, in turn, activates the miR-155 promoter. This upregulation of miR-155 contributes to tumor development.

When the researchers inactivated miR-155 in tumor cells in mice, tumor growth slowed down. If the *BRCA1*-associated tumors in humans are confirmed to also be dependent upon miR-155, it may be possible to treat hereditary *BRCA1*-mutated breast and ovarian cancers by challenging them with agents that can inactivate miR-155. In fact, expression levels of miR-155 may be a useful biomarker for *BRCA1*-deficient human tumors.

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

Fighting Fire with Fire, Immunologically

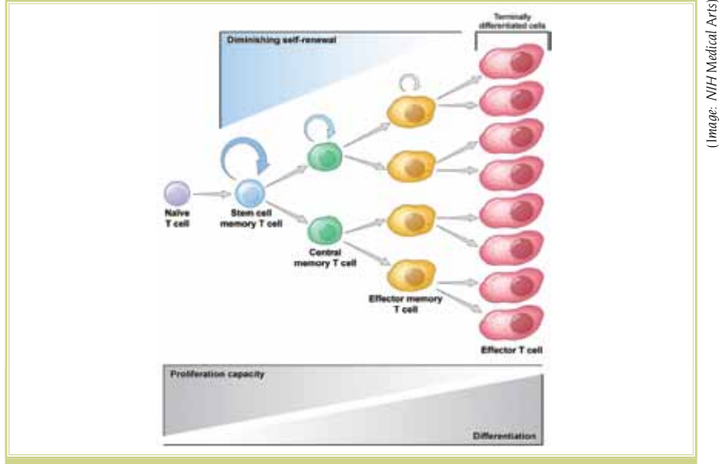
CCR scientists describe a new stem-cell-like memory T cell with potential to enhance and prolong immune responses against tumor cells.

Immunological memory refers to the ability of certain immune cells to "remember" an encounter with an antigen and to then react more swiftly and effectively to that antigen should it return. The complex mechanisms by which this occurs are not fully understood, but it has long been speculated that a subpopulation of memory lymphocytes with stem-cell-like attributes must be involved.

For the first time, Luca Gattinoni, M.D., and Nicholas Restifo, M.D., of CCR's Surgery Branch, and colleagues have documented this putative memory T subpopulation. These human stem-cell-like memory T cells displayed enhanced self-renewal and the ability to differentiate into diverse, mature immunological cell types, including memory T cells. The findings were published in the September 18, 2011, online issue of *Nature Medicine*.

Stem-cell-like lymphocytes were previously described in mice, but the key marker used to identify these cells had no counterpart in humans. To overcome this roadblock, Gattinoni and Restifo artificially created a population of human T cells by activating a key developmental pathway named "Wnt" that had previously enabled the scientists to generate stem-cell-like T cells in mice.

The next challenge was to determine if their artificially constructed stem-cell-like human memory cells had a counterpart among *naturally occurring* human lymphocytes. The researchers analyzed samples from both healthy human donors and cancer patients, and found that around two to three percent of all circulating T lymphocytes expressed the same markers as the artificially created stem-cell-like T cells. Upon stimulation, these cells demonstrated the ability to retain "memory" and to rapidly proliferate and acquire effector functions, but importantly, they also exhibited the classical stem-cell-like properties of self-renewal and multipotency. Tests of adoptive transfer into immunodeficient mice showed that the newly characterized memory stem-cell-like T cells had enhanced replication and survival capabilities compared to fully differentiated memory T cells, and they exhibited potent antitumor activity. In fact, the stem-cell-like memory T cells triggered enduring tumor regressions in mice that would otherwise have died within two to three weeks.



(*Image: NIH Medical Arts*)

Stem-cell-like memory cells have physical characteristics of very young immune cells. They still have the potential to differentiate and become many different types of immune cells, making them extremely valuable.

The identification of a human stem-cell-like memory T cell population is an exciting step in the rapidly growing fields of regenerative medicine and immunotherapies for cancer.

"Many current therapies are short-lived in nature, but using modified immune cells that are capable of continually refreshing themselves and fully integrating with the patients' own immune system provides potential for far more sustained assaults on tumor cells in the future," said Gattinoni.

The team is currently working towards the goal of creating stem-cell-like memory T cells to enhance immune responses against tumors. "Tumors are in many ways similar to stem cells—both self-renew and can adapt quickly to environmental changes—so fighting tumors with immune cells that function similarly is like fighting fire with fire," concluded Restifo.

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

Staff News at CCR

Announcements

(*Photo: E. Branson*)

Andre Nussenzweig, Ph.D.

Nussenzweig has been appointed Chief of CCR's newly formed Laboratory of Genome Integrity. He received his Ph.D. in physics from Yale University. He completed his postdoctoral training at the École normale supérieure in Paris and at Memorial Sloan-Kettering Cancer Center prior to joining CCR's Experimental Immunology Branch. During his 13-year career at NCI, he has made major contributions to our understanding of how the integrity of the genome is maintained. He has made a series of incisive discoveries in the fields of DNA repair and oncogenesis, including: establishing that the major non-homologous end-joining pathway acts as a genomic "caretaker" that protects against cancer; determining the etiology of chromosomal translocations associated with lymphomas; finding that a core histone, the basic unit utilized by cells to compact their genomes, can act as a tumor suppressor; and discovering pathways that prevent genetic damage from being passed on from one generation to the next.

(*Photo: R. Baer*)

Shiv Grewal, Ph.D.

Grewal has been named Chief of CCR's Laboratory of Biochemistry and Molecular Biology. He obtained his Ph.D. from the University of Cambridge, United Kingdom, where he studied as a prestigious Cambridge-Nehru Scholar. He then came to NCI-Frederick in 1992 as a Postdoctoral Fellow and extended his research on the mechanisms that enable epigenetic control of gene expression and development. In 1998, Grewal chose to join the Cold Spring Harbor Laboratory as a faculty member and rose to Associate Professor before returning to NCI in 2003 as a Senior Investigator in CCR's Laboratory of Molecular Cell Biology where he led the Chromosome Biology Section. His work to understand the epigenetic control of chromosome structures continues. *Science* magazine cited his discovery of a connection between RNAi and heterochromatin formation as a "Breakthrough of the Year 2002." His focus area is critically important to cancer research because it addresses important questions about epigenetic mechanisms that are essential for the maintenance of genomic integrity. And defects in genomic integrity can lead to cancer and other human diseases.

(*Photo: F. Barr*)

Frederic Barr, M.D., Ph.D.

Barr joins CCR's Laboratory of Pathology as the Deputy Laboratory Chief. He received his M.D. and Ph.D. degrees from the Washington University School of Medicine. He completed residency training in anatomic pathology at the Hospital of the University of Pennsylvania and performed postdoctoral research in the Division of Human Genetics and Molecular Biology at the Children's Hospital of Philadelphia. Before coming to the NIH, Barr was a faculty member in the Department of Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine. His research interest is molecular genetics of cancer with a focus on recurrent chromosomal alterations in sarcomas.

Newly Tenured CCR Scientists

James L. Gulley, M.D., Ph.D.
Laboratory of Tumor Immunology and Biology

Yikang Rong, Ph.D.
Laboratory of Biochemistry and Molecular Biology

New Tenure-Track Scientists

(*Photo: E. Branson*)

Eric Batchelor, Ph.D.

Batchelor joins CCR's Laboratory of Pathology as an NIH Earl Stadtman Investigator. He received his Ph.D. in physics from the University of Pennsylvania where he studied two-component signal transduction in bacteria in the laboratory of Mark Goulian. Batchelor then pursued postdoctoral training in the Department of Systems Biology at Harvard Medical School. He studied p53's dynamic response to DNA damage in the laboratory of Galit Lahav. Batchelor's research focuses on quantitatively understanding the regulation and function of mammalian stress responses and on understanding the tumor suppressor protein p53.

(*Photo: D. Sone*)

Heidi Kong, M.D.

Kong is now a tenure-track investigator in CCR's Dermatology Branch. She received her M.D. from Baylor College of Medicine. She then completed her dermatology residency at Duke University. After completing a clinical research fellowship in the Dermatology Branch and the Duke-NIH Masters Program in Clinical Research, Kong became an Assistant Clinical Investigator and worked with collaborators to establish the NIH Intramural Skin Microbiome Consortium. Her research focuses on the skin microbiome in health and in skin diseases with the goal of expanding understanding of host-microbe interactions.

(*Photo: J. Summers, SPCM, NCI-Frederick*)

Jadranka Loncarek, Ph.D.

Loncarek joins CCR's Laboratory of Protein Dynamics and Signaling as an NIH Earl Stadtman Investigator. She obtained her Ph.D. from the Faculty of Sciences at Zagreb University, Croatia, in cell and molecular biology. She completed her postdoctoral training in the laboratory of Alexey Khodjakov at Wadsworth Center, Albany, New York, where she studied the mechanisms of centriole duplication and mitotic spindle formation. Her current research focuses on elucidating the molecular mechanism of centrosome biogenesis and its function, with particular attention on numerical control of centrosome formation in nontransformed and cancerous human cells.

(*Photo: C. Palena*)

Claudia M. Palena, Ph.D.

Palena is now a tenure-track investigator in CCR's Laboratory of Tumor Immunology and Biology. She received her Ph.D. degree in biochemistry from the National University of Rosario, Argentina. She subsequently joined the NIH as a Postdoctoral Fellow and served as a Staff Scientist in the Laboratory of Tumor Immunology and Biology. Palena's current research is focused on the development of novel immunotherapeutic approaches aimed at targeting critical events in tumor progression with the ultimate goal of designing vaccine platforms and combinatorial therapies for the prevention and/or treatment of metastases in human cancer.

(*Photo: J. Summers, SPCM, NCFFrederick*)

John (Jay) Schneekloth, Jr.

Schneekloth joins CCR's Chemical Biology Laboratory. He received his Ph.D. from Yale University where he studied natural product total synthesis and chemical biology relating to the ubiquitin-proteasome pathway with Craig Crews. He then pursued an NIH postdoctoral fellowship with Prof. Erik Sorensen at Princeton University, where he worked on the development of a new multicomponent reaction and the synthesis of analgesic natural products. He returned to Yale where he worked as a medicinal chemist at the Yale Small Molecule Discovery Center. Schneekloth's research involves using synthetic chemistry and screening techniques to develop small molecule probes of signal transduction pathways, specifically related to ubiquitin-like protein signaling.

Recent CCR Awards

Elected to the Association of American Physicians

Ronald Gress, M.D.
Chief, Experimental Transplantation and Immunology Branch

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

2011 Samuel Heyman Service to America Homeland Security Medal

Partnership for Public Service

For his achievements in developing a blueprint for the U.S. to deal with the health consequences of a radiological or nuclear incident, and helping the Japanese respond to radiation from earthquake and tsunami-damaged nuclear power plants

C. Norman Coleman, M.D.
Radiation Oncology Branch

2011 Abbott-ASM Lifetime Achievement Award

American Society of Microbiology

For sustained contributions to the microbiological sciences

Susan Gottesman, Ph.D.
Co-Chief, Laboratory of Molecular Biology

2011 Fellow of the Biophysical Society Award

For her extraordinary contributions to advances in computational biology on both nucleic acids and proteins

Ruth Nussinov, Ph.D.
CCR *Nanobiology Program*

Waldenstrom's Award for Myeloma Research – 2011

The International Myeloma Foundation

For lifetime achievement in myeloma research

Michael Kuehl, M.D.
Genetics Branch

2011 William B. Coley Award for Distinguished Research in Tumor Immunology

Cancer Research Institute

For pioneering work bringing adoptive T cell transfer from the laboratory, through proof of concept, to the clinic, as a treatment for cancer

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

Making a Difference Award

American Academy of Dermatology Leadership Circle for Volunteerism Program

For compassionate care and volunteer work for patients with the rare, cancer-prone genetic disease Xeroderma pigmentosum

Kenneth Kraemer, M.D.
Dermatology Branch

2011 Honorary Professorship Award

Fudan University

Xin Wei Wang, Ph.D.
Laboratory of Human Carcinogenesis

Jane C. Wright, M.D., Young Investigator Award

Conquer Cancer Foundation of the American Society of Clinical Oncology

Jung-min Lee, M.D.
Medical Oncology Branch

2011 ASCO Young Investigator Award

American Society of Clinical Oncology

John Hays, M.D., Ph.D.
Medical Oncology Branch

Young Investigator Award

Kaleidoscope of Hope Foundation

Andrea McCollum, Ph.D.
Medical Oncology Branch

Bill Gates Millennium Scholarship

Awarded, in part, for his work at NCI

Phung Tran
Genetics Branch

An Early Career Off to a Stellar Start

James Gulley receives prestigious Presidential Award.

(Photo: White House Office of Science and Technology Policy)

NIH Director Francis S. Collins, M.D., Ph.D., HHS Secretary Kathleen Sebelius, M.P.A., James L. Gulley, M.D., Ph.D., White House Office of Science and Technology Policy Director John P. Holdren, Ph.D.

The White House recently named James L. Gulley, M.D., Ph.D., Deputy Chief of CCR's Laboratory of Tumor Immunology and Biology, a recipient of the Presidential Early Career Award for Scientists and Engineers, the highest honor bestowed by the United States Government on science and engineering professionals in the early stages of their independent research careers. Gulley was selected for using randomized, controlled studies to test novel, recombinant vaccines to reduce the progression of prostate and other cancers and to increase patients' survival.

This award has been given annually since President Bill Clinton commissioned the National Science and Technology Council to create it in 1996. Sixteen Federal departments and agencies, including the Department of Health and Human Services (HHS), join together annually to nominate the most meritorious candidates. Awardees are selected for their pursuit of innovative research at the frontiers of science and technology and their commitment to scientific leadership, public education, or community outreach.

Gulley was one of 20 HHS employees honored this fall, and one of 94 recipients overall. The scientists and engineers selected received their awards in a ceremony on October 14, 2011, in Washington, D.C.

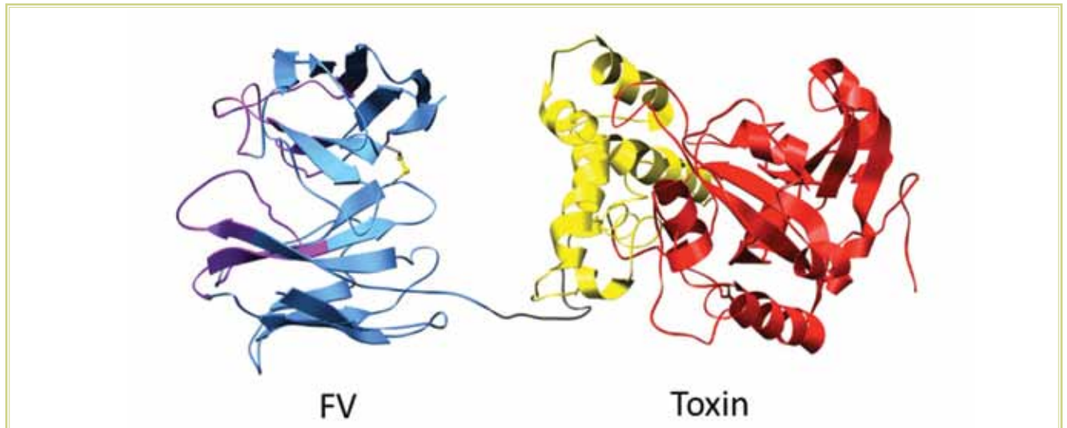
"It is inspiring to see the innovative work being done by these scientists and engineers as they ramp up their careers—careers that I know will be not only personally rewarding but also invaluable to the Nation."

President Barack Obama
White House Press Release, September 2011

Improving an Immunotoxin

Immunotoxins are chimeric proteins that comprise a targeting domain (e.g., the Fv portion of a monoclonal antibody or ligand) and a toxin domain that is capable of causing cell death.

(*Image: I. Pastan, CCR*)



The targeting domain includes the Fv portion of monoclonal antibodies specific for antigens preferentially expressed on human cancers.

When targeted specifically to a diseased cell, immunotoxins can be effective treatments for disease. An ideal immunotoxin should be active so that only small amounts need to be given to cause tumor regressions, small in size so that it can penetrate into cancers, stable so it remains functional during the five to ten hours required to reach the interior of a cancer, and low in immunogenicity so it can be given repeatedly. Until recently, this was difficult to achieve due to the inherent immunogenicity of immunotoxins when administered to humans.

Ira Pastan, M.D., Co-Chief of CCR's Laboratory of Molecular Biology, has designed and produced recombinant immunotoxins with these desirable properties through his work on *Pseudomonas* exotoxin A (PE). PE was first improved by removing portions of the toxin that were not required for its cell-killing activity. This modification decreased the size of the molecule while also removing undesirable protease sites, resulting in a more stable molecule. PE was further improved by reducing its immunogenicity by identifying and removing B cell and T cell epitopes, through deletion and point mutation of key amino acids. These modifications allow the repeated administration of a PE that retains its cell-killing activity.

The modified PE has been attached to several targeting domains, and the resulting immunotoxins are being investigated in clinical trials. The targeting domains used so far include the Fv portion of monoclonal antibodies to antigens that are preferentially expressed on human cancers, such as mesothelioma and ovarian and pancreatic cancer (anti-mesothelin antibodies), and several types of B cell leukemias (anti-CD22 antibodies). These improved immunotoxins are very promising prospects as treatments for patients suffering from cancer. Notably, the toxin can be attached to any targeting domain for use as a treatment of a number of different diseases.

The NIH currently is pursuing patent rights that cover these immunotoxins. Many of them are available for licensing. For more information, please contact Dave Lambertson, Ph.D., (lambertsond@mail.nih.gov) in the NIH Office of Technology Transfer.

In Conversation:
Clinical Fellow Jung-min Lee

CCR connections recently met up with Jung-min Lee, M.D., winner of the Jane C. Wright, M.D., Young Investigator Award, from the American Society of Clinical Oncology, honoring outstanding early-career researchers in the final two years of their subspecialty training. We took the opportunity to discover more about Jung-min's current research, and her future goals.

(*Photo: E. Branson*)

Jung-min Lee, M.D.

CCR: Welcome, and thanks for taking the time to speak with us today. Congratulations on your recent award—can you please tell us a little about your ongoing research interests?

Jung-min: I work with Elise Kohn, M.D., Head of the Molecular Signaling Section and the Women's Cancers Clinic in CCR's Medical Oncology Branch, and I'm a clinical fellow in medical oncology and hematology. The award was for my work on translational research related to poly (ADP-ribose) polymerase (PARP) inhibitors in women's cancers. PARP inhibitors are promising therapies in women with *BRCA1/2* mutations, and my work investigates this in two ways: I'm examining the differential effects of sequential administration of the PARP inhibitor olaparib in combination with carboplatin chemotherapy in preclinical and clinical models and I'm looking at mechanisms of DNA damage that occur as a result of different schedules of these drugs in preclinical models.

CCR: Olaparib is supposed to make chemotherapy more effective, so some research has suggested that it should be given first. Can you share with us what your research suggests?

Jung-min: Yes, our preclinical work actually gave a very different result—we observed that when carboplatin is given first, followed by olaparib, you get more DNA damage in *BRCA1*-mutated breast and ovarian cancer cell lines. We hypothesized that administering carboplatin followed by olaparib will cause greater DNA damage than olaparib presensitization of carboplatin in patients as well. So we've now launched a clinical trial to test this hypothesis generated by our preclinical work. We will look at differential drug exposure and clinical benefit based on correlative end points and scheduling differences.

CCR: Are you working on other research questions related to *BRCA1/2* in your laboratory?

Jung-min: Yes, recent data suggest that certain women with cancer can benefit from PARP inhibitors even though they don't carry the *BRCA1* and *BRCA2* mutations—this may represent homologous recombination dysfunction in the DNA damage repair pathway—and we're currently investigating this in the lab.

CCR: How do you go about selecting which patients might benefit the most from PARP inhibition therapy?

Jung-min: We know that *BRCA1* and *BRCA2* mutation carriers are sensitive to PARP inhibitor, but we don't know which patients with high-grade, serous ovarian cancer might respond to it. So, part of my research is to investigate possible predictive biomarkers for PARP inhibitor therapy—so far, they include RAD51 and gamma H2AX.

CCR: How does your research help you as a clinician?

Jung-min: It helps me tremendously. Even though I'm at the early stage of my career, understanding molecular mechanisms and working on hypothesis-driven clinical trials has made me mature as a physician. The majority of patients who come here are knowledgeable, and fully understand their disease, so I take the time to share the rationale of our clinical trials and advances in cancer research with them. I think it's critical to communicate our knowledge and experience with patients because it really makes a difference in how we care for them. Dr. Kohn sets a good example of how important it is for physicians to share the understanding and advances of research with patients. I'm also part of a great clinical and laboratory team.

CCR: In terms of your future, both as a clinician and researcher, what do you see as some of your next steps?

Jung-min: I'm looking for a faculty position as a physician-scientist in women's cancer and I'm particularly interested in rare, under-studied subgroups in ovarian and breast cancer. I want to take what I've learned here to the next phase of my career, carrying out hypothesis-driven clinical and translational research.

IL-15 Prepares for Its Clinical Debut

It's been nearly two decades since CCR researcher Thomas Waldmann, M.D., *co-discovered interleukin-*15 (IL-15), *a cytokine and potent stimulator of antitumor memory* CD8+ T *cells. Now, in a major step towards the molecule's clinical development,* Waldmann—Chief *of CCR's* Metabolism Branch—*and his CCR colleagues have started the process of testing* IL-15 *in human cancer patients for the first time.*

It took several years and a major collaboration at the NIH between NCI and the National Institute of Allergy and Infectious Diseases (NIAID) to produce clinical-grade IL-15 for human cancer research. "This is a watershed moment," Waldmann said. "IL-15 is one of the most promising new candidates in cancer immunotherapy. For us to have reached this point is hugely gratifying, considering the long scientific odyssey that might never have happened without the cooperation of CCR's Robert Wiltrout, Ph.D., and Clifford Lane, M.D., Deputy Director for Clinical Research and Special Projects at NIAID."

The IL-15 being used by Waldmann was produced by NCI's Biopharmaceutical Development Program (BDP), part of the Division of Cancer Treatment and Diagnosis (DCTD). During a series of NCI-sponsored workshops, investigators put IL-15 at the top of a list of the most compelling new immunotherapies for cancer treatment. The Cancer Immunotherapy Trials Network (CITN), a newly organized multicenter research consortium funded by NCI and headquartered at the Fred Hutchinson Cancer Research Center in Seattle, Wash., has made IL-15 studies a priority. In addition to Waldmann and Lane, other investigators are beginning clinical research with IL-15 including Steven Rosenberg, M.D., Ph.D., Chief of CCR's Surgery Branch, and a number of extramural scientists, including Jeffery Miller, M.D., the Associate Director of Experimental Therapeutics at the Masonic Cancer Center, University of Minnesota.

> "IL-15 holds great promise for exploiting the immune system to treat cancer and infectious diseases"

"IL-15 holds great promise for exploiting the immune system to treat cancer and infectious diseases," said Wiltrout. "The ability to fully understand its possible benefits to patients has been limited by a lack of commitment from the private sector to develop it for clinical use. Thus, the decision by NCI's CCR and DCTD to partner with NIAID has now resulted in the production of clinical grade IL-15 and the initiation of novel clinical trials that would otherwise not have been possible."

(*Photo:* B. Branson)

Robert H. Wiltrout. Ph.D.

(*Photo:* R. Baer)

Thomas A. Waldmann, M.D., and colleagues.

Potentially Better Than IL-2

Much of the excitement surrounding IL-15 concerns its ability to stimulate natural killer (NK) and CD8+ T cells without inducing capillary leak syndrome. This reaction, typically associated with a related immunotherapy in clinical use today—IL-2—heightens the risk for organ failure in some patients. What's more, unlike IL-2, IL-15 doesn't trigger regulatory T cells (Tregs or suppressor cells) that might otherwise put the brakes on its therapeutic benefits.

Waldmann co-discovered IL-15 in 1994, at about the same time that Kenneth Grabstein, Ph.D., a scientist with Immunex Research and Development Corporation, in Seattle, Wash., was making the same discovery. Working independently, the scientists found that IL-2 shares its T cell receptor with a related molecule—later called IL-15—with which it has some similarities, but also some important differences. Both IL-2 and IL-15 stimulate T cell proliferation, activate NK cells, and induce immunoglobulin synthesis by human B cells. However, unlike IL-15, IL-2 also participates in activation-induced cell death (AICD) of helper CD4+ T cells, is critical in the maintenance of Tregs, and blocks the persistence of memory CD8+ cells. According to Waldmann, this is how IL-2 helps to eliminate lymphocytes that target self-antigens in autoimmune illness. IL-15, on the other hand, inhibits IL-2's role in AICD, has a positive effect on memory CD8+ cells, and, therefore, favors long-term responses against foreign pathogens.

"So we postulated that IL-15 might be useful for cancer treatment," Waldmann said. "And we and others were able to demonstrate this in a number of mouse models, while also showing that IL-15 had relatively low toxicity. This is what convinced NCI to stimulate funding for the production of clinical-grade IL-15 for further research."

The IL-15 subsequently produced by the BDP (in an *E. coli* expression system) under the direction of Stephen Creekmore, M.D., Ph.D., Chief of NCI's Biological Resources Branch, was then tested in a primate model through a collaborative project involving scientists throughout the NIH. Results published in the journal

(*Photo*: B. Branson)

Kevin Conlon, M.D.

Blood, on May 5, 2011, confirmed what Waldmann and other researchers saw in mouse models: Given by bolus infusion, at doses ranging from 10-50 µg/kg/day for 12 days, IL-15 stimulated NK and memory CD8+ cells with minimal toxicity. Buoyed by these findings, Waldmann and co-authors submitted 2,700 pages of supporting data to the Food and Drug Administration (FDA), along with their Investigational New Drug Application to sponsor a clinical trial in humans with metastatic cancer.

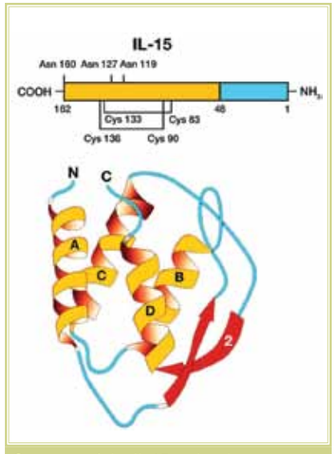
Another Immunotherapy Moves Into the Clinic

With Waldmann as Principal Investigator, a Phase I study of IL-15 is under way. Mirroring dosing protocols from the primate study, the CCR researchers enrolled patients with either metastatic melanoma or metastatic renal cell carcinoma—two illnesses with long-standing, unmet needs for new treatment—and gave them bolus, intravenous infusions of IL-15 at various doses for 12 consecutive days. Unexpectedly, however, human patients proved to be much more sensitive to the cytokine than primates, Waldmann said. "Even at the lowest dose, some patients developed fever and other side effects anywhere from two to four hours after a 30-minute infusion," he said. "So we had to reduce the dose substantially."

According to Kevin Conlon, M.D., a Metabolism Branch clinical collaborator working with Waldmann on the trial, the plan is to complete the bolus infusion study at the lower dose, and then progress to two other exposure scenarios: continuous, low-dose intravenous infusions for ten days, and then subcutaneous injections scheduled Monday through Friday, once a day, for two weeks in a row. Primate data published online November 8, 2011, in the journal *Blood*, suggested that these exposure routes substantially modulate the immune system with fewer side effects.

"We think the problem with bolus infusion has to do with IL-15's pharmacokinetics," Waldmann said. "It has a half-life of 30 minutes, so it drops rapidly to undetectable levels after the peak, which is when we see most of the toxicity. With continuous infusion, we think we'll be able to maintain a more desirable dose level that yields the highest activated lymphocyte count with less fever and hypotension. Subcutaneous dosing might also be appropriate, given that continuous infusion might not be practical, even in this academic, clinical hospital setting."

Howard Streicher, M.D., a Senior Investigator in the NCI's Cancer Therapy Evaluation Program (CTEP), the clinical program in DCTD, is also gearing up for clinical studies with IL-15. He said that CTEP plans to support investigations with the CITN and extramural investigators to focus on finding safe, biologically effective doses over longer time frames, in the order of months. "What we're hoping to do is extend Dr. Waldmann's pioneering work on IL-15 by taking advantage of some new opportunities that are now available in immunotherapy," he said. Indeed, several new approvals have galvanized cancer immunotherapy, Waldmann added, and IL-15 could ride on their success.



(*Image*: T. Waldmann, CCR)

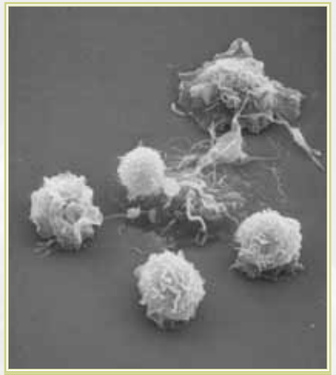
Structure of the IL-15 molecule.

CCR Center of Excellence in Immunology

CCR Director Robert Wiltrout, Ph.D., describes IL-15 as a major theme for NCI's Center of Excellence in Immunology (CEI). One of five centers of excellence in NCI's Intramural Research Program (IRP), the CEI comprises immunology investigators from throughout CCR. Wiltrout chairs the organization, which aims to foster discovery, development, and delivery of novel immunologic approaches for the treatment of cancer and cancer-associated viral diseases.

The CEI's steering committee—made of branch and laboratory chiefs, and key principal investigators—meets once a month to discuss ongoing initiatives and opportunities for further research and hosts a biweekly seminar series to stimulate collaborations. The CEI also provides an interactive forum through which industry representatives can discuss partnership opportunities and material transfer agreements with NCI. Every year, the CEI hosts an annual meeting, with roughly 1,200 to 1,400 registrants, geared towards one of three rotating subject areas: basic immunology, cancer inflammation, and clinical immunotherapy. The most recent meeting, held in Bethesda, Md., on September 22-23, 2011, focused on immunotherapy. In addition, the CEI hosts biannual minisymposia on a range of current subjects such as IL-15's potential in pediatric oncology.

"The CEI provides the infrastructure to develop and support research projects that would be difficult for individuals to accomplish on their own," Wiltrout said. "And it creates opportunities to unite external scientists with our highly skilled and very accomplished, NCI investigators so they may engage in big-picture projects."



(*Image*: K. Nagashima, CCR)

Scanning electron micrograph of immune cells using their projections called microvilli to attach to a target protein.

To learn more about the Center of Excellence in Immunology, please visit its CCR Web site at https://ccrod.cancer.gov/confluence/display/COEI/Home.

> "...the story of IL-15 illustrates the ability of the NIH intramural program to make distinctive and important contributions to biomedical research."

Il-15 and Vaccines in Combination

Streicher's view, shared by others in the field, is that while IL-15 will ideally prove effective as a stand-alone treatment, it might also be useful in combination with other therapies, including monoclonal antibodies or vaccines. "We're not there yet, but it's the kind of thing we envision," he said. "As an NCI program devoted entirely to new therapies, our role is to take a pioneering lead and provide a coordinated development for clinical trials that often would not be done without NCI support. We're just starting to do this with IL-15 now."

Waldmann added that his group has also begun to incorporate IL-15 into vaccines for HIV, anthrax, tuberculosis, human papilloma virus, and other threats. "These are situations in which vaccines alone aren't fully adequate," he said. "There's always a limitation in how long the immune system can be stimulated, and that's where IL-15 provides an advantage: it helps the recall response."

"It's become obvious that this molecule has a lot of potential and that's why it's a central theme in immunology here at CCR. In many ways, the story of IL-15 illustrates the ability of the NIH intramural program to make distinctive and important contributions to biomedical research," Wiltrout said. "Intramural scientists from different institutes worked together to understand the basic biology, colleagues at NCI produced it, and now our NIH clinical center is enrolling the first patients onto clinical trials." The story of IL-15 started a long time ago, but it certainly doesn't end here.

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

To learn more about CCR's clinical trial on IL-15, please visit its Web site at http://bethesdatrials.cancer.gov/clinical-research/search_detail.aspx?ProtocolID=NCI-10-C-0021.

CCR Nurses:

Collaborative, Committed, and Caring Amidst Complexity

A welcoming presence at the door and a familiar face going forward—in addition to the best chances for treatment, this is also what cancer patients seek when they enroll in one of CCR's many clinical trials. As consistent points of contact during treatment, CCR nurses Mary Ann Yancey, Megan Mackey, Melissa Walker, *and* Marcia Mulquin *meet that obligation, even as they tend to the administrative details of managing research. As caregivers, CCR nurses provide a reassuring presence for patients. And as liaisons among patients, physicians, and pharmaceutical companies, they manage the critically important flow of clinical information from the bedside to the research database.*

As in other medical settings, cancer treatment at CCR is a collaborative effort directed by a team of doctors and nurses who oversee a patient's daily care—but working in a research environment adds even more responsibility: to aid in the development of safe, potentially better cancer therapies. As highly trained professionals in oncology, CCR nurses aim to make a patient's cancer journey as successful as possible. During cancer treatment, patients experience side effects and anxiety, which contribute to a reduced quality of life. CCR nurses work tirelessly to address these complicating factors using the most advanced techniques available.

> As highly trained professionals in oncology, CCR nurses aim to make a patient's cancer journey as successful as possible.

The Nurse Practitioners

Some nurses have "hands-on" jobs that tend to the clinical aspects of patient care, while others have administrative responsibilities ensuring that the many clinical trial protocols are carried out seamlessly. Mary Ann Yancey's job is among the former. A former Peace Corps volunteer, Yancey came to CCR's Medical Oncology Branch Multiple Myeloma Section, headed by Ola Landgren, M.D., Ph.D., in 2007 after finishing her master's degree in oncology nursing at George Mason University. She compares the workings of various research teams at CCR to the parts of a bicycle. "The principle investigators are like the bike frame," she said. "They bring us the science, the hypotheses, and the protocols. The research nurses are the hub of the bicycle wheels. They connect the needs of the protocol with the rest of CCR to make sure the protocol is followed as written and keep the whole thing running." Her greatest pleasure is seeing how new treatments developed through CCR's clinical trial programs can really help patients,

(*Photo: R. Baer*)

Mary Ann Yancey, R.N., C. Ola Landgren, M.D., Ph.D., and Marcia Mulquin, R.N.

not just physically, but also by giving them hope. She recalls one patient, a 26-year-old woman with cutaneous T cell lymphoma, a type of non-Hodgkin's lymphoma that manifests in the skin. Nothing had worked until the patient was enrolled in a Phase I clinical trial with intravenous fenretinide, a drug related to vitamin A. "That was four years ago, and as of today she continues to be free of disease and able to care for her two children," Yancey said. "This type of experience makes my job very rewarding."

Yancey said that most CCR patients arrive fearful and confused, and what she has learned over time is how listening to what patients say often helps to calm their fears. "I try to be as communicative as possible; I try my best to listen to their concerns and answer their questions as quickly and as best as I can. When I don't have the answer, I will look to other sources to help them better understand their disease as well as what we are trying to accomplish here," she said. The entire research collaboration is focused on the patient, Yancey added. "Everyone at CCR tries their best to make this as easy an experience as possible for the patient. Without the patient and their time and commitment, there would be no research," she said.

As a research nurse, Yancey also participates in administrative duties: she schedules labs and clinical procedures, and helps to guide patients in their journey through the clinical trial protocol. Yancey acknowledges the hardships that come with her job. One patient—a 56-year-old male truck driver who was treated six years earlier for breast cancer—arrived in the clinic with his family and with a new diagnosis of pancreatic cancer. "He enrolled in a Phase I trial of gemcitabine, cisplatin, and an experimental drug and responded well initially. It was very gratifying to see the treatment work, at least for a while," Yancey said. "But sadly, after six months, his disease progressed. Talking about this with him and his family was extremely difficult."

> "The principal investigators are like the bike frame... The research nurses are the hub of the bicycle wheels."

Megan Mackey, a nurse practitioner in CCR's Neuro-Oncology Branch, headed by Howard Fine, M.D., also describes her work as highly rewarding because it occurs in an environment where new treatments are constantly evolving. Mackey came to the NIH in 1999 for an internship after graduating from nursing school at the University of Rochester and

"We work together with our patients in every way that we can, from diagnosis until they leave the program."

decided to stay on full-time—working first in a stem cell transplant unit, and then in the Oncology Day Hospital. After earning a Master's Degree in Nursing (MSN) in 2006 from the University of Maryland, she was offered the position she holds today, tending to patients with primary brain tumors and tumors of the spinal cord. "These are such terrible illnesses with such a great need for new therapies," she said. "And that makes it a great field for research and a huge part of why I enjoy being part of that process."

The Branch team—five nurse practitioners, two patient care coordinators, three clinical fellows, three research nurses, and four attending physicians including Fine—have seen, or consulted on, thousands of patients. Some of them are newly diagnosed, others are waiting for diagnosis, and others are in the midst of treatment. "We work together with our patients in every way that we can, from diagnosis until they leave the program," she said. "The patients we see here are really looking to us for guidance and are very appreciative of the wealth of knowledge that our Branch can offer."

(*Photo: D. Sone*)

Megan J. Mackey, C.R.N.P., working in the clinic.

Most of the patients undergoing treatment in the Branch participate in drug trials, and for them, Mackey takes medical histories, performs physical exams, orders tests and medications, confirms that patients enroll in the right studies, reviews imaging scans and laboratory results, and monitors side effects. "Doctors make most of the major decisions when it comes to imaging, patient stability, and treatment," she explained, "but we do the exams, report back findings, and help to decide what's needed next." The Neuro-Oncology Branch also works with hundreds of enrollees in a separate, natural history protocol. In this group, Mackey and her colleagues work with local doctors throughout the country to guide patient treatment. "Primary brain tumors are relatively rare and that's our main focus," Mackey said. "Word of mouth travels quickly among those faced with this disease, and many of our referrals are actually from current patients."

While nurse practitioners take on clinical responsibilities, research nurses focus on clinical study protocols.

The Research Coordinators

As a research nurse in CCR's Surgery Branch, Melissa Walker's job is less clinical, yet equally important in ensuring every possible success for patients who come to the Surgery Branch. Walker first came to the NIH in 2001 after graduating from nursing school at Florida State University, and has been working in the Surgery Branch since 2007. She now works with Itzhak Avital, M.D., who heads the Surgery Branch's Gastrointestinal and Hepatobiliary Malignancies Section, and whose research focus is on solid organ cancer stem cells.

"Patients are often fearful, and our job is to bring them hope and keep them safe."

While nurse practitioners take on clinical responsibilities, research nurses focus on clinical study protocols. Patients typically interact more with research nurses than with others at CCR, and Walker said these routine contacts are what motivates her in her job. "Working with another research nurse and a clinical coordinator, together we see patients from their initial call until they complete therapy or leave the study," she said. "Interacting with patients for that length of time really allows us to establish close relationships with them."

Like Walker, Marcia Mulquin, a research nurse with the Medical Oncology Branch's Multiple Myeloma Section, headed by Ola Landgren, M.D., Ph.D., coordinates patient care and research participation for the duration of treatment. Mulquin came to the NIH in 1983, after receiving her nursing degree from East Carolina University. She specialized in oncology but also worked in other areas, including intensive care, infectious disease, immunology, and behavioral health, where she treated patients with depression, substance abuse problems, and other issues. She said she enjoys being part of a research team, describing it as unique to the NIH work environment. "It's the best of both worlds," she said. "One day you're working closely with other nurses in the clinic, and the next day you might be amending trial protocols with a sponsoring drug company." Mulquin also emphasizes her role as an educator. Patients tend to be savvy about their illness, she said, but not about the drugs. "You have to get them up to speed fast," she explained. "Patients are often fearful, and our job is to bring them hope and keep them safe. For example, certain drugs can lower white cell counts, so patients need to be aware of what that means, and to be on the lookout for low-grade temperatures or any signs of infection."

(*Photo: R. Baer*)

Melissa Walker, R.N., with patient Norman Holmes.

Mulquin emphasizes the collaborative nature of her work, both in terms of treatment and research. She said she collaborates closely with clinic nurses and other team members who routinely update one another on each patient's status. On the research side, Mulquin and her team work hard to ensure that databases remain error-free, and she describes toxicity monitoring as a "huge" part of the job, with a responsibility to determine if adverse events could be related to the experimental treatment, and if so, how that might influence dosing. "I've been here 28 years, and I love the research side of it," she said. "As a nurse, it's extremely rewarding to see new therapies advance in a research setting, and we often see patients who've had complete remissions. By the time they get here, patients may feel like they don't have many options left—so to be even a small part of a potential solution, to provide a better quality of life, those are the goals. We all do everything we can to make that happen."

"By the time they get here, patients may feel like they don't have many options left—so to be even a small part of a potential solution, to provide a better quality of life, those are the goals."

Man's Best Friend in More Ways Than One

Cancer drug development typically begins with in vitro research before proceeding through varying degrees of investigation in cell lines and laboratory animals, eventually culminating in human clinical trials. However, this often arduous development path may now find an ally in a relatively new branch of oncology research, referred to as comparative oncology. Initiated and directed by Chand Khanna, D.V.M., Ph.D., the CCR Comparative Oncology Program complements translational research through the characterization of relevant and naturally occurring cancer models that develop in pet animals as a window to evaluate novel therapies.

(Photo: Frances and Peter Way)

Prince.

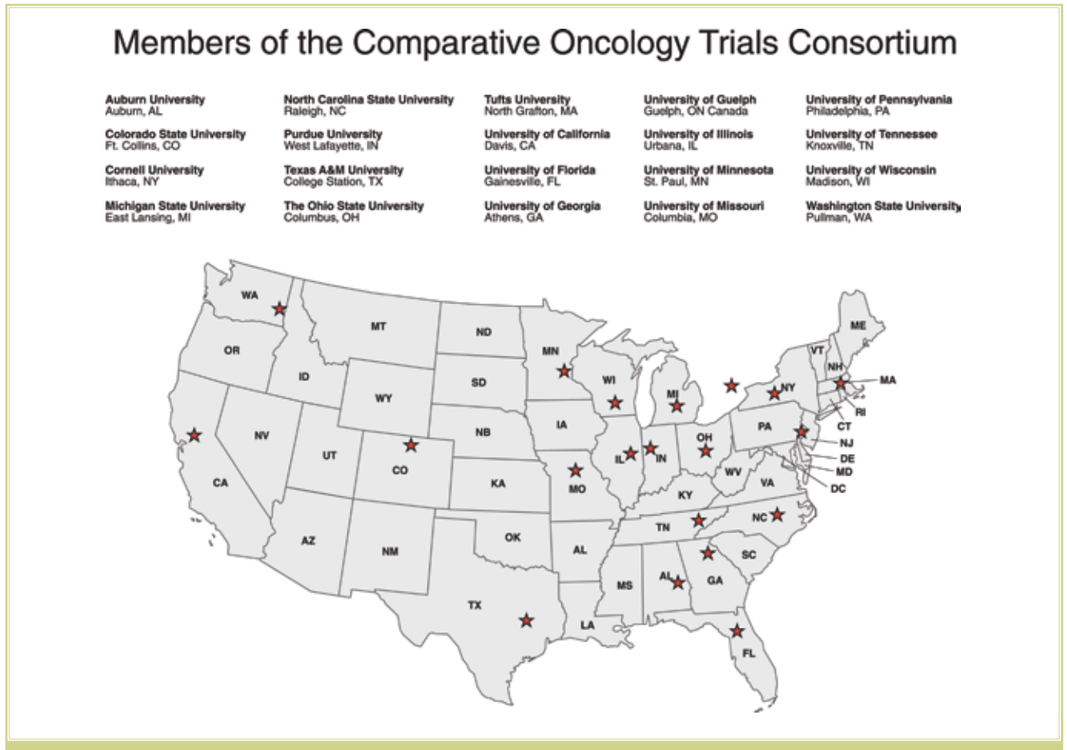
It was nearly Christmas when Prince, an amiable, nine-year-old Labrador retriever was diagnosed with oral malignant melanoma. His owners, Frances and Peter Way, wanted the best treatment for their companion, so when veterinarians at Colorado State University's (CSU) Animal Cancer Center suggested a clinical trial, the Ways readily agreed. Prince's melanoma was very aggressive, similar in many ways to human melanoma, and the Animal Cancer Center was part of a consortium, sponsored by CCR's Comparative Oncology Program (COP), through which dogs are treated with experimental therapies to prepare for their use in human clinical trials. Prince was treated with a new immunotherapy and achieved a robust response, living for almost a year. "That's a lot longer than we think he would have otherwise," said Peter Way, from Fort Collins, Colo. "We were hoping for a cure, but we also knew this would help the science, and I was glad that Prince could participate. It fit his spirit and his attitude; he was a great dog."

Addressing a Lost Opportunity

To date, the COP has treated more than 150 dogs over the course of nine clinical trials conducted by its leadership of the Comparative Oncology Trials Consortium (COTC), a collaborative network of 20 veterinary schools from around the country overseen by Melissa Paoloni, D.V.M. Khanna conceived of the COP while he was a postdoctoral fellow in CCR, in the lab of Lee J. Helman, M.D. At the time, he was working on tumor metastasis and osteosarcoma, a bone cancer that occurs in pediatric patients and is also common in dogs. "It became clear to me that there would be great value in including pet animals with cancer within our CCR studies," Khanna said. "There are very few human cancers that don't also occur naturally in pet animals. To me, not learning from their cancers to help inform human clinical trials seemed like a lost opportunity."

> "It became clear to me that there would be great value in including pet animals with cancer within our CCR studies."

In 2004, with backing from Helman and Carl Barrett, Ph.D., then Chief of CCR's Pediatric Oncology Branch and CCR's Director, respectively, Khanna established the COP. Its mission, Khanna explained, is to improve the assessment of novel treatments for humans by treating pet animals—primarily dogs with naturally occurring cancers—while also trying to give these animals better quality of life by offering them the benefit of cutting-edge research and experimental therapies. "The ultimate goal was for NCI to engage



(Image: VSB Associates)

The Comparative Oncology Trials Consortium (COTC) is an active network of 20 academic comparative oncology centers, centrally managed by CCR's Comparative Oncology Program.

(Photo: R. Baer)

Chand Khanna, D.V.M., Ph.D.

the drug development community, and to lead a consortium of veterinary schools in a program to supply clinical research results that might accelerate new human treatments, and simultaneously help pet animals with cancer," Khanna said.

> ...human and canine cancers are very similar with respect to their genetics and biological features.

A Logical Partnership

According to Khanna, enrolling pet dogs in clinical trials has the potential to offer some key insights into human drug research. Dogs routinely develop osteosarcoma, lymphoma, bladder cancer, head and neck cancer, malignant melanoma, and mammary cancer. Moreover, human and canine cancers are very similar with respect to their genetics and biological features. On a practical level, the compressed life span of a dog and the more rapid course of cancer progression makes it easier for scientists to evaluate the benefit of novel cancer therapy in a short period of time. Repeat biopsies are often easier to perform in dogs than they are in people, making canine pre- and post-dosing tissue studies to investigate drug-target interactions feasible. Additionally, thanks to the public release of the canine genome sequence in 2005, scientists can now understand the molecular changes that are associated with a treatment response and a treatment failure. And finally, the COTC studies spontaneously occurring tumors in pet dogs, which are far more similar to human cancer than tumors induced artificially in mouse models of cancer. "It's also important to note that the COTC provides cutting-edge cancer treatment to pets whose owners might not otherwise be able to help them," said Paoloni. "It can cost thousands of dollars to pay for cancer therapy in a dog, and unfortunately that puts it out of reach for many families, so our efforts can help both human and veterinary cancer patients."

According to Khanna, COTC projects emerge from collaborations with the drug industry and other research organizations involved in cancer drug development. The COP then chooses participating sites based on their availability, access to advanced technology (such as PET scans, for instance), and other factors. Once approved, researchers at chosen sites enroll dogs according to eligibility criteria that—mirroring the situation with human clinical trials—can be very strict. For instance, many studies exclude dogs with other co-morbidities, such as renal failure or cardiac disease.

COP Trials

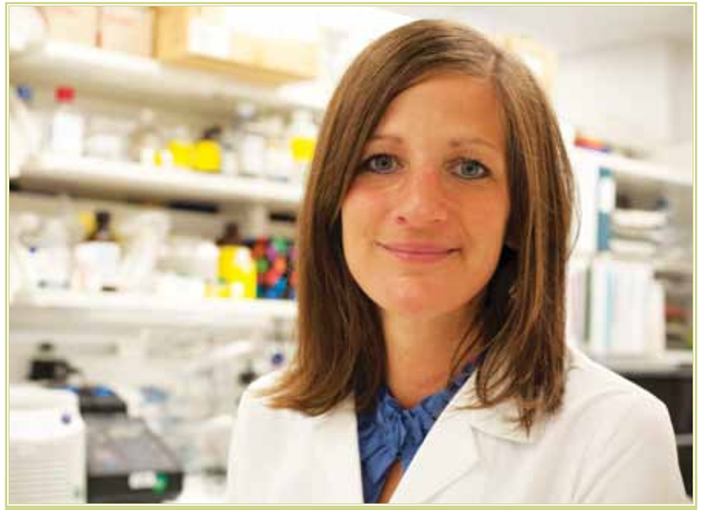
Prince was enrolled in a study of two immunocytokine fusion proteins in tumor-bearing dogs, which aimed to define optimal doses and early indications of antitumor activity. "He'd get a monthly injection and then periodic bloodwork," said Susan Lana, D.V.M., Chief of Clinical Oncology at Colorado State University Veterinary Teaching Hospital and Animal Cancer Center. "And we would follow the tumor as he progressed. Prince was a good candidate because, although his tumor was large, he was healthy in all other respects."

The first of the COTC's studies was sponsored by CCR and conceived through a collaboration of CCR's Surgery Branch and COP, and individuals at M.D. Anderson Cancer Center in Houston, Tex. Led by Paoloni, that study investigated the safety and effectiveness of using a bacterial phage to deliver tumor necrosis factor (TNF)—an inflammatory cytokine—directly into tumor blood vessels by intravenous dosing. TNF-alpha was already approved for local administration in melanoma and soft tissue sarcoma, but with systemic dosing, human patients ran a high risk of hypotension and other cardiovascular reactions. The phage was designed to avoid that problem by targeting a molecular marker on tumor blood vessels. Thus, the phage would theoretically spare normal vasculature and avoid cardiovascular and other toxicities. However, it was not practical to test the phage in healthy animals without cancer because animals lacking the tumor marker would simply excrete the phage. During the trial, COTC scientists at five participating universities tested the phage in a pair of sequential studies. That data is now being used as a basis for the first Phase I clinical trial in humans, Paoloni said.

According to Khanna, many COTC studies strive to predict appropriate human doses of new cancer drugs. "We want to know the relationship between dose and effect at a molecular level." Furthermore, he said, "There is a great need to answer simple questions that would optimize the success of future human trials. This can be as simple as: When is the best time to conduct a biopsy after dosing to see if a drug did what it was intended to do?"

In a pilot study that is nearing completion, the COTC is investigating the feasibility of collecting, processing, and analyzing tissues from multiple sites for molecular markers in under a week, a clinically necessary timeline. According to Paoloni, this particular study, which is sponsored by the Translational Genomics Research Institute (TGEN), a non-profit organization in Phoenix, Ariz., will confirm COTC's future ability to participate in large-scale, molecular research in personalized medicine. "It's crucially important that we can process tissues and generate accurate molecular readouts quickly," Paoloni said. "There are many examples of failures that may occur with sample processing in the era of targeted cancer therapy. You cannot underestimate how important it is to be able to collect samples correctly—otherwise the promise of personalized medicine will be difficult to realize." Lana agrees, pointing out that in a "non-clinical world" it might take months to generate that kind of information. "Patients with cancer can't wait that long," she said.

(Photo: R. Baer)



Melissa Paoloni, D.V.M.

> Many COTC studies strive to predict appropriate human doses of new cancer drugs.

This pilot study, Paoloni said, precedes a trial planned for next year—also sponsored by TGEN—to determine if appropriate therapies can be prescribed on the basis of molecular profiling to support the clinical utility of individualized cancer care. Two additional trials planned for the coming year involve new areas of research for COTC investigators: one aims to determine if molecular results obtained from tumor biopsies can be predicted by advanced imaging technologies, and a second is focused on discovering the novel mechanism of action for a compound currently in the clinic. "These are both exciting studies," Paoloni said.

Asked about long term plans for COTC, Paoloni envisions the future this way, "Essentially, we want to demonstrate the utility of this model so effectively that large pharmaceutical companies begin to develop their own comparative oncology programs. In that case, the COTC's role would be to oversee and advise on trials run by the pharmaceutical industry."

The Way family's opinion is that Prince was an active partner in the COP's research. "It wasn't easy," they said. "Although we worked with great veterinarians in the program, it was emotionally hard to go through it. But we thought the program could benefit a lot of people, and that was important to us. We all have friends or family who have struggled with or died of cancer and this was a positive thing for us and our dog to do."

To learn more about Drs. Khanna and Paoloni's research, please visit Dr. Khanna's CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=khanna.

To learn more about the Comparative Oncology Program, please visit the Web site at https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home.

The Time for Continued Investments in Cancer Research Is Now

Currently an American Cancer Society Professor at Dartmouth Medical School, Ethan Dmitrovsky, M.D., was a fellow at NCI during the 1980s. During that time, he began the research that still defines his career today: using pharmacological agents to induce terminal differentiation in tumor cells for cancer therapy. This year, Dmitrovsky became chair of the NCI's prestigious Board of Scientific Counselors for Clinical Sciences and Epidemiology. In that capacity, he will guide efforts by this Board's 22 extramural member scientists from cancer centers and universities across the U.S. to advise NCI on future directions for intramural cancer research. Dr. Dmitrovsky graduated from Harvard College and Cornell University Medical College, and completed an internal medicine residency at Memorial Sloan-Kettering Cancer Center (MSKCC) in New York. He was a faculty member at MSKCC for more than a decade prior to joining the Dartmouth faculty as the Andrew G. Wallace Professor and Chair of the Pharmacology and Toxicology Department.

I recall my fellowship at NCI as a transformational experience that allowed me to combine in-depth science in clinical trials with laboratory-based research. Ever since, I've been impressed with the public-spirited nature of NCI—and CCR in particular—and how its leadership is extraordinarily devoted to the prudent use of public funds to combat the daunting problem of cancer.

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Cancer Cell Differentiation

My interest in differentiation therapy began with a landmark publication from Charlotte Friend, M.D., at Mt. Sinai Hospital in New York. During the 1970s, she showed that dimethyl sulfoxide (DMSO) could trigger mouse leukemia cells to become terminally differentiated hemoglobin-producing cells that stopped growing. At NCI, I set out to identify what regulated that process.

My collaborators and I found that expression of the *c-myc* oncogene falls and rises precipitously following DMSO treatment, and we therefore hypothesized that *c-myc* might regulate differentiation. So, we set about engineering mouse erythroleukemia cells to over-express *c-myc*—the idea being that if we could block this fluctuation, we might prevent mouse leukemia cells from differentiation in response to DMSO. We confirmed this was the case, and published those findings in *Nature*. The finding was replicated by several other groups, leading to the conclusion that oncogenes can control a tumor cell's differentiation state.

But, as a clinician and a scientist, I didn't want to *prevent* tumor cells from differentiating; I wanted to do the opposite, and, from a clinical perspective, the DMSO dose needed to induce tumor cell differentiation in patients was too high. However, we also knew from the work of Theodore Breitman, M.D., at NCI, that all-trans-retinoic acid—a natural derivative of vitamin A—could induce maturation of acute promyelocytic leukemia (APL) cells at levels that are a thousand times lower than the millimolar doses needed with DMSO.

(*Photo: Dartmouth Medical School*)

Ethan Dmitrovsky, M.D.

As a clinician and a scientist, I didn't want to *prevent* tumor cells from differentiating; I wanted to do the opposite.

Moving Towards Retinoic Acid

This formed the basis for my decision to study retinoic acid in differentiation therapy. At around the same time, two research teams—one headed by Pierre Chambon, M.D., from INSERM, in Strasbourg, France, and another by Ronald Evans, Ph.D., from the Salk Institute in San Diego, Calif.—reported the discovery of retinoic acid receptors. I also became aware of a discovery from Chinese investigators showing that retinoic acid could produce remissions in APL patients. These findings led us to conduct the first U.S. clinical trial with retinoic acid in APL patients, which we reported in the *New England Journal of Medicine*. That trial produced two important findings: first, that retinoic acid could stimulate leukemia cell maturation; and second, that the patients who responded to the drug had an aberrant retinoic acid receptor.

I regard it as a privilege to care for patients who have life-altering medical conditions, and I take great pleasure in being a small part of the many research groups working tirelessly toward the creation and testing of better therapeutics.

This was, in many ways, an early example of targeted therapy, and upon coming to Dartmouth, I decided to expand my research by investigating whether retinoic acid might function as a *preventative* agent in cancer. That research resulted in a paper, published in *Proceedings of the National Academy of Sciences*, showing that retinoic acid can prevent lung cancer *in vitro* via destruction of the G1 cyclins. A hallmark of retinoic acid response in all contexts is, therefore, G1 arrest. I set out to identify the precise mechanisms involved in that process, and launched a decade-long effort to determine if triggering the G1 arrest and subsequent cellular growth inhibition pathway could have clinical benefits for patients with lung cancer.

The Present and Beyond

Meanwhile, despite *in vitro* data suggesting the opposite, clinical trials have clearly shown that classic retinoids, carotenoids, and other vitamin A derivatives are unable to prevent lung cancer in patients. My research and that of other laboratories has shown that a specific retinoic acid receptor—the RAR-beta receptor—triggers G1 arrest. However, this receptor is often silenced in lung cancer and in the bronchial epithelial cells of smokers, which may explain in part why these clinical trials haven't been successful. RAR-beta partners with retinoid X receptor (RXR) to form a complex, and we've now shown *in vitro* that by targeting RXR with rexinoids, namely, bexarotene, it's possible to activate G1 arrest by inducing degradation of G1 cyclin proteins. During the last 10 years, we've focused on this finding and have used bexarotene to engage the RXR pathway in cooperation with a second pathway that we are able to modulate with the epidermal growth factor receptor (EGFR) inhibitor erlotinib. By combining these two drugs, we broaden their pharmacological activity. Phase 0, Phase 1, and Phase 2 clinical data have since shown that they produce objective responses in lung cancer patients ***with*** k-ras mutations, and also in patients ***without*** "activating" EGFR mutations in their lung cancers. This work has now been independently replicated by a team at M.D. Anderson Cancer Center, and our future work continues to investigate the potential for this treatment regime in lung cancer.

I regard it as a privilege to care for patients who have life-altering medical conditions, and I take great pleasure in being a small part of the many research groups working tirelessly toward the creation and testing of better therapeutics. It's an honor to work with the Board of Scientific Counselors in its efforts to move new discoveries from NCI into the broader cancer-care enterprise. With new discoveries emerging on an almost daily basis from the human genome, the time is right for continued investments in cancer research, and especially for the translational work that brings revolutionary science from the bench to the bedside.

The NCI *is* the nation's cancer center, and so it is with a tremendous sense of responsibility that I look forward to continuing my work with the distinguished members of the Board, thus ensuring that NCI remains very squarely at the forefront of the nation's cancer research efforts.

Imaging

Minimally Invasive Therapy

As clinicians and collaborators, Peter Choyke, M.D., and Peter Pinto, M.D., have combined their skills in advanced imaging techniques and laser therapy to potentially revolutionize prostate cancer treatment. Their approach, which ultimately aims to remove only cancerous portions of the organ while leaving healthy tissues intact, could result in more men being successfully treated for their illness while retaining normal prostate functioning. A radiologist, Choyke set up CCR's Molecular Imaging Program shortly after arriving at NCI in 2004. Pinto, a Staff Clinician in CCR's Urologic Oncology Branch, pioneers minimally invasive treatments for prostate cancer, including laser ablation and robotic prostatectomy. In a new clinical trial that launched in July 2011, the researchers are testing the safety and effectiveness of magnetic resonance imaging-guided laser therapy in men whose prostate cancer has not yet spread to other parts of the body.

Pinto. The motivating factor for my research is an essential question: How can we offer better prostate treatment? With the standard diagnostic tests of today, that is, the random 12-core biopsy of the prostate, which uses ultrasound guidance to direct a prostate tissue collection needle into the prostate a dozen times, there is always a possibility of missing some cancerous cells, or of misjudging the size of the tumor. So, here at CCR, Dr. Choyke and I are collaborating on testing a platform that aims to tell us more precisely where a tumor is located within the gland, which, in turn, allows me, as a clinician, to operate with more precision, and to identify with more accuracy those patients who need immediate surgery and those who do not.

> [We] are collaborating on testing a platform that aims to tell us more precisely where a tumor is located within the gland, which, in turn, allows [us] to operate with more precision, and to identify with more accuracy those patients who need immediate surgery and those who do not.

Choyke. We use an endorectal coil multiparametric magnetic resonance imaging (MRI) to visualize the prostate. This is different from standard MRI in that we combine multiple MR scans in ways that allow us to stratify lesions by risk category—low, moderate, or high—depending on how many parameters are positive. We can then fuse the MR imaging data—which establishes coordinates for the lesion within the prostate—to an ultrasound device.

(*Photo: R. Baer*)

Bradford Wood, M.D., and Peter A. Pinto, M.D., perform a prostate biopsy.

Pinto. Again, this is quite different from how urologists typically use ultrasound today. Most urologists use it to define the borders of the prostate, but not to identify specific lesions. What's novel about our approach, which is being developed through a Cooperative Research and Development Agreement with Philips Medical Systems, is that we transfer MRI coordinates for the lesion directly to an ultrasound machine. Then, when I'm performing the biopsy, I use an electromagnetic tracker—which is part of this platform—to guide the biopsy needle in real time towards the tumor. If we locate a well-defined tumor surrounded by healthy tissue, then we treat the tumor only, and leave the rest of the prostate intact. This new trial represents the first time that we have attempted to treat just the tumor nodule based on MRI information. And because of the accuracy of the imaging, we are now able to use a laser to destroy cancer in the prostate while avoiding the nerves that control erectile functioning and continence. The technique is called MR image-guided focal laser ablation.

Choyke. One of the many benefits is that MR-guided laser ablation of the tumor makes it possible to monitor temperature changes in real time—you can see if critical structures, nerves, or the urethra are receiving dangerous levels of heat, and therefore avoid damage to otherwise healthy areas surrounding the prostate.

> This new trial represents the first time that we have attempted to treat just the tumor nodule based on MRI information.

Pinto. Another advantage of this technology is that nodule treatment happens under sedation, like a colonoscopy. Patients are comfortable, but they are not under general anesthesia, so they can come in for treatment and go home on the same day. That makes prostate tumor treatment an outpatient

procedure for some patients. It won't replace surgery, but not every patient requires surgery.

Choyke. We have also found that the MR method generates useful clinical information in just about every situation. For some patients, the doctor may look for a trend of rising prostate-specific antigen (PSA) measurements over time rather than a single elevated PSA level. For these patients, you can use MRI to identify lesions before the biopsy. For a patient with a previously negative biopsy who has a rising PSA, the MRI can be helpful in detecting occult lesions.

> ...the MR method generates useful clinical information in just about every situation.

(*Photo: R. Baer*)

Peter L. Choyke, M.D., and Peter A. Pinto, M.D., examine an image of the prostate.

(*Photo: R. Baer*)

Bradford Wood, M.D., Jochen Kruecker, Ph.D., Peter A. Pinto, M.D., and Peter L. Choyke, M.D.

Pinto. Alternatively, we could encounter a situation in which the radiologist tells me that a suspicious area might be cancerous, but that the risk level is low. In that case, we could recommend to the patient that we not biopsy immediately but use the imaging data to guide follow-up. So, MRI is also useful for "watchful waiting" in patients who do not want to be treated at all. It defines the volume of cancer and the true extent of disease.

MRI also improves how I perform robotic surgery in patients with confirmed cancer. Nerve sparing is always the goal during surgery—my job is to peel the nerves away from a tumor. This MRI imaging technique improves how I do that procedure, and it gives insights into whether a tumor might break out of the prostate, and if so, on what side. Under current treatment protocols, in patients with high-grade cancer we remove the whole prostate and the surrounding nerves and tissues, which often renders the patient impotent. But if MRI shows that the tumor is far enough away from the nerves, we might not have to do that. What is significant about this treatment is that it parallels what we've been doing in breast cancer for years. With breast cancer, it is possible to remove a mass by lumpectomy instead of removing the whole breast. For patients with well-defined prostate tumor nodules, in the right location, and with the right shape, we can now offer a similar option.

Of course, what we're undertaking in the clinical trial is at the very early stages of this type of treatment. We're trying to find out first if the approach is feasible, and second, whether it's safe. The protocol is complicated, but in a nutshell, it is offered to patients with a diagnosis of prostate cancer that corresponds with a lesion that we can see on MRI, and that we can also treat with a laser. We do not want to treat the most aggressive cancers at this stage and are, therefore, limiting enrollment to patients with MR-visible nodules confirmed on biopsy to have mild to moderately aggressive tumors.

We are able to offer this type of novel treatment because the imaging method developed by Dr. Choyke allows us to visualize the tumor so precisely. We still need to define the best candidates for the procedure, and this is what we will be working on going forward. It's incredibly exciting for us as clinicians because this really does feel like the dawn of a new treatment era for prostate cancer.

(*Photo: R. Baer*)

Peter A. Pinto, M.D., and colleagues in surgery.

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

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

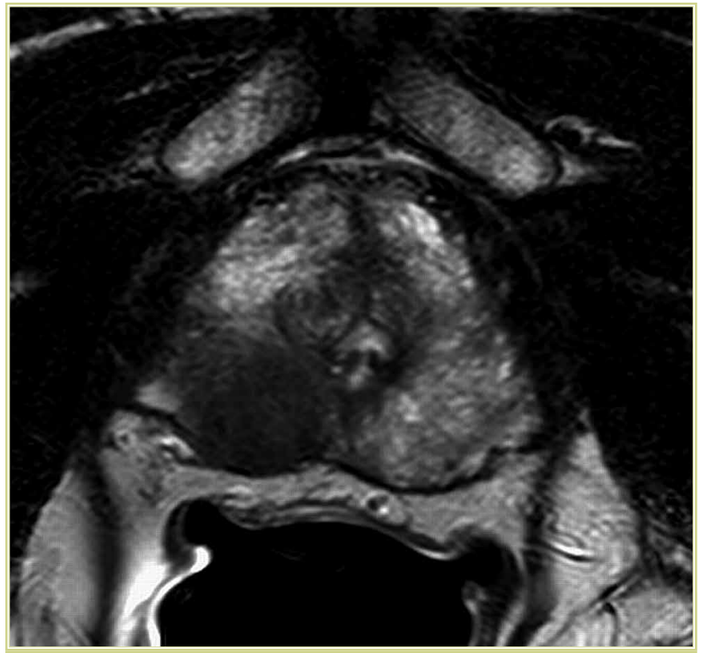
To learn more about the imaging trial described in this article, please visit the following Web site http://bethesdatrials.cancer.gov/clinical-research/search_detail.aspx?ProtocolID=NCI-11-C-0158.

Multiparametric MRI in Action

Gary Fisher, M.D., a cardiologist from Chevy Chase, Md., was 64 years old when, after his annual prostate-specific antigen (PSA) test, he noticed a worrying rise in PSA levels.

Although he more commonly referred his own patients for follow-up, Dr. Fisher referred himself to one urologist who recommended a biopsy, and a second urologist who told him to wait and follow up with a new PSA test in four to six months. Dr. Fisher chose the latter option, and six months later, his PSA levels were higher still. Having heard about the new multiparametric magnetic resonance imaging (MRI) being offered at NCI, Dr. Fisher opted to have his biopsy done using this new technique. Multiparametric MRI integrates traditional T2-weighted imaging with one or more functional techniques. "The procedure was very straightforward," said Dr. Fisher, "and the MRI clearly showed an abnormality, which the biopsy confirmed as cancer."

(*Photo: P. Choyke, CCR*)



An example of a T2 weighted MRI of the prostate showing a prostate cancer on the left side of the image (patient's right). This image guided a targeted biopsy done at NCI.

Dr. Fisher credits the MRI imaging technique pioneered by Peter Choyke, M.D., as contributing to his quick and accurate diagnosis. Of the 14 cores taken at biopsy, 12 random cores came back negative, and only the two cores guided by the multiparametric MRI came back positive. "Were it not for this informative MRI, I may well have left the clinical center with a negative biopsy and a continued recommendation of active surveillance," said Dr. Fisher.

The imaging also helped the clinical team to visualize the unusual placement of the tumor, so that when Peter Pinto, M.D., performed Dr. Fisher's surgery, a robotic radical prostatectomy, there were no surprises.

One year after surgery, Dr. Fisher remains very positive about his experience—he is thankful for the care and attention he received at the clinical center and extremely happy with the cancer-free outcome of the surgery—but it is his continued advocacy and many patient referrals over the last 12 months that are perhaps the most telling example of how he feels about the revolutionary prostate imaging and treatment options being pioneered at CCR.

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

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