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CCR connections

CENTER FOR CANCER RESEARCH

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The Key Ingredient:

A commonly found and inexpensive ingredient in many food and lubricant products—carrageenan—may protect women from HPV infection.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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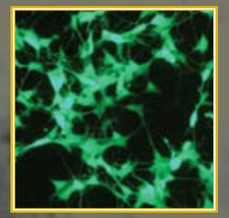

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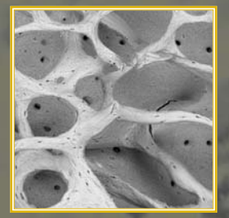

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We invite your comments and suggestions about *CCR connections*.
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Center for Cancer Research

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CENTER FOR CANCER RESEARCH

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The mission of CCR is:

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

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

Making Connections, Finding Solutions

We can accomplish more, and make every dollar go farther, if we communicate and collaborate.

In our culture, tales of scientific discovery often celebrate a lone investigator—Archimedes in his bath or Newton under an apple tree—in a moment of epiphany. Indeed, many great leaps of innovation and discovery have been, and will continue to be, driven by creative individuals. Thus, the present day mechanisms for rewarding scientific performance place special emphasis on the individual, whether it is the first author of a paper or the invited guest lecturer. In reality, and as usually acknowledged by the individuals so distinguished, science is more often a collaborative effort.

At CCR, we are constantly looking for ways to encourage and reward collaborative science. Increasingly, the complexity of modern biomedical research requires skills and expertise that a single person simply cannot master in one lifetime. We recognize the need to build teams of scientists and clinicians that cut across traditional boundaries to solve the health problems we are facing.

As we learn in "Collaboration Reigns," CCR is working across national boundaries on several projects with Thailand's Chulabhorn Research Institute. A two-day symposium sponsored by CCR featured a visit by the Princess of Thailand, herself a research scientist as well as founder of the Chulabhorn Institute. Programs like the Collaborative Research and Graduate Partnership Program in Cancer Technology with the University of Maryland, described here in "Teaming Up to Fight Cancer," are building bridges between academia and government scientists. And our Cooperative Research and Development Agreements (CRADAs) with industry continue to reap rewards (see "HIV Vaccine Strategy Is Not Dead").

One factor that is not always noted in strategic discussions of team science, however, is the success that derives from the simple fun and passion of people working together to solve problems. Two articles in this issue of *CCR connections* tell the stories of NIH investigators who have enjoyed years of fruitful collaboration. In "Putting Heads Together," Yardena Samuels, Ph.D., at NHGRI and Steven Rosenberg, M.D., Ph.D., at CCR describe their work on the genetics of melanoma. In "All in Good Fun," CCR investigators Ira Pastan, M.D., and Michael Gottesman, M.D., talk about the inception of their pioneering work on the molecular basis of chemoresistance. As important as these scientists' research accomplishments have been, the articles' most striking revelation is the obvious pleasure that both men have derived from doing science in good company.

Sheue-Yann Cheng, Ph.D., notes in "The Interconnectedness of All Things" that science is not nine-to-five work and that the enjoyment of research is a necessary component of success. Cheng has developed mouse models with impaired thyroid hormone signaling that are the basis for multiple collaborations around the world to study physiological mechanisms underlying cancer, lipid metabolism, skeletal development, and more.

(*Photo:* B. Branson)

Robert Wiltrout, Ph.D.

As Gabriela Kramer-Marek, Ph.D., describes in our "In Conversation" series, her work to visualize breast cancer and its metastases through positron emission tomography (PET) imaging has only approached fruition through the combined efforts of the chemists, physicists, and oncologists with whom she has worked at CCR and abroad. Collaborations not only reap scientific advances that would otherwise not be possible, they also train the next generation of scientists to solve problems together.

The Key Ingredient

A commonly found and inexpensive ingredient in many food and lubricant products—carrageenan—may protect women from HPV infection.

(Photo: iStockphoto.com)

Gelatinous extracts called carrageenans that come from the *Chondrus crispus* red seaweed—commonly used as food additives and in lubricants—may have anti-HPV properties.

Virtually all cervical cancers are caused by persistent human papillomavirus (HPV) infections, the most common sexually transmitted infection in young adults. According to the World Health Organization, cervical cancer is the second leading cause of female cancer deaths worldwide. But despite significant advances in the fight against HPV, many women are not yet reaping the benefits. HPV vaccines, for example, are too expensive for most women in developing countries. However, a safe, reliable, and inexpensive topical microbicide could protect women around the world from HPV infection and its potentially deadly consequences.

Recent studies have shown that the common, low-cost gelling agent carrageenan (found in many food products) is an extremely potent inhibitor of HPV infection *in vitro* and in animal challenge models. Retrospective clinical data announced at the 2010 International Papillomavirus Conference held in Montreal, Canada, indicated that a carrageenan-based personal lubricant called Carraguard, developed by The Population Council, is effective for preventing HPV infection in women.

Chris Buck, Ph.D., Head of the Tumor Virus Molecular Biology Section at CCR, had more than a passing interest in these findings when he first learned of them. Dr. Buck did much of the pioneering basic research to first identify carrageenan as an HPV entry inhibitor. He began his research under CCR mentors John Schiller, Ph.D., and Douglas Lowy, M.D., investigating HPV entry into cells and screening compounds that might interfere with that process. "I actually ended up testing some food additives just out of curiosity, and carrageenan has long been known to look chemically similar to the known primary attachment receptor for HPV, heparan sulfate," said Dr. Buck. "Carrageenan was so powerful that I had to do the experiment three times, each time diluting more and more because I was getting 100 percent inhibition."

Making the connection between its therapeutic potential and its gelling properties, Dr. Buck determined that carrageenan was found in several personal lubricant products. "The reason I'm so excited about the clinical data is because I think it's conceivably a cheap and easy method for women to protect themselves," said Dr. Buck. As with Carraguard, he believes the use of other carrageenan-based personal lubricants—such as Divine No 9, BIOglide, or Oceanus Carrageenan—may also prevent HPV infection. Prospective trials are now under way to give a clearer picture of carrageenan's efficacy against HPV, including trials with HPV typing and sampling at the start of the trial.

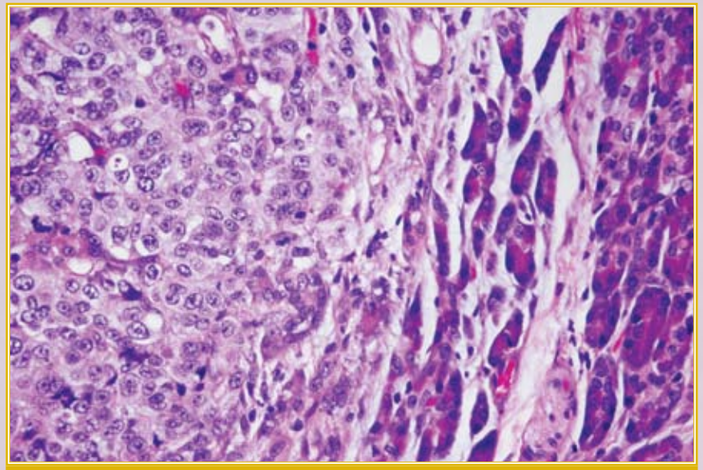
Although Dr. Buck's research has moved beyond topical microbicides, he and Dr. Schiller still support the development of carrageenan trials by testing candidate preparations and placebo controls to make sure the best possible candidates enter the trials. "We're sort of cheerleaders and we do some of the basic science bench work to support those trials," said Dr. Buck. "And we're excited because we had already gotten to the point where we felt it was okay to tell people that if they were already using a lubricant product, they might as well choose one that has carrageenan in it because—who knows—maybe it would help. And that recommendation is even more legitimate now."

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

Putting Heads Together

NCI Chief of Surgery Steven Rosenberg teams up with NHGRI researcher Yardena Samuels against melanoma.

(Photo: R. Lee, M.D., Ph.D.)



Micrograph of metastatic melanoma cells, *left*, that have invaded pancreatic tissue, *right*.

Sometimes, two heads are better than one. The vigorous collaboration between Steven Rosenberg, M.D., Ph.D., Chief of Surgery at CCR, and Yardena Samuels, Ph.D., an Investigator in the Molecular Cancer Genetics Section of the National Human Genome Research Institute (NHGRI), is a case in point.

"It began with a phone call from Dr. Francis Collins, who was then Head of NHGRI," recounted Dr. Rosenberg on how their collaboration began in November 2006, "telling me that he was interested in recruiting a postdoctoral fellow from Johns Hopkins to come to NHGRI, that she was very interested in somatic mutations in melanoma and I should meet with her. And that person was Yardena Samuels."

The two met the very next day. "After talking for approximately 15 minutes, Dr. Rosenberg agreed to collaborate with me," said Dr. Samuels. Dr. Rosenberg's team was studying melanomas from an immunologic standpoint, and Dr. Samuels wanted to study the genomics of the disease. Immune responses are largely the result of genetic mutations within the melanoma, so by identifying these mutations, they hoped to identify potential new therapeutic targets.

Before their collaboration, Dr. Samuels' research into the genomics of melanoma was limited by lack of access to melanoma patients or samples. Dr. Rosenberg had accumulated just such samples over decades in the course of his immunotherapy studies. "We had put away melanoma digests that came out of the operating room from 467 different patients," said Dr. Rosenberg. "We had 329 different tissue culture lines and we had 562 fresh frozen blocks of melanoma, so we had what's probably the largest compendium of melanoma samples in the world."

"Dr. Rosenberg had the ingenuity to preserve not only the tumors, but also derive cell lines from the tumors as well as keep the patient's blood. Few melanoma clinicians or scientists ever had such insight," added Dr. Samuels. He also had the clinical history of his patients so that they could make correlations between the genetic changes and clinical outcome. Dr. Rosenberg gave Dr. Samuels access to the entire collection of melanoma samples to begin her genomic study of the disease.

"That began what has been a wonderful and very productive collaboration where many new genetic changes have been identified in the tumors from melanoma patients," said Dr. Rosenberg. "And it has been the perfect example of going from the patient to the laboratory to study, and then back to the patient, because one of the mutations that we observed—a mutation in the *ERBB4* gene present in about 19 percent of melanomas—was something that we could specifically target with a drug called lapatinib."

Both Drs. Rosenberg and Samuels continue to learn from each other and share information about genomic changes in melanoma and how they relate to the course of the disease. They have published many papers together (in *Cancer Biology & Therapy, Pigment Cell Melanoma Research, Nature Genetics, Cancer Research,* and *Biochemical and Biophysical Research Communications*) and have submitted several more for publication. "It's a wonderful collaboration and exactly what the NIH is about," remarked Dr. Rosenberg, "this marriage of basic science and clinical medicine that is quite unique here."

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

Collaboration Reigns

Princess Chulabhorn of Thailand visits the NIH to discuss collaborations with NCI.

(*Photo: E. Branson*)

Her Royal Highness Princess Chulabhorn Mahidol of Thailand

A long-established collaborative culture at the NIH has produced important scientific breakthroughs by connecting not only different groups within the NIH, but also institutions across the country and even across continents. Thus, a two-day visit to the NIH by Her Royal Highness Princess Chulabhorn Mahidol of Thailand only seems unusual without the knowledge that she is a committed research scientist as well as founder and president of Thailand's Chulabhorn Research Institute. Its mission focuses on global collaboration and applying translational discoveries to improve the quality of life for the people of Thailand.

The princess attended a CCR-sponsored meeting June 15-16, 2010, to discuss recent findings and new studies aimed at the discovery and development of natural products that could yield treatments for cancer. The meeting explored ongoing and potential research collaborations between the NCI and Chulabhorn Research Institute.

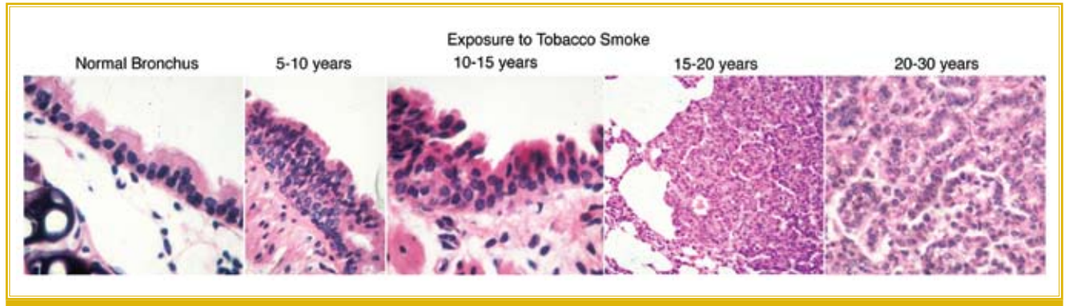
The Thailand Initiative on Genomics and Expression Research for Liver Cancer (TIGER-LC)—a collaborative project between Thailand, CCR, and other international institutions—was the topic of discussion on the first day of the meeting. Director of CCR Robert Wiltrout, Ph.D., and CCR investigators Curtis Harris, M.D., and Xin Wei Wang, Ph.D., as well as other CCR scientists involved in TIGER-LC, presented new findings in lung and liver cancer research relevant to the TIGER-LC project, including studies investigating cancer biomarkers that could guide decisions for appropriate treatment choices. At the close of the day, H.R.H. Princess Chulabhorn toured CCR's Genomics Center and imaging laboratories, where some of this research takes place.

The following day served as a forum for sharing data on natural products, an area of research underway at both NCI and the Chulabhorn Research Institute to discover disease-fighting compounds in natural resources. H.R.H. Princess Chulabhorn, who has a Ph.D. in organic chemistry and has taught courses in natural products chemistry, presented the latest data on natural products research in Thailand. David Newman, Ph.D., of NCI's Division of Cancer Treatment and Diagnosis, discussed NCI's Natural Products Branch and Repository, which contains plant samples, marine animals, and microbes from more than 35 countries. Jim McMahon, Ph.D., Chief of CCR's Molecular Targets Laboratory (MTL), described the natural products screening efforts currently taking place in the MTL. Barry O'Keefe, Ph.D., also from the MTL and a former teaching assistant of H.R.H. Princess Chulabhorn, discussed an antiviral protein that his team isolated from red algae called griffithsin that has shown activity against several viruses including HIV (see "By Land or by Sea" in Vol. 3, No. 1 of *CCR connections*). Finally, Yves Pommier, Ph.D., of CCR's Laboratory of Molecular Pharmacology, presented two types of natural products studied at CCR with anticancer properties called camptothecins and ecteinascidins.

The two-day meeting between Her Royal Highness and CCR fostered a rich exchange of information on cancer research and natural products, and plans are in place for further collaboration in the months ahead. "The Chulabhorn Research Institute does excellent work in natural products, and Thailand is home to numerous endemic species and biodiversity with potential for both bioprobe and drug discovery," said Dr. O'Keefe. "Since we already have a good track record of interacting in a productive manner through TIGER-LC, we are optimistic that future collaborations between the two groups in the area of natural products will also be successful."

Teaching an Old Drug New Tricks

A drug regularly used to treat diabetes may be effective in preventing lung cancer in smokers.



(*Image: J. Weddle*)

Exposure to tobacco smoke over a lifetime causes normal cells from a lung bronchus to progress to hyperplasia (5-10 years later), dysplasia (10-15 years later), carcinoma *in situ* (15-20 years later), and, eventually, malignant adenocarcinoma (20-30 years later).

For about 15 years, metformin has been a safe, inexpensive, and widely used treatment for Type 2 diabetes. The drug decreases levels of insulin-like growth factor-1 (IGF-1) and circulating insulin, explaining its role in a disease where the normal insulin response is impaired. Recent research suggests, however, that metformin may also be used as an anticancer drug to prevent lung carcinoma in smokers. IGF-1 is believed to play a crucial role in this form of cancer, and a variety of studies have suggested that, by blocking IGF-1's activity, metformin may inhibit cancer.

Phillip A. Dennis, M.D., Ph.D., Head of the Signal Transduction Section of the Medical Oncology Branch at CCR, and colleagues tested this idea by administering metformin to mice for 13 weeks following exposure to the most prevalent tobacco carcinogen, nicotine-derived nitrosamine (NNK). They found that mice treated with an oral form of metformin showed a reduction in lung tumor burden by about 55 percent compared to untreated mice. This effect was even more profound when the treatment was administered by injection with higher levels of metformin: the drug reduced lung tumor burden by almost 75 percent compared with no treatment. These findings were published in the September 1, 2010 issue of *Cancer Prevention Research*.

The researchers further evaluated the effects of metformin on a series of biomarkers for lung tumorigenesis. They showed a marked inhibition of the cell signaling protein mTOR—which promotes lung tumor growth—related to decreased levels of circulating insulin and IGF-1. "What's interesting is that it didn't appear that lung tissues were responding directly to metformin, but that liver tissues were," said Dr. Dennis. "This showed us metformin works through an unusual mechanism that wouldn't have been discerned if we hadn't used an animal model because an intact liver is needed to respond to metformin and change the circulating levels of IGF-1 and insulin."

Metformin for lung cancer prevention has several advantages, noted Dr. Dennis. "It's oral, it's inexpensive, and it has almost no side effects in nondiabetics." With any chemopreventive agent, minimal toxicity is critical, and metformin was well tolerated in the mice. In fact, the livers of the treated mice not only showed no signs of toxicity, but they actually appeared healthier than those of untreated mice since fewer cases of fatty liver were observed. Although oral dosing is feasible in humans, reaching levels achieved by injection would require development of more potent derivatives of metformin.

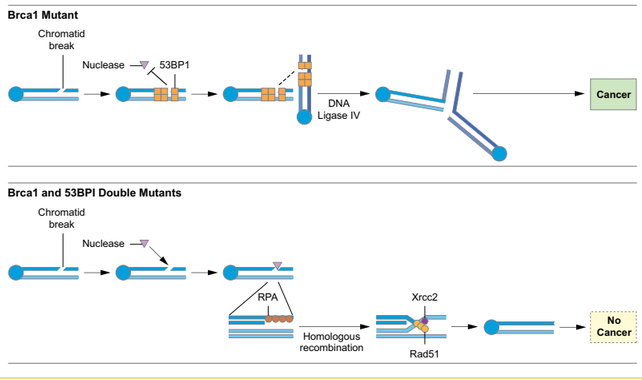
This study may be the first to show the potential for metformin to prevent lung cancer. Next, Dr. Dennis' group plans to combine metformin with other preventive agents. "In fact, because metformin and rapamycin work through different mechanisms to inhibit mTOR, we think it might be very interesting to combine those two drugs." The team also plans to test metformin in other model systems and develop a clinical trial to test the drug in people at the highest risk of developing lung cancer.

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

Choose Your Pathway Wisely

Inhibition of the 53BP1 gene restores an error-free pathway for DNA repair that is lost due to a mutation in the oncogene BRCA1.

(*Image: J. Kelly*)



The 53BP1 protein impairs homologous recombination in BRCA1-deficient cells by blocking a nuclease from generating single-strand DNA necessary for recombination between two homologous DNA molecules. As a result, error-prone DNA repair is mediated by ligase IV. When BRCA1 and 53BP1 are defective, the break is not "clogged," the nuclease can generate single-strand DNA, and homologous recombination can proceed.

Double-strand breaks (DSBs) occur naturally several times a day in every human cell. If left unchecked, they are one of the most mutagenic kinds of DNA damage and have been implicated in many cancers. Homologous recombination (HR)—in addition to producing new combinations of DNA sequences during meiosis—is widely used by cells to accurately repair harmful DSBs. In humans, mutations in the *BRCA1* gene increase the risk of breast and ovarian cancers by impairing HR through incompletely understood mechanisms. Women who carry a harmful mutation in the *BRCA1* gene have up to an 85 percent greater lifetime risk of developing breast cancer than other women, and up to a 40 percent greater chance of developing ovarian cancer.

Mouse *BRCA1*-associated mammary tumors have significant similarities to human *BRCA1*-associated breast cancer in regard to tumor aggressiveness, high incidence, mutations, and genetic instability. In a study published in the April 16, 2010 issue of *Cell*, Andre Nussenzweig, Ph.D., Head of CCR's Molecular Recombination Section of the Experimental Immunology Branch, and fellow NIH investigators as well as colleagues from Rockefeller University and the Spanish National Cancer Research Institute, have compensated for cancer-causing mutations in the *BRCA1* gene in mice by deleting a second gene.

The researchers found that, when a gene known as *53BP1* was also defective, formation of the mammary tumors that normally develop in *BRCA1* mutant mice was suppressed. Moreover, they found that inactivation of *53BP1* restored the DNA repair function that is lost when *BRCA1* is mutated. Using a strain of mice with a defective *BRCA1* gene, the team observed that the mice frequently developed mammary tumors similar to human breast cancers, but tumor formation was largely suppressed when the mice also were lacking the functional protein 53BP1. "This was very unexpected because it was previously believed that BRCA1 was absolutely essential for DNA repair by HR," said Dr. Nussenzweig.

Mechanistically, the team also discovered that both BRCA1 and 53BP1 are capable of binding to replication-associated chromosome breaks; so when both proteins are present, BRCA1 displaces 53BP1, the HR machinery has full access to the breaks, and HR proceeds. In BRCA1-deficient cells, the binding of 53BP1 to the site of DNA damage interferes with the DNA repair activity of HR proteins, so the damage is repaired instead by an alternative pathway that is more prone to producing mutations. When 53BP1 is absent, however, BRCA1 is not needed to displace it so HR can take place normally.

Because BRCA1-deficient tumor cells are forced to turn to other, less faithful DNA repair pathways, the researchers suggest that they may become resistant to chemotherapy by acquiring additional mutations. Such resistance may one day be overcome by therapies to affect pathway choice. "What we've found here is that the choice of DNA repair pathway determines whether the repair is error-free or not," said Dr. Nussenzweig. "This opens the possibility of using drugs to inhibit mutagenic DNA repair pathways and tumor formation."

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

In Conversation:

Research Fellow Gabriela Kramer-Marek, Ph.D.

CCR: Gabriela, we understand that you traveled to Kyoto this fall to present your work at the 2010 World Molecular Imaging Congress. How did that come about?

Gabriela: Yes, I was honored to receive an invitation to speak at the biggest meeting in our field. The work I presented is the result of studies we have been pursuing since I came to the laboratory of Jacek Capala, Ph.D., in CCR's Radiation Oncology Branch, five years ago.

CCR: Can you tell us about your research?

Gabriela: We have developed a new approach for the noninvasive assessment of HER2/neu expression in breast cancer, using positron emission tomography (PET) imaging. It allows for detection of HER2/neu status in both the primary tumor and distant metastases and could also be used for monitoring the response to therapeutic intervention.

CCR: So is this a technology that is ready for patients?

Gabriela: Not yet, but our collaborators will begin clinical trials next year. When I came to the lab, the project was just a proposal. First, we had to develop the tracer. We used different positron emitters to label a relatively small protein (Affibody) that tightly binds to HER2/neu. After that we tested different tumor models as well as experimental conditions to acquire high-contrast PET images for quantification of receptor expression. At every step, there were challenges. Fortunately, we have a great multidisciplinary team of researchers and very supportive collaborators.

CCR: What background did you bring to the work?

Gabriela: I trained as a medical physicist at Silesian University in Poland, which is where I am from originally. So I had a solid background in physics and I knew the theory of PET, but, of course, reading about something and working with it are two very different things. I had done my doctoral research on light-activated drugs as a cancer therapy, so I was immersed in oncology. But I don't think I had ever seen a laboratory mouse before I came here.

CCR: What made you come all the way to the NCI from Poland to continue your research?

Gabriela: Honestly, I never wanted to come to America. I had decided to continue my postdoctoral research in Portugal when I got an email from a friend saying that Jacek was looking for a fellow. After my interview with Jacek, I think I made the decision to come here within 24 hours. During my doctoral studies, I had conducted research in some well known institutions across Europe, but none had the breadth of opportunity I saw here.

CCR: And has the experience lived up to your expectations?

Gabriela: Definitely. The facilities are, of course, amazing. But so is the network of people here at NIH. You can find and count on the support of experts in any field of medicine. And the NIH Office of Intramural Training and Education has courses on how to give a good presentation, or write a grant. Especially as a foreigner, it is a great opportunity to get a lot of experience and improve your skills.

(*Photo: B. Branson*)

Gabriela Kramer-Marek, Ph.D.

Staff News at CCR

announcement


(Photo: E. Branson)

Ronald E. Gress, M.D.

Ronald Gress has been named a Deputy Director of CCR. He received his M.D. from Baylor College of Medicine and completed his residency and clinical oncology training at The Johns Hopkins Hospital and further fellowship training in oncology and basic immunology at the NCI. In 2000, he became Chief of CCR's Experimental Transplantation and Immunology Branch and has established a highly successful bone marrow transplant program with an active clinical component and state-of-the-art bone marrow transplantation unit in the NIH Clinical Center. His research focuses on transplantation immunology with emphasis on the regulation of allogeneic responses and the mechanisms by which peripheral lymphocyte populations are generated and maintained. As a Deputy Director, he will help guide the CCR clinical program.

newly tenured CCR scientists

Vineet KewalRamani, Ph.D.
HIV Drug Resistance Program

Terry Yamaguchi, Ph.D.
Cancer and Developmental Biology Laboratory

Di Xia, Ph.D.
Laboratory of Cell Biology

new tenure-track scientists


(Photo: E. Branson)

Federico Bernal, Ph.D.

Federico Bernal joins CCR's Metabolism Branch. He received his Ph.D. from The Scripps Research Institute after performing work on the development of synthetic methodologies for the construction of complex marine natural products in the laboratory of Dr. K.C. Nicolaou. He completed his postdoctoral training in chemical biology under the supervision of Dr. Gregory Verdine at Harvard University followed by additional studies in the same area in the laboratory of Dr. Loren Walensky at the Dana-Farber Cancer Institute. His research investigates the use of synthetic molecules to manipulate cancer pathogenesis pathways.


(Photo: E. Branson)

Terry Fry, M.D.

Terry Fry rejoins CCR's Pediatric Oncology Branch after serving as Chief of the Division of Blood and Marrow Transplantation at Children's National Medical Center since 2007. Dr. Fry obtained his M.D. from Georgetown University. After completing a pediatric residency at Georgetown, he received fellowship training in pediatric hematology and oncology at The Johns Hopkins University and worked in the CCR laboratory of Dr. Crystal Mackall as part of that training. He continued working in Dr. Mackall's laboratory as a Postdoctoral Fellow and was a Staff Clinician in the Pediatric Oncology Branch from 2004 until 2007, when he left for Children's National Medical Center in Washington, DC.


(Photo: E. Branson)

James Gulley, M.D., Ph.D.

James Gulley has been promoted to tenure-track scientist in CCR's Laboratory of Tumor Immunology and Biology, where he serves as the Director of the Clinical Trials Group. He is also a Principal Investigator in the Medical Oncology Branch. He received his medical training at Loma Linda University in its medical scientist training program where he obtained an M.D. and Ph.D. for his work in tumor immunology. He completed his residency in internal medicine at Emory University and then came to NCI for a fellowship in medical oncology. Following his fellowship, he was retained as a staff clinician at NCI. Since 1999, Dr. Gulley has run multiple clinical trials in immunotherapy for prostate cancer. He has received numerous awards and has authored over 130 original articles, reviews, and book chapters on immunotherapy and cancer treatments. His research focuses on the use of cancer vaccines and other immunostimulatory molecules to modulate the immune response in cancer patients and to enhance vaccine-mediated killing.

Recent CCR Awards

Elected to the Institute of Medicine in 2010

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

Carl Wu, Ph.D.
Chief, Laboratory of Biochemistry and Molecular Biology

International Society for Biological Therapy of Cancer Team Science Award

For ongoing clinical and basic research that has advanced the understanding of the biological response to cancer therapies designed to modulate the human immune response
NCI-Frederick Biological Response Modifier Program

For their pioneering role in the development of T cell therapies for patients with cancer
NCI Surgery Branch

International Society for Biological Therapy of Cancer Exceptional Service Award

For lifetime service to iSBTc
Robert Wiltrout, Ph.D.
Director, Center for Cancer Research
Steven Rosenberg, M.D., Ph.D.
Chief, Surgery Branch

The International Cytokine Society 2010 Lifetime Membership Award

In recognition of outstanding achievements in cytokine research
Giorgio Trinchieri, M.D.
Chief, Laboratory of Experimental Immunology

2009 Arthur S. Flemming Award

Shyam Sharan, Ph.D.
Mouse Cancer Genetics Program

Association of Residents in Radiation Oncology 2010 Educator of the Year Award

Kevin Camphausen, M.D.
Chief, Radiation Oncology Branch

Clinical Cytometry Foundation 2010 Wallace Coulter Award

In recognition of "...her lifetime contributions to the science, education, and practice of Clinical Cytometry."
Maryalice Stetler-Stevenson, M.D., Ph.D.
Laboratory of Pathology

2010 Karl Landsteiner Memorial Award and Lectureship

American Association of Blood Banks
Steven Rosenberg, M.D., Ph.D.
Chief, Surgery Branch

Elected to Fellowship in the American Academy of Microbiology

Douglas R. Lowy, M.D.
Deputy Director, National Cancer Institute
Chief, Laboratory of Cellular Oncology

Sriram Subramaniam, Ph.D.
Laboratory of Cell Biology

Elected to the American Academy of Arts and Sciences

Michael Gottesman, M.D.
Deputy Director for Intramural Research, National Institutes of Health
Chief, Laboratory of Cell Biology

2011 Biophysical Society Fellow

For extraordinary contributions to advances in computational biology on both nucleic acids and proteins
Ruth Nussinov, Ph.D.
CCR Nanobiology Program

The International Society of Interferon and Cytokine Research Distinguished Service Award

Howard Young, Ph.D.
Laboratory of Experimental Immunology

American Thyroid Association's 2010 Van Meter Award

Electron Kebebew, M.D.
Surgery Branch

American Medical Association's 2010 Nathan Davis Award

"Outstanding Member of the Executive Branch in Career Public Service"
Ira Pastan, M.D.
Chief, Laboratory of Molecular Biology

The International Society of Interferon and Cytokine Research Milstein Young Investigator Award

Ram Savan, Ph.D.
Laboratory of Experimental Immunology

HIV Vaccine Strategy Is Not Dead

A CRADA between NCI and Sanofi Pasteur leads to the first clinical trial for a preventive HIV vaccine regimen to show efficacy.

(Photo: E. Branson)

Genoveffa Franchini, M.D.

Once thought to be a dead end for tackling HIV, vaccines are showing the first signs of success in clinical trials. Through a Cooperative Research and Development Agreement (CRADA) with Sanofi Pasteur, NCI scientists, in the largest HIV intervention trial to date, have helped to develop a vaccine approach that reduced the risk of HIV infection.

The novel two-pronged regimen known as RV144 uses Sanofi Pasteur's canarypox vector-based candidate ALVAC-HIV to prime the immune response, followed by a boost to the system with AIDSVAX B/E, a genetically engineered version of HIV's gp120 surface protein. Genoveffa Franchini, M.D., Senior Investigator in the Vaccine Branch at CCR, led the preclinical studies that spawned this vaccine approach, which has been patented by the NCI.

Researchers from the U.S. Military HIV Research Program, the Ministry of Public Health in Thailand, and the NIH published the results of their phase 2b trial—involving more than 16,000 Thai volunteers—in the December 3, 2009 issue of *The New England Journal of Medicine*. At the conclusion of the six-year trial, the prime-boost regimen reduced the risk of HIV infection by about 31 percent.

Dr. Franchini's group plans to investigate the immune system factors that correlate with RV144's protective effects in nonhuman primate models in order to identify and boost the beneficial immune response. "Then," said Dr. Franchini, "we will perform more direct experiments that determine the contribution of different arms of the immune system and test methods to improve these vaccines."

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

Teaming Up to Fight Cancer

The University of Maryland and CCR collaborate on integrative and systems biology and biological physics research.

With the goal of bringing together the distinct expertise of their respective institutions to tackle the most pressing issues in cancer research, CCR Director Robert Wiltrout, Ph.D., and University of Maryland Vice President for Research Melvin Bernstein, Ph.D., signed a formal Memorandum of Understanding on May 19, 2010, at the UMD College Park campus.

The agreement involves the Departments of Physics, Mathematics, and the Institute for Physical Sciences and Technology at UMD in a Collaborative Research and Graduate Partnership Program in Cancer Technology with CCR. The vision is that the application of cutting-edge mathematical, physical, and engineering tools—both experimental and theoretical—will contribute significantly to the understanding of biological systems in health and disease.

UMD graduate students had already been working in NCI laboratories for several years, and the fruits of their research convinced administrators that an official program would benefit both institutions. The program will allow qualified UMD graduate students to conduct research under the joint supervision of CCR and university faculty and includes a series of lectures, workshops, and meetings.

The agreement also brings together cancer researchers, physical scientists, mathematicians, and engineers on the two campuses. "We are very enthusiastic about this partnership with the University of Maryland," said Dr. Wiltrout. "I believe it will make both institutions stronger and ultimately advance cancer research for the benefit of patients. We can accomplish more by working in interdisciplinary teams."

(Photo: J. T. Consoli)

Robert Wiltrout, Ph.D., and Melvin Bernstein, Ph.D., sign a formal Memorandum of Understanding.

All in Good Fun

A brief walk through the history of science uncovers many famous collaborators whose discoveries have changed the world. Pierre and Marie Curie, James Watson and Francis Crick, Michael Bishop and Harold Varmus, to name just a few. Some came together for one brief shining discovery; others had a lifetime commitment to a shared research agenda. Asking what makes for a successful collaboration may be like asking what makes for any successful relationship, and may be as difficult to answer. Michael Gottesman, M.D., and Ira Pastan, M.D., came together at the NCI in the 1970s to uncover the molecular basis for the multidrug resistance (MDR) that develops in cellular diseases ranging from cancer to bacterial infections. Whether it is because "opposites attract" or because of "shared mutual interests," according to Gottesman and Pastan, the hallmark of a successful collaboration is that it is just plain fun.

"Michael was interested in somatic cell genetics and I was interested in biochemistry," recalled Pastan, who is now Chief of CCR's Laboratory of Molecular Biology. "And, you know, genetics plus biochemistry equals molecular biology."

Pastan was interested in the role of the intracellular signaling molecule cyclic adenosine monophosphate (cAMP) in limiting the growth of cancer cells. Gottesman, who is now Chief of CCR's Laboratory of Cell Biology, suggested that as a new way into the problem, they make mutant cell lines (from Chinese hamster ovary, or CHO, cells) that did not respond to cAMP and study their properties.

"At that point, Ira had gotten some space and I hired a postdoc and we began to isolate mutants that were resistant to cAMP," explained Gottesman.

At this juncture, so the story goes, Bruce Chabner, M.D., who was the Head of the Cancer Chemotherapy Division of the NIH, pointed out that the phenomenon of multidrug resistance was a major reason that chemotherapy fails in patients. Chemotherapy itself was a relatively new innovation pioneered by the NIH and was universally hailed as being highly successful until people became resistant to the treatments. Chabner, therefore, asked: "Why don't you actually study resistance in a model relevant to human cancer?"

"Resources were limited," remembered Gottesman, "but the clinical relevance of drug resistance was clear at a time when Ira was moving away from very basic biology to much more focused applications to cancer. This seemed like a good transition. So we started to study how cancer cells become resistant to chemotherapy."

Different Styles Can Be Complementary

Gottesman and Pastan both have medical degrees but had decided early on to move into more basic research. In preparing to tackle resistance to chemotherapy, they decided to participate in clinical rounds once a week and to ask questions. "NIH was the place where chemotherapy was initiated, so this was a great re-education," said Gottesman.

The goal was to develop tools for the clinical evaluation of drug

(*Photo: R. Baer*)

Michael Gottesman, M.D., and Ira Pastan, M.D.

> This was not a shotgun marriage; we found each other and a mutual interest.

resistance, which meant using human cell lines. Furthermore, they wanted the reagents, antibodies, and whatever else they developed to be relevant to the clinic.

"I tried to define in the beginning what our contributions were," said Pastan, "but basically we just liked working together. It was a problem we were both clearly interested in. This was not a shotgun marriage; we found each other and a mutual interest. We enjoyed talking to each other."

"I was in the lab 100 percent of the time in those days and we just had a good time," said Gottesman. "We were exactly on the same wavelength. We came up with a plan, usually quickly. But neither of us is entrenched in our way of doing science."

"We had somewhat different orientations in terms of science but each appreciated the other's discipline," added Pastan. "Although you can't be an expert in every field, you have to be conversant in the language. We just had a lot of fun. We both enjoy a certain kind of humor and both enjoyed interacting with the fellows."

"One of the major principles I learned from Ira is that you shouldn't be technically limited," said Gottesman. "One great advantage of the NIH is that there is always somebody down the hall or across the campus that has the knowledge you need. We depended a lot on people at the NIH."

"We had weekly data meetings," remembered Pastan. "Michael was the good guy, and I was the bad guy. I am very systematic in the practice of science and try to teach people to organize their thoughts accordingly. If I ask 'Why did you do this?', then 'I thought it would be interesting,' is not a good answer. Doing science, you have to be pretty focused. After all, life is short."

"And after the meeting, I'd tell them that Ira was just trying to help and explain what he meant," said Gottesman. "Ira is enormously capable of figuring out the shortest distance; I'm a little more grandiose. Everyone needs two parents."

"I have heard that there are some people who work together who do not have as comfortable a relationship. I think there are different styles," noted Pastan.

Successful Scientists Make Successful Teams

"I think we would have been successful or not in different ways regardless of whether we worked together," said Pastan. "How do you identify a person who will be successful? It is wonderful to be smart and focused, but it isn't sufficient. Some people have the knack of seeing problems and solving them—maybe they have a bit of luck—and some people don't, even though they may be smarter."

Pastan pointed out that usually there is an excess of problems to solve. "Somehow, people who like to do research—they look around and they can sort of figure out something that nobody else is doing that would be of some interest. I have people who come to me and say, 'I can't think of what to do.' Some people have a knack of finding important, novel things to study and if they don't, they should be doing something else."

Pastan concedes that there is luck involved, too. "You can be focused and hard working, but life is full of these chances. You go to a seminar and you hear something and say 'Oh my God, maybe…'"

The fellows that Pastan and Gottesman worked with over the years form an illustrious cadre. "Ira's first postdoc was Harold Varmus!" said Gottesman, referring to the Nobel Laureate and current NCI Director. "And when I interviewed Harold, I turned down Mike Brown!" retorted Pastan, referring to another Nobel Laureate.

> How do you identify a person who will be successful?

"We had a postdoc application from a well-established scientist in Japan, Shin-Ichi Akiyama, who developed multidrug resistant cell lines that became the basis of most of our studies," said Gottesman. "In fact, the entire pharmaceutical industry uses these cell lines to pretest their drugs."

"If you look at the 50 to 60 postdocs who worked on MDR over the years, virtually all are still working scientists," concluded Pastan.

What, When, and Where Also Matter

"And then there's when to get in a field," said Pastan. "My feeling is that I wouldn't want to work in a field filled with bright people, because it would be kind of boring. What I think is fun is identifying a new area—that's why the MDR project was fun. It was a new area and clearly important, but not much was known.

"And I would expand that to include much of the intramural program—it's always easy to do incremental science and it could be extremely important, but it is much more fun to work in a completely unknown area and make really seminal discoveries."

As he once taught Gottesman long ago, Pastan continues to stress the need to not be limited by technology. "Be broad in your reading of basic biological and clinical literature, and find the people who can help you—for one person to tackle an important problem, it is virtually impossible.

"When I first came to the NIH, I worked in a group studying thyroid hormones," added Pastan. "There were seven people each with a project and they were doing everything I could think of to do. So instead of working on the thyroid gland itself, I decided to work on thyroid stimulating hormone. There was a guy down the hall purifying it, so I had rare access."

(*Photo: R. Baer*)

Michael Gottesman, M.D., and Ira Pastan, M.D.

> It is much more fun to work in a completely unknown area and make really seminal discoveries.

Moving On

Pastan and Gottesman no longer have any joint projects.

"I had to decide whether to work on immunotoxins where I thought we could get drugs into the clinic in my lifetime or MDR where we'd have to rely on companies to develop the drugs," said Pastan. "So one day I said I can't do MDR anymore. It seems like yesterday, but it was 12 years ago."

"I haven't formed the same kind of partnership again," said Gottesman, who has less time to devote to his lab now that he is also Deputy Director for the NIH Office of Intramural Research.

Pastan speaks of a few past collaborations that had some of the same close feeling. For example, he worked closely with Robert Perlman, Ph.D., on cAMP gene regulation in the bacterium *Escherichia coli*. "Bob and I were like two high school kids on the phone late at night, talking about science."

Despite the lack of joint projects, Pastan and Gottesman remain fast friends. "One of the reasons that we so enjoyed the collaboration is that we actually liked each other," said Pastan. "The role that plays in developing long-term collaborations is not a trivial one."

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

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

A Problem of Disordered Development

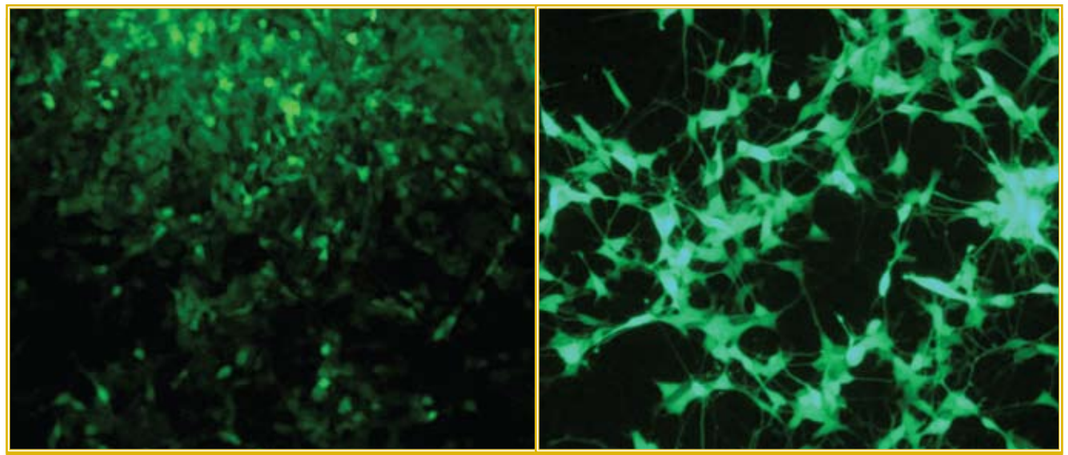
Neuroblastoma is a notoriously heterogenous disease. A neuroendocrine tumor, it is the most common extra-cranial solid tumor in children. It is known to spontaneously regress and disappear in infants. But it can also metastasize and develop chemoresistance, in which case the options for treatment are limited, toxic, and seldom curative. Carol Thiele, Ph.D., Head of the Cell and Molecular Biology Section in CCR's Pediatric Oncology Branch, has been studying the molecular mechanisms that determine whether neuroblastoma cells proliferate or differentiate since she joined the Pediatric Oncology Branch in 1983. Her insights have led to new therapeutic approaches and the discovery of a novel human gene that is likely to be fundamental both to tumor suppression and to normal development.

On the bulletin board next to Carol Thiele's desk is a black and white photomicrograph with four panels labeled with the kind of adhesive lettering that was painstakingly applied to such images before the advent of computerized image processing. Each panel shows a collection of neuroblastoma cells that are growing under different conditions. Thiele will eagerly take it down to show visitors the cellular phenomenon that spurred her research career.

Mark Israel, M.D., was Head of the Molecular Genetics Section of the Pediatric Oncology Branch when Thiele was first working at the NCI as a Damon Runyon Scholar and looking for a full-time position. "Mark presented me with this phenomenon," Carol explained. "If you look at neuroblastoma cells in culture, they are round, undifferentiated, rapidly proliferating cells. But, if you simply add retinoids (a derivative of vitamin A) to the mix, they stop dividing and start to look more neuron-like." Neuroblastoma cells have a number of genetic alterations that render them cancerous. "That you could impose growth control and induce differentiation with retinoids suggested to me that the compound was acting to bypass or complement the defective mutations." Thiele, a molecular biologist by training, was hooked into delving deeper into this process to understand its mechanisms.

The first use of vitamin A to differentiate cancer cells was pioneered in acute promyelocytic leukemia (APL) by Theodore Breitman, M.D., at the NCI in the 1980s. "They actually put retinoids into a clinical protocol to treat APL," Thiele explained, "but the response wasn't as good as in cell culture." It turns out that only a subset of the APL patients—those with a particular chromosomal translocation—respond to retinoids. Once that was understood, retinoids became frontline therapy for those patients.

Meanwhile, other investigators were testing the effects of retinoids on different cancer cell lines without knowing how they might work. "It was definitely considered something of a touchy-feely area of clinical research," recalled Thiele. Researchers have since demonstrated that retinoids bind to a family of retinoic acid receptors, which are actually nuclear binding proteins that regulate diverse transcriptional programs relating to cell growth and differentiation. It is also now known that retinoic acid (RA) is important for the development of certain neural subtypes. But many questions still remain about how retinoic acid can compensate for the genetic alterations that occur in neuroblastoma cell lines to produce differentiation and how to optimally translate the mechanisms observed in culture into tools for the clinic.



(*Image: C. Thiele*)

Neuroblastoma cells labeled with green fluorescent protein, before *(left panel)* and after *(right panel)* treatment with retinoic acid. Retinoic acid treatment arrests tumor cell growth and induces differentiation.

Deconstructing Vitamin A

To follow up on the effect of retinoic acid on differentiation of neuroblastoma cells, Thiele conducted collaborative studies to define the underlying mechanisms. "We were really lucky because one of our collaborators, Pat Reynolds, was able to insert retinoic acid into a neuroblastoma clinical trial that involved high-dose chemotherapy and bone marrow transplantation," said Thiele. The patients were randomized to receive retinoic acid after the intensive treatments and the study showed that the kids who received retinoic acid had a longer event-free survival. As a result, retinoic acid is now part of the standard of care for patients with high-risk neuroblastoma. "It's invigorating and motivating for basic scientists when they can actually see how their research can contribute to the development of a clinical trial."

Continuing with their studies, Thiele and her colleagues found a subtlety in the effects of retinoic acid. They realized that at concentrations normally found in the body (much lower than the pharmacological doses), retinoic acid was turning on another receptor on the neuroblastoma cell surface—TrkB—which, in normal neurons, is a receptor for brain-derived neurotrophic factor (BDNF). Furthermore, researchers discovered that TrkB and BDNF were expressed in neuroblastoma tumors that had poor prognoses.

BDNF is a survival factor for neurons and when they are physically or chemically disrupted, neurons respond by turning on the TrkB receptor system. "We hypothesized that the expression of TrkB may be how the neuroblastoma fights back against chemotherapy and develops drug resistance," said Thiele.

In 1996, Thiele and her colleagues first showed that BDNF and TrkB could affect the way the cells process cytotoxic drugs—the common chemotherapeutic agents vincristine and vinblastine. They then showed that if a neuroblastoma cell line is incubated with progressively higher levels of a cytotoxic drug, TrkB levels stayed the same but the cellular expression of BDNF increased as the cells became more drug resistant.

The team also took cells that had either low or high expression of TrkB and incubated them with different concentrations of BDNF and then chemotherapy. They found

that high levels of TrkB and low concentrations of BDNF had the same effect as low levels of TrkB and high concentrations of BDNF. In either case, drug resistance increased.

"So we knew that the TrkB receptor was attenuating the effects of chemotherapy in our cell cultures," said Thiele. "The question was how."

And the answer was the AKT signaling pathway, a major common denominator for survival factors like BDNF. Thiele and her Research Fellow Zhijie Li, M.D., adopted a strategy to restore chemosensitivity by targeting AKT with drugs. In a recent paper in the *Journal of the National Cancer Institute*, Thiele's team has shown that neuroblastoma cells are very sensitive to an AKT inhibitor alone. But they now have data demonstrating that the combination of AKT inhibition and a standard chemotherapeutic agent is highly synergistic.

"We're also excited by a recent phase I clinical trial of the AKT inhibitor perifosine in pediatric cancers," added Thiele. Among the patients tested, there were four responders, which included two of the three neuroblastoma cases included in the trial. "So our hope is this kind of strategy—high-dose chemotherapy up front integrated with an AKT inhibitor—will get more kids into a complete response. We know that the complete responders have a much lower chance of relapsing over time."

Thiele notes that they deliberately chose not to work on the translation

> Our hope is this kind of strategy... will get more kids into a complete response.

(Photo: R. Baer)

Zhijie Li, M.D., and Carol Thiele, Ph.D.

of TrkB inhibitors into the clinic. "In studying the biology of neuroblastoma, I realized there were probably several growth factors that could have a similar effect on chemosensitivity. So instead of a therapy that combines multiple specific targets, I felt that the best bet was to go downstream to a common survival signaling node like AKT." This approach, Thiele hopes, will bypass any relapse due to alterations in a related survival factor.

Hunting for Tumor Suppressors

Alfred Knudson, M.D., Ph.D., is credited with the development of the hypothesis that somatic loss of both alleles of a gene could lead to cancer. The now-famous two-hit hypothesis later merged with the concept of tumor suppressor genes when it became clear that the development of retinoblastoma was associated with mutations in both alleles of the retinoblastoma gene *RB*. "Less well known," said Thiele, "is that neuroblastoma is the second cancer for which Knudson postulated a tumor suppressor."

"One of the genetic alterations in neuroblastoma cells is the loss of chromosomal region 1p, and the kids with that signature have a very poor prognosis," explained Thiele. Garrett Brodeur, M.D., postulated that this loss of 1p could be associated with the loss of a tumor suppressor gene. "So people have been looking for *the* neuroblastoma tumor suppressor gene for 25 years."

Two years ago, Brodeur published the identification of a candidate neuroblastoma tumor suppressor gene, *CHD5*, in the right region of chromosome 1. And while that may have seemed the long-awaited end of the search, Thiele and her colleagues have also identified an entirely different tumor suppressor candidate in that same vicinity.

"It was the kind of discovery that arises out of knowing people around the NIH campus who are doing interesting things," said Thiele. Her colleague, Beverly Mock, Ph.D., Deputy Chief of CCR's Laboratory of Cancer Biology and Genetics, was sequencing genes on chromosome 4 of the mouse in a region that is syntenic (corresponds in location) with human 1p when she came across a neural gene. "Bev asked if I would be interested in studying it," said Thiele. "Meanwhile, Ward Odenwald, Ph.D., at NINDS had studied the same gene in *Drosophila* and thought it was important from a neurodevelopmental perspective."

"What we knew from the fly," explained Odenwald, "was that the gene *Castor* is expressed during nervous system lineage development but only in a very narrow time window within the neural precursor cell." *Castor* is part of a dynamic temporally regulated network of genes that are markers for particular developmental programs.

(Photo: M.Li)

Zhihui Liu, Ph.D.

"We all got together and talked about cloning the human homolog of *Castor*," said Thiele. "I had a research fellow coming to the laboratory and I thought this would be a really easy project for him to get started on." But it took the work of two talented fellows, Xuezhong Yang, Ph.D., and Zhihui Liu, Ph.D., to actually perform the critical experiments to characterize *CASZ1*.

"At the time," remembered Liu, who had just arrived from China in 2004 to work in the Thiele laboratory, "cloning the gene was really hard." The Human Genome Project was in progress, but that particular region was not well annotated. Furthermore, it is a large gene and even once it was cloned, it was difficult to transiently transfect it into neuroblastoma cell lines to test its tumor suppressor activity. Liu persevered and eventually succeeded in cloning *CASZ1* and making stable neuroblastoma clones in which *CASZ1* gene expression could be induced.

"Now we think we have developed a very nice story," said Thiele. Her team has found that *CASZ1* is deleted in 98 percent of neuroblastoma tumor cells that have the 1p deletion. Their studies also show that transfecting *CASZ1* back into neuroblastoma cells reimposes growth control and induces differentiation of these cells. More recently, they have shown that the remaining copy of *CASZ1* in neuroblastoma cells undergoes epigenetic remodeling to suppress its expression.

Working with neuroblastoma tumor samples from the Children's Oncology Group, they have also seen a correlation between the levels of *CASZ1* expression and disease prognosis. High *CASZ1* expression is associated with a very good prognosis and with differentiated cells when examined histologically, consistent with its putative role as a tumor suppressor.

Liu is currently writing up several papers for publication describing what they have learned so far about *CASZ1* and their plans to continue working on this gene.

FEATURE

> Nobody has really studied this gene in the mammalian system.

They are in the process of generating genetically engineered mice with *CASZ1* deletions. "There are so many things to do," he explained. "Nobody has really studied this gene in the mammalian system."

"*CASZ1* must be playing some basic fundamental role in vertebrate nervous system development," added Odenwald. "In all the vertebrates we have examined, there are remarkable pockets of sequence conservation—both in the DNA binding domain and outside the open reading frames."

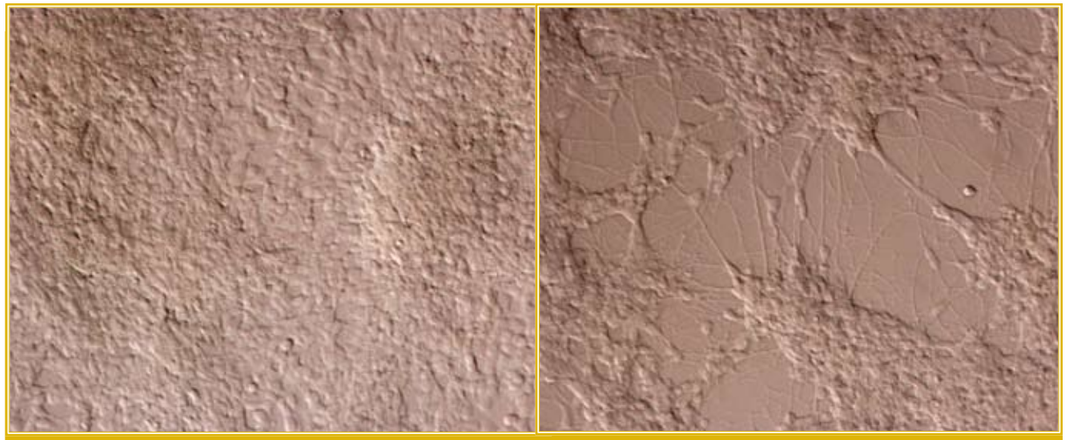
"It is a fun project because we can bring a lot of NCI resources to bear on studying this completely novel human gene," said Thiele. "We have worked with the CCR's Office of Science and Technology Partnerships to develop antibodies against CASZ1 and with the NCI Core Facilities in Frederick to identify CASZ1 interacting proteins." It also turns out that there are *CASZ1* mutations in the germline, so Thiele is working with her colleague in the Pediatric Oncology Branch, Javed Khan, M.D., Head of the Oncogenomics Section, and Stephen J. Chanock, M.D., Chief of the Laboratory of Translational Genomics in NCI's Division of Cancer Epidemiology and Genetics, to determine the frequency of the polymorphisms and functionally analyze them.

Putting It All Together

Intriguingly, retinoic acid turns on *CASZ1* expression in neuroblastoma cells. But researchers are still a long way from understanding how retinoic acid differentiates these cells.

"Our biggest limitation," noted Thiele "is not having access to good

(Image: C. Thiele)



Induction of *CASZ1* gene expression in neuroblastoma cell lines *(right panel)* arrests tumor cell growth and induces differentiation.

> There is an interaction with development and neuroblastoma that we don't quite understand.

primary tumor tissue because of the rarity of the disease. I value *in vitro* models, but we need to develop more physiologically relevant *in vitro* models. That would also help with drug discovery." She also noted that although there is one transgenic mouse model of neuroblastoma, it is specific to one particular subtype of the disease.

In collaboration with her colleague Chand Khanna, Ph.D., Thiele and a Clinical Fellow, Amy McKee, M.D., were able to develop an orthotopic model of the disease by placing neuroblastoma cells under the adrenal fat pad in mice. "Amy was able to put as few as five cells into a fat pad and recapitulate the tumor. So these cells are highly tumorigenic."

On the other hand, there is intriguing epidemiological data to suggest that many potential neuroblastomas resolve themselves during the course of development. When doctors have autopsied the adrenal glands of children for other reasons, they find wrests of neuroblasts that look for all intents and purposes like neuroblastoma. The incidence is about one in 500 children. But the incidence of neuroblastoma itself is only one in 500,000, suggesting that the body is mostly capable of regulating developing cells that go awry. Furthermore, in children under one year of age, very dramatic cases of neuroblastoma appear and then spontaneously regress. As long as there are no bone metastases, it seems that the skin lesions are able to resolve on their own.

"There is an interaction with development and neuroblastoma that we don't quite understand," said Thiele. She cited additional evidence from a screening procedure instituted in Japan. "Neuroblastoma was one of the first tumors to be screened," Thiele explained. It was discovered that in children with neuroblastoma, catecholamines were secreted in the urine. As a result, the Japanese were screening every infant for the disease. "The idea was to catch the disease as early as possible," said Thiele. However, these very early cases would simply go away and there was no decrease in the incidence of late-stage disease, suggesting that they were seeing two distinct diseases over time.

"For me it is really fun to see how many people have gotten into this field," said Thiele. "When I first entered the field, the neuroblastoma scientific meetings included maybe 30 people. Now, at this year's meeting on Advances in Neuroblastoma Research in Stockholm, there were over 500 people." Among the attendees was Nobel Laureate Elizabeth Blackburn, Ph.D. "She really learned about neuroblastoma. I welcome great minds getting really interested in unraveling the enigmatic biology of neuroblastoma. I know this will be key for us to develop more effective and less toxic therapies."

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

The Interconnectedness of All Things

Approximately ten years ago, Sheue-Yann Cheng, Ph.D., Head of the Gene Regulation Section in CCR's Laboratory of Molecular Biology, teamed with an unusual lab partner—a mutant mouse. She made this mouse to study a rare inherited disease, resistance to thyroid hormone (RTH), caused by a mutation in one of two thyroid hormone receptor genes. RTH had been recognized for many years as a paradoxical deficit in thyroid hormone signaling that is seen despite elevated levels of thyroid hormone itself. But the disorder was only relatively recently traced to a receptor mutation that prevents hormone binding and the resulting transcriptional regulation. The mice she used to study the effects of this mutation turned out to be an important window into multiple physiological systems, including cancer.

Normally, laboratory mice live quite happily for 18 to 24 months, and yet Cheng's mice were starting to die at six months. "Our mice were dying!" exclaimed Cheng, "so we did autopsies." The Cheng team discovered that these mice had massively enlarged thyroid carcinomas.

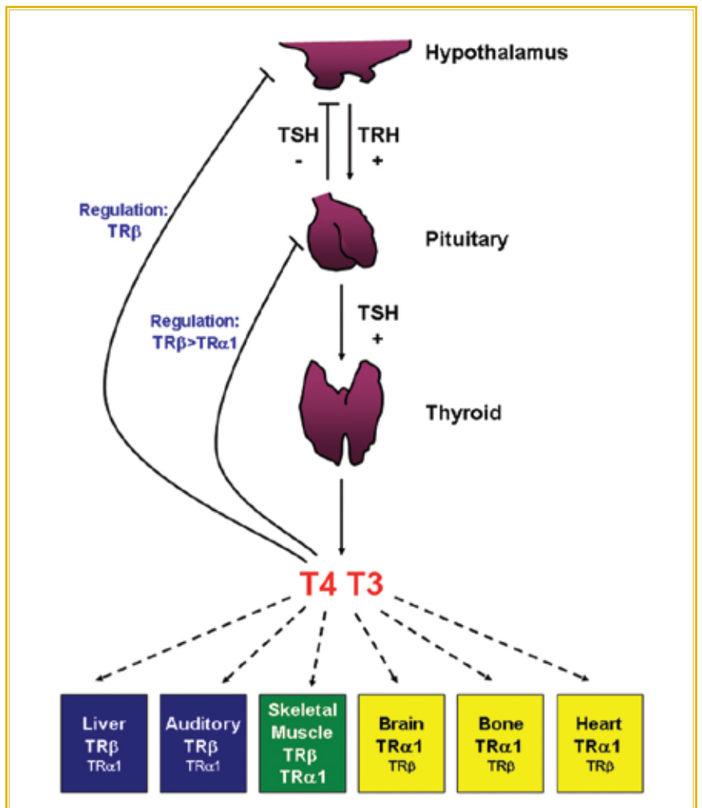
Cheng immediately jumped on the opportunity to study what proved to be a model of follicular thyroid cancer. Thyroid cancers are one of the few cancers with a rising incidence around the world, particularly in women. Follicular thyroid cancer accounts for about 15 percent of the total thyroid cancer disease burden but has a poorer prognosis as compared to the dominant papillary variety. "Cancer is such a devastating disease. I felt that since I had been in the thyroid hormone field for so long, perhaps I could make a unique contribution."

> Thyroid cancers are one of the few cancers with a rising incidence around the world.

Spontaneous Tumor Generation

Thyroid hormones operate in a tightly controlled feedback loop involving the hypothalamus, pituitary, and thyroid glands. Activation of normal thyroid hormone receptors encoded by one of two genes—*THRA* or *THRB*—results in the downregulation of thyroid-stimulating hormone (TSH). TSH, as its name implies, encourages the growth and activation of cells in the thyroid gland.

The PV mutation that Cheng studies was derived from an RTH patient and encodes a mutation that shifts the translation of DNA by a single base pair near one end of *THRB*. This frameshift mutation results in a complete loss of binding of thyroid hormone receptor β to the thyroid hormone T3. In addition, the PV mutation acts in a dominant negative fashion, suppressing the function of the remaining normal TRβ receptor. Mice bearing this mutation, like people with RTH, have growth retardation and other hallmarks of reduced thyroid hormone signaling. RTH patients typically only have one mutated copy of the *THRB* gene; there has only been one report of a patient who had mutations in both copies. But homozygous mice bearing two copies of the PV mutation develop thyroid cancer.



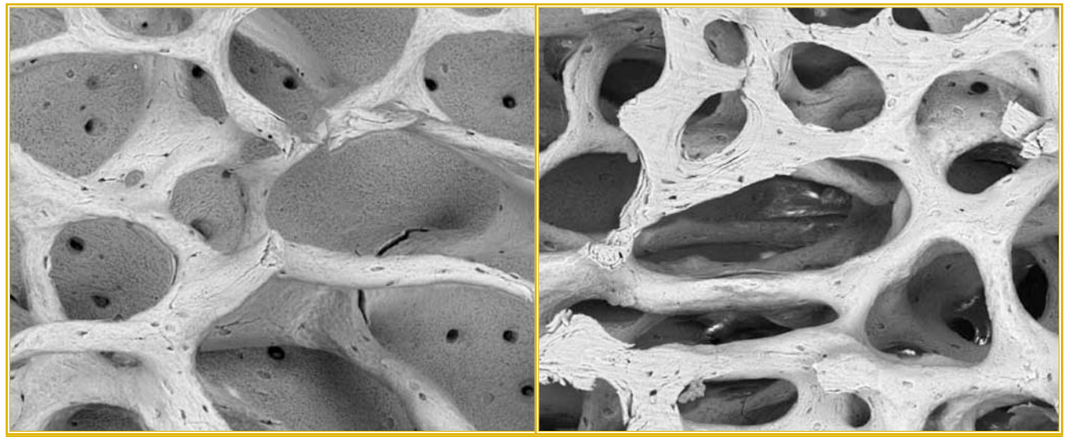
The hypothalamic-pituitary-thyroid (HPT) axis consists of a complex signaling network that regulates multiple organ systems. TSH = thyroid-stimulating hormone; TRH = thyrotropin-releasing hormone; T3, T4 = thyroid hormones; TR = thyroid hormone receptor. Image adapted from O'Shea et al., *Nuclear Receptor Signaling* (2006) 4, e011.

"When we developed this mouse model, there wasn't any other spontaneous mouse model of metastatic thyroid cancer," said Cheng. "As they got older, they just developed cancers. Eventually 100 percent of these mice develop thyroid cancer."

Several studies later, Cheng and her colleagues have characterized the progression and molecular changes associated with their mouse model of thyroid cancer. The cellular progression of the disease resembles the human situation. "We have studied so many of these mice," said Celine Guigon, Ph.D., a Postdoctoral Fellow in Cheng's laboratory. "And they all develop goiter around two months of age and then go on to develop cancer." Remarkably, even metastatic progression is observed reliably in these animals. "This mouse has a similar frequency of metastases to that seen in humans—around 25 to 30 percent for follicular carcinoma," said Cheng.

They have also found very strong correlations between the additional mutations observed in human follicular thyroid carcinoma and those seen in their PV mice. For example, they have established that the tumor suppressor PPARγ has reduced expression and activity in their mice and, intriguingly, that administration of a PPARγ agonist, rosiglitazone, blocked the development of metastasis.

Having validated their model as recapitulating many of the hallmarks of follicular thyroid carcinoma, Cheng and her colleagues hope to use the power of mouse genetics and molecular biology to gain novel insights into the disease. For example, their work has already shed light on the controversial role of TSH in these cancers. "Some patients who have elevated TSH have a high incidence of thyroid cancer; however, some patients with aggressive cancer express lower levels of TSH receptor," explained Cheng.



Scanning electron micrographs (SEM) of trabecular bone architecture in normal mice *(left panel)* and mice with mutations in the thyroid hormone receptor TRα. Image adapted from Bassett et al., *Scanning*, (Author manuscript; available in PMC 2009 July 6).

Cheng's team has found that in mice that are homozygous for the PV mutation, TSH levels are elevated about 200 times above levels in mice with only a single copy of the mutation. "TSH stimulates the proliferation of thyrocytes," said Cheng. "Together with the thyroid hormone receptor β mutation, these two altered signals stimulate cancer." Cheng and her colleagues have shown that elevated levels of TSH alone do not cause cancer, nor does the dominant negative action of the PV receptor mutation alone. Both are required. "As you know, cancer is a multigenetic disease," said Cheng. Her goal is to dissect the multiple molecular interactions critical to thyroid cancer formation and progression and bring the findings back to the clinic.

Fat and Bones

"Why do we need two thyroid hormone receptor genes?" asked Cheng. *THRA* and *THRB* encode TRα and TRβ receptors, respectively. TRα and TRβ are known to have different distributions in the body, and mice lacking the genes for each of these receptor subtypes have distinct functional deficits. But no case of RTH had ever been reported to be the result of a *THRA* mutation. "So we decided to target the PV mutation to the *THRA* gene and see what happens in the mouse," explained Cheng.

The lab is now studying lipid metabolism in these mice conferred by either *THRA* or *THRB* mutations; they are finding differences in regulation of both white adipocytes and lipid content of the liver. "We are focusing on lipid metabolism not only because our mice have distinct phenotypes, but because it is very important to know how thyroid hormone regulates lipid metabolism," said Cheng. Drug companies are interested in developing thyroid hormone analogs to accomplish therapeutic goals like lowering cholesterol. However, because of its pleiotropic actions on different receptor subtypes, a simple thyroid hormone analog would have too many side effects. "So there is a drive to devise analogs that are TR-subtype specific." Cheng hopes that her work will shed light on the potential effects of such specific analogs.

Meanwhile, Cheng has several collaborators who are using her mice to study diverse topics. "I cannot study everything in these mice," said Cheng. "They have a phenotype of interest to lots of investigators."

Graham Williams, Ph.D., Professor of Endocrinology at Imperial College in London, has worked with Cheng for several years.

"We had identified TRα as the major thyroid hormone receptor expressed in bone, and I developed an interest in *in vivo* models to investigate the molecular and physiological mechanisms of thyroid hormone action in bone," said Williams. "After seeing the early phenotype descriptions of the PV mutants, I was sure they would be very informative to our understanding of the skeleton. So, at one of the American Thyroid Association meetings, I introduced myself to Dr. Cheng and suggested that we work together on analyzing the skeletal phenotypes."

The differences in skeletal development between the two mice are marked—mice with PV mutations in the α receptor have retarded bone development characteristic of thyroid hormone deficit (hypothyroidism) whereas those with PV mutations in the β receptor develop an osteoporosis that is characteristic of elevated thyroid hormone (thyrotoxicosis). By comparing these mice, the investigators are able to tease apart the molecular mechanisms that regulate the skeletal response to thyroid hormone.

"Dr. Cheng and I have discussed and designed experiments, and members of her laboratory have provided bone samples from appropriate groups of mice to our laboratory so that we can perform the skeletal analyses," said Williams, describing the closeness of their collaboration.

In addition, their work together has resulted in some unique training opportunities. "One of my graduate students, Patrick O'Shea, was able to spend two years as a postdoctoral fellow in Dr. Cheng's lab at the NCI before returning to my lab in London for the third year through a European Union fellowship obtained as a direct result of our collaborations. His work with Dr. Cheng complemented his prior pharmacology background to enable him to secure an independent scientific career."

> It is clinical and epidemiological data that no one understands.

Gender Matters

When not in the operating theater, Electron Kebebew, M.D., Head of the Endocrine Oncology Section in CCR's Surgery Branch, has been trying to understand why the incidence of thyroid cancer is about four times as great in women as in men. He also wants to know why, paradoxically, men tend to have a more aggressive disease—typically presenting with metastases in the lymph node or lung at diagnosis—with higher rates of recurrence and reduced overall survival. "It is clinical and epidemiological data that no one understands," said Kebebew.

Given the gender disparity, some investigators have suggested that sex hormones may play a role in thyroid cancer. Studies in cell culture models have shown that estrogen administration leads to higher rates of growth in thyroid cancer cells. Kebebew's group has also done genomic studies in human thyroid cancer samples to demonstrate gene expression differences in papillary thyroid cancer in women as compared to men. Kebebew is collaborating with Cheng to see if they can study gender differences in her mouse models.

Guigon, Cheng, and their colleagues have "improved" upon their original model of follicular thyroid cancer by combining the homozygous PV mutation with a mutation in one copy of the *PTEN* gene. *PTEN* mutations are also observed in human follicular thyroid carcinomas and the addition of this mutation to the PV mutations appears to accelerate the formation and progression of tumors. In addition, there is a gender difference in the manifestation of thyroid cancer in these mice, for example in the rate of metastasis.

"In conjunction with some *in vitro* experiments, we're using Cheng's model to see if sex hormones influence the rate at which the mice get follicular thyroid cancers, as well as their aggressiveness," said Kebebew. In order to study these questions, Kebebew's group is taking the mice at an early age and replacing the ovaries or testes with estrogen or testosterone implants, respectively. Half the mice receive sham implants without sex hormones so that the researchers also can compare the development of cancer with and without sex hormone. They will also study the gene expression differences in the mouse tumors and compare them with profiles of human disease.

"Other transgenic mice exist that develop thyroid cancer, but in none of those studies have people noticed a difference in gender. Hers is the first and only model that I am aware of in which gender differences have been reported, which made it a natural model for us to use," explained Kebebew.

Back to Cancer

"Later on, we looked back in the literature and found there are correlative studies to indicate that abnormal expression and somatic mutations of the *THRB* gene are found in human cancers," said Cheng, describing the evolution of her entry into the field of cancer research after generating the first PV mutant mice. "We realized that the thyroid hormone receptor β could be a tumor suppressor."

(*Photo:* R. Baer)

Sheue-Yann Cheng, Ph.D.

There and Back Again

(*Photo:* S. O'Neal)

Celine Guigon, Ph.D.

Celine Guigon, Ph.D., is Dr. Sheue-Yann Cheng's most senior Research Fellow, whose five-year tenure in the Gene Regulation Section has almost ended. She will be returning to France to begin independent research on the role of estrogen receptors in ovarian cancer at the University of Paris, Diderot.

"During my doctoral work, I was interested in female fertility. So I studied ovarian physiology and pathology. And I was curious about the role of estrogens in ovarian cancer. First, however, I felt it would be important to gain some broader experience," said Guigon. Guigon found Cheng's advertisement for a research fellow on the NIH Web site and realized that the opportunity was a perfect match with her interests.

> You never know where life—or science—will take you.

Guigon is testing that hypothesis by looking at the propensity of PV mutant mice to develop mammary cancers. "We knew that *PTEN*-deficient female mice are a good model for mammary gland tumors," said Guigon. They also found that the homozygous PV mutation alone could enhance abnormal growth (hyperplasia) of mammary glands, although mammary gland tumors were only found in about eight percent of mice homozygous for the PV mutation. Hypothesizing that the homozygous PV mice were dying before they could develop mammary tumors, Guigon added a single *PTEN* mutation to heterozygous PV mice, which normally do not develop tumors or die prematurely.

"With the two mutations together, the frequency of mammary gland tumors is much greater than with the *PTEN* mutation alone," said Guigon. Their evidence to date suggests that the two mutations interact through critical intracellular signaling pathways. They are currently working to elucidate those mechanisms and further establish the role of the thyroid hormone receptor β in tumor suppression.

Serendipity in Science

"I came to the NIH because I was following my husband, who was recruited from Chicago to work for the Nuclear Regulatory Commission in Washington, DC," recalled Cheng. "But I was very excited by the opportunity." Cheng transitioned from working on estrogen receptors at the University of Chicago to working on thyroid hormones at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

"I became a Principal Investigator at the NCI in 1979," said Cheng, "But at the time, I worked more on hormone actions than cancer research. I thought I would be studying thyroid hormones, and now I am studying cancer.

"You never know where life—or science—will take you," added Cheng. "But you've got to embrace it. In science, you can't proscribe what people do; they need to have a passion for it. Research is not that easy—it is not nine-to-five work. The NIH is a great place for people who have a passion for science, who are interested in what they are doing, who love what they are doing. A lot of times, I just can't wait to come to work. Every morning, I wonder what I'm going to hear from my fellows or what I'm going to discover."

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

Guigon created mutant mice that combined the PV mutation of *THRB* with a *PTEN* mutation, to study their synergistic action in the development of multiple cancer types."Meanwhile, I was involved in about five different projects," said Guigon. With nine coauthored publications to date from the Cheng laboratory, Guigon has no shortage of evidence to back up her claims to productivity. She is also the proud mother of a two-year-old son.

Returning to France, Guigon will be able to establish an independent research agenda without having to build her own laboratory. "Now, I want to use the background of my doctoral and postdoctoral work to develop a new direction for my research. I applied for several grants this year and was funded to begin some studies of my own."

Expanding Clinical Impact

For 14 years, Patrick Hwu, M.D., worked in the Tumor Immunology Section of CCR's Surgery Branch developing novel immunological approaches for the treatment of cancer. When he was recruited by the University of Texas M.D. Anderson Cancer Center to lead a new Department of Melanoma Medical Oncology, he saw it as a golden opportunity to bring the therapeutic advances he and his colleagues had made into a brand new clinical setting. Seven years later, Hwu is seeking funding to begin a multicenter randomized clinical trial that he hopes will be pivotal in introducing tumor infiltrating lymphocyte (TIL) therapies as a new standard of care for the treatment of melanoma.

An Immunologist Finds Melanoma

I am now the head of a large melanoma department, but my first interest was immunology. One of the early successes of cancer immunology was the discovery that interleukin-2 (IL-2)—a soluble signaling molecule important for the proliferation of T cells and other lymphocytes that carry out the immune response—had a therapeutic effect in melanoma and kidney cancer patients. So melanoma was an obvious place to start to develop the principles of immunotherapy.

Working with Steven Rosenberg, M.D., Ph.D., at CCR, we developed methods to treat patients with T lymphocytes grown from their own tumors (TILs) and eventually achieved a 50 percent response rate. In a subset of patients, the cancer was effectively cured. The therapeutic potential was clear, but the combination of expertise and infrastructure required for its implementation made it difficult for extramural researchers to easily pick up the gauntlet we had thrown down to develop similar clinical research programs. So I accepted the self-imposed challenge to take this technology out to other centers in an attempt to make an impact across the country.

> It was a wonderful fit for me to bridge these two strong programs.

(*Photo: B. Smith*)

Patrick Hwu, M.D., and members of the Department of Melanoma Medical Oncology at the University of Texas M.D. Anderson Cancer Center.

Building a Team

In 2003, M.D. Anderson had just started expanding its basic immunology department and had built a new four-story building with a vision of bringing in translational researchers like myself. In addition, there was a very large clinical program in the melanoma department, but it lacked a matching laboratory research component. It was a wonderful fit for me to bridge these two strong programs. The resulting Melanoma Medical Oncology Department now has a full team of investigators including faculty members devoted to laboratory research, those focused on the clinic, and a few physician-scientists, like me, that straddle both worlds.

The physician-scientist perspective is really critical, in my opinion, for developing a translational program like ours in which you bring insights from the clinic back into the laboratory. Physician-scientists provide the glue to hold the pieces of such a program together and make connections to accelerate translation. For example, we have been able to build a program where we generate T lymphocytes for patient therapy, administer and monitor the therapy through biopsies and unique assays to determine who does and doesn't respond. If I didn't know both the "bench" and "bedside," I wouldn't have been able to organize this program effectively.

Yet, as Chair of a department with trainees at all levels, I realize how extremely challenging it is to train physician-scientists. We are

> We are really asking people to learn two totally different fields.

really asking people to learn two totally different fields. We are asking people who have already gone through medical school and done their fellowships, "Are you ready to be an intern again?" I'm not sure people realize what a gem CCR really is for training future physician-scientists.

What I appreciate most, especially now that I am no longer there, is that at CCR, there are no pressures to see a lot of patients or get grants. You can focus on the patients that are a part of your research. Investigators are free to perform science without having to worry about many other issues that might fill the day. CCR offers vital protected time for the physician-scientist to mature.

Improving Standard of Care

At M.D. Anderson, we have now treated 30 melanoma patients with TIL therapy and have achieved a 50 percent response rate—identical to what we observed at the NCI. Now we are talking with other centers about doing a multicenter randomized clinical trial in which we can assess the impact of combining TIL with IL-2 therapy, as compared to IL-2 alone. If we can get that trial funded and performed successfully, the cancer treatment we pioneered at CCR will change the standard of care.

Of course, that's not the end of the story. Half the patients we treat don't respond to TIL therapy, and we need to improve those odds. We are also pursuing another line of investigation that we hope to bring to human trials soon. Melanomas produce chemical signals—chemokines—that T lymphocytes don't normally recognize. We are trying to engineer TILs with a receptor that recognizes these chemokines so they can follow the signal to the tumor source. If successful, this is a principle that can be generalized to many other kinds of tumors.

Our chemokine work actually also began at CCR. At the time, I didn't know much about chemokines, but Philip Murphy, M.D., one of the world's experts in chemokines, was just an elevator ride away. All that preliminary data we generated at CCR got me the initial NIH grant to continue this work at M.D. Anderson. Now we're moving it into people.

We do a lot of fantastic clinical trials at M.D. Anderson, but having been "on the outside" for a few years, I do miss the ease of having the NIH's massive Clinical Research Center on my doorstep, where I didn't have to worry about things like insurance approval before putting patients on a protocol or giving them an x-ray. It truly facilitates clinical research. The Center is a major opportunity for the intramural scientists, but it could also be as valuable to extramural scientists who don't have a chance to translate their ideas to the clinic. Think of a sabbatical program in which the Center could host people to develop their idea, start a trial, and spend a couple of years running that trial.

I think there are a lot of opportunities for intramural/extramural collaboration that should be encouraged. For instance, because they don't have a high patient volume, the NIH Clinical Research Center can find it difficult to recruit patients. That is definitely not a problem for places like M.D. Anderson, where patients are seen regardless of the need to fit into a research protocol upfront. So some additional integration might be the best of both worlds.

He Always Responds

A majority of children with acute lymphoblastic leukemia are cured. But for the rest, the treatment journey can be long and uncertain.

(Photo: M. Spencer)

Avery Lachapelle and Alan Wayne, M.D.

> I am forever grateful that we live in a world where this kind of research is possible.

Avery Lachapelle is 11 years old. He is an average student, he hangs out with his cousins, he enjoys playing video games, and he hasn't yet decided what he wants to be when he grows up. His mother, Trish Daly, describes him as an amazingly resilient child. The truth of that description becomes apparent upon learning that Avery was diagnosed with leukemia at the age of 14 months and has been in and out of hospitals to combat its recurrence ever since.

"Our family doctor knew right away," Trish said of the initial diagnosis. "They sent us for blood work on a Thursday; Friday was the bone marrow biopsy. Saturday, they started chemo." Although the idea of cancer was terrifying, Trish will never forget the doctor saying, "Oh, it's not as bad as you think." Acute lymphoblastic leukemia (ALL) is considered the good cancer.

And in fact, ALL is routinely trotted out as a success story in the war on cancer—a disease that was nearly universally fatal in the 1960s now has a cure rate of over 80 percent. But for those children who are not in that fortunate majority, the future is much less bright.

"He had two years of standard chemotherapy after the diagnosis," recalled Trish, "and then relapsed a year later." After the relapse, Avery had a bone marrow transplant in March 2004. "The doctors were shocked when it came back in November 2007," added Trish, explaining that there is only a five percent chance that ALL will recur two years after a transplant.

> A disease that was nearly universally fatal in the 1960s now has a cure rate of over 80 percent.

Since then, Avery has had a second (blood stem cell) transplant and an experimental therapy (rituximab). "That's the thing about Avery," said his mother. "He always responds to therapy, but for some reason, the cancer keeps coming back." When the cancer again showed signs of recurrence in February 2010, his doctors in Canada had reached the limit of their therapeutic options.

"Our doctors are wonderful at home," said Trish, "They did some research and found out about this place." Avery was the ninth child enrolled in a clinical trial run by Alan Wayne, M.D., at the NIH Clinical Center to test a second-generation investigational immunotoxin (HA22 or moxetumomab pasudotox) developed by Ira Pastan, M.D., Chief of CCR's Laboratory of Molecular Biology.

"It did wonders for him," said Trish. But once again the cancer showed signs of returning, so his NCI care team decided to enroll Avery in a new trial of a cancer vaccine approach to ALL that Dr. Wayne and colleagues have developed at the NIH. Avery's mother is not thinking about the long-term prognosis. "I just do one day at a time," she said "Today is a good day; let's focus on that."

Trish stressed that despite the years devoted to Avery's cancer and the unexpected life changes it has wrought, she considers herself and her son lucky that "all these new therapies are available." She thanks the Canadian healthcare system and places like the NIH Clinical Center. "I am forever grateful that we live in a world where this kind of research is possible."

Dr. Wayne's Clinical Trials

A Pilot Trial of WT1 Peptide-Loaded Allogeneic Dendritic Cell Vaccine and Donor Lymphocyte Infusion for WT1-Expressing Hematologic Malignancies [NCI-08-C-0051]

Hematologic Malignancy Biology Study [NCI-04-C-0102]

A Phase I, Multi-Center, Dose Escalation Study of CAT-8015 in Pediatric Patients With Refractory CD22+ Acute Lymphoblastic Leukemia (ALL) or Non-Hodgkin's Lymphoma (NHL) [NCI-08-C-0123]

Phase II Study of UCN-01 in Relapsed or Refractory Systemic Anaplastic Large Cell and Mature T-Cell Lymphomas [NCI-04-C-0173]

Phase I Trial to Evaluate the Safety, Activity and Pharmacokinetics of Marqibo® (Vincristine Sulfate Liposomes Injection) in Children and Adolescents With Refractory Cancer [NCI-10-C-0220]

Short-Course EPOCH-Rituximab in Untreated CD-20+ HIV-Associated Lymphomas [NCI-01-C-0030]

To learn more about Dr. Wayne's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?profileid=5603.

Childhood Cancers
in Translation

Despite the fact that approximately 80 percent of children with acute lymphoblastic leukemia (ALL) are cured with current chemotherapy regimens, ALL remains a leading cause of death from cancer in childhood. Furthermore, side effects of standard therapies have lifelong consequences for many survivors of childhood ALL. Alan Wayne, M.D., Head of the Hematologic Diseases Section of CCR's Pediatric Oncology Branch, works with a team of investigators to develop novel targeted approaches to treat ALL and other childhood leukemias and lymphomas in hopes that these new therapies will prove more potent and less toxic than those currently available.

When asked how he got his start in leukemia research, Alan Wayne explains that early in childhood he was given a microscope along with glass slides of cases of acute lymphoblastic leukemia (ALL) from his father, an internist and hematologist. A National Science Foundation-sponsored high school summer internship at the Roswell Park Memorial Institute with the late Jun Minowada, M.D., who developed many of the modern leukemia cell lines, further steered his interests toward blood cancers. Years later, he worked as a resident, fellow, and then faculty member at the Children's Hospital and Dana-Farber Cancer Institute in Boston where the late Sidney Farber, M.D., achieved the first remissions in childhood ALL.

Decades after those formative experiences, Wayne serves as the Clinical Director of the CCR's Pediatric Oncology Branch. He directs a clinical research program focused on ALL, including two ongoing clinical trials for relapsed ALL that were developed as a result of translational research conducted by CCR investigators.

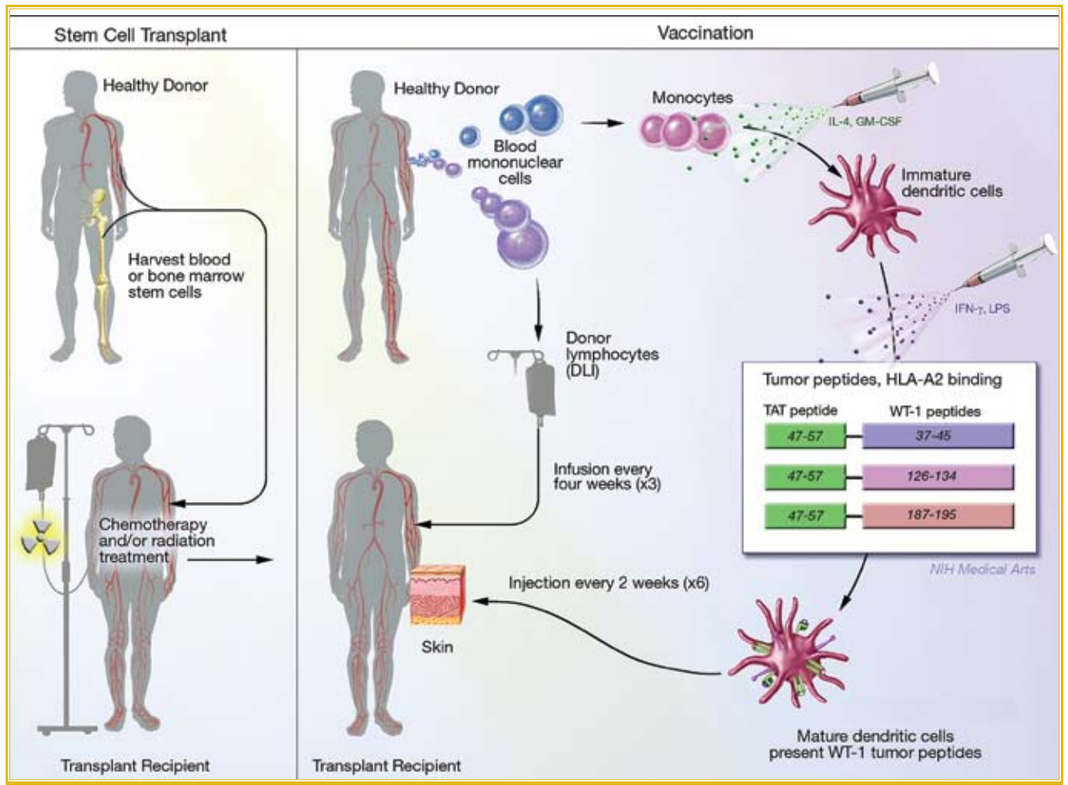
(*Photo: iStockphoto.com*)

In leukemia, abnormal white blood cells proliferate and circulate.

A Targeted Toxin

Moxetumomab pasudotox, also known as CAT-8015 or HA22, is the product of work undertaken in the laboratory of Ira Pastan, M.D., Chief of the Laboratory of Molecular Biology at CCR. [see "A Better Immunotoxin," *CCR connections* Vol. 2 No. 1]. It is a recombinant immunotoxin—an engineered drug made up of two components. One component is an antibody-derived molecule that binds to a protein called CD22 on the surface of cells. The other component is a potent bacterial toxin known as pseudomonas exotoxin A that causes cell death by inhibiting protein synthesis.

Moxetumomab pasudotox is a second generation anti-CD22 immunotoxin that was derived from a predecessor agent, CAT-3888 or BL22. MedImmune, LLC is sponsoring a multicenter phase I clinical trial of moxetumomab pasudotox that is being conducted at the NIH, the Dana-Farber Cancer Institute/ Children's Hospital Boston, and St. Jude Children's Research Hospital.



(*Image: NIH Medical Arts*)

A standard approach in the treatment of many blood-based cancers that have relapsed after transplantation is to transfuse lymphocytes (a type of white blood cell) from the transplant donor. In the WT-1 immunotherapy trial, white blood cells called mononuclear cells are also isolated from the donor. These cells are matured in the laboratory into immune cells called dendritic cells, and then exposed to protein molecules that are found primarily in cancer cells. The dendritic cells will take up the tumor peptides, process them, and display them on the cell surface, where they will be "visible" to other immune cells. Then, the dendritic cells are injected into the patient, where it is hoped they will stimulate an immune response that selectively targets and kills cells that have the target molecules.

A Fortified Immunotherapy

Another ongoing clinical trial that includes children with relapsed ALL is a vaccine-based approach that is designed to boost an anticancer immune response after stem cell transplantation. A standard approach in the treatment of many blood-based cancers that have relapsed after transplantation is to transfuse lymphocytes (a type of white blood cell) from the transplant donor. Such donor lymphocyte infusions (DLI) are extremely effective in some forms of leukemia, but are only rarely useful in the treatment of relapsed ALL. This new trial represents an attempt to try to improve the efficacy of DLI for ALL and other blood cancers.

Relapsed ALL, like a number of other cancers, is often associated with the expression of a protein known as Wilms Tumor-1 or WT-1. The immune response includes a class of cells known as dendritic cells that process proteins and present them to other cells of the immune system. This directs immune effector cells to recognize and destroy target cells. In this trial, dendritic cells derived from the donor of a prior stem cell transplant are programmed to present WT-1. These cells are

(Photo: A. Wayne)

Research Nurses Sharon Mavroukakis *(left)* and Cindy Delbrook *(right)* display the documentation on WT-1 immunotherapy submitted to the Food and Drug Administration for the Investigational New Drug application.

then administered to the transplant recipient in an attempt to direct a DLI-associated immune response against ALL cells that express WT-1. The phase 1/2 WT-1 vaccine trial, which began in 2008, is open to children and adults from age 1 to 75.

Rare and Resistant

Children with relapsed ALL make up approximately 25 percent of the pediatric cancer patients that are currently participating in clinical trials conducted by the Pediatric Oncology Branch. But the Branch is pioneering multiple treatments for refractory childhood cancers.

Lee Helman, M.D., Head of the Molecular Oncology Section in CCR's Pediatric Oncology Branch, has recently completed an international phase 2 study of a human monoclonal antibody against IGFR1, a molecule expressed in several cancer types. The trial included more than 140 patients with Ewing's sarcoma (EWS), and although the overall response rate was very low, some patients had durable complete remissions.

"Compared to anything else available to treat refractory EWS, we haven't seen anything like it," reported Helman. "We have a 14-year-old girl who came to us last Christmas time who was told there was nothing left to do for her. Now she is in complete remission, coming to get this antibody every three weeks. The multiple pulmonary nodules we saw are just completely gone and she has regained her normal weight."

The challenge and frustration for Helman is that they don't yet understand why only certain patients responded so well. "We still don't know how to stratify them. And the frustration has been that the community really feels that this is worth pursuing but the companies that make these antibodies have seen much less activity in adult cancers and aren't so interested in EWS."

Helman notes that most patients that showed a response to the drug did relapse after a few months. However, the treatment involved only a single agent. As Crystal Mackall, M.D., Head of the Pediatric Oncology Branch observed, "We'd love to find a magic bullet that could be used as a single agent. But the ultimate success is going to come from a combination of approaches that together reduce the tumor burden and prevent recurrence."

> The [Pediatric Oncology] Branch is pioneering multiple treatments for refractory childhood cancers.

(Photo: E. Branson)

Crystal Mackall, M.D., working in the clinic.

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

Web Sites with More Information about CCR

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

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

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

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

Patient Information on Cancer and Clinical Trials

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

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

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

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

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

Additional Links

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

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

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

Correction

CCR connections Vol. 4 No. 1 page 29 photo caption should have read Ola Landgren, M.D., Ph.D., and Mary Ann Yancey, R.N., working in the clinic. Yancey is the lead research nurse for the Multiple Myeloma Section at CCR.

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