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.

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Rare, But Not Forgotten

Cancer is not a rare disease. In the United States, more than one in three individuals will be diagnosed with cancer over a lifetime. Advances in treatments—even cures—come from studying both the commonalities within patient populations and from our recognition of the molecular differences among cancers that seem similar upon initial observation. As we learn in "RAS Takes Center Stage," detailed basic research is required to understand and successfully treat even the most common cancer mutations. However, clinicians are beginning to successfully stratify and treat many common cancers, e.g. breast, lung, and skin cancers, based on the molecules that they express.

The same principle holds true for the less common cancers, but sheer paucity in numbers make it difficult for researchers to uncover the molecular principles that govern them. The NIH Clinical Center specializes in studying and treating rare diseases—cancers among them—because of our unique capabilities to bring in and treat a critical mass of patients with cancers that might otherwise be seen only sporadically in more regional facilities. In this issue of *CCR connections*, we highlight several examples of how this capacity is paying off for understudied cancers.

In "Dramatic Responses in a Rare Type of Sarcoma," Shivaani Kummar, M.D., and her colleagues in CCR and NCI have seen highly encouraging results in the treatment of alveolar soft part sarcoma, which is often misdiagnosed and treated (unsuccessfully) as a more common sarcoma. Based on the results they observed with cediranib, an inhibitor of vascular endothelial growth factor receptors (VEGFRs), in only 46 patients, a multicenter open-label phase 2 trial is testing the drug in a much larger patient population.

Diffuse large B-cell lymphoma (DLBCL) affects less than 1 in 10,000 and is typically studied as a uniform population, even though heterogeneous pathologies and treatment outcomes have been recognized for years. As we learn in "Radiating Good Health," through years of careful study of this patient population, Wyndham Wilson, M.D., and his team in CCR's Lymphoid Malignancies Branch formed and tested a therapeutic hypothesis for a subpopulation with primary mediastinal B-cell lymphoma that has thus far effectively cured greater than 90 percent of patients.

Meanwhile, research efforts led by CCR's Genetics Branch, have led to new insights into a rare subtype of gastrointestinal stromal tumors (GIST). In adults, these tumors harbor activating c-kit or PDGFR mutations and respond to kinase inhibitors targeting these receptors, but pediatric patient populations have proved more resistant to these therapies. Several years ago, CCR set up a pediatric GIST clinic to study and treat this rare subpopulation. In "The GIST of One Cancer: Two Distinct Molecular Diseases," we learn that Keith Killian, M.D., Ph.D., Paul Meltzer, M.D., Ph.D., and their colleagues have identified distinct epigenetic changes in the tumors

(Photo: R. Baer)

Lee J. Helman, M.D.

of these patients associated with succinate dehydrogenase mutations.

Bladder cancer might not be rare, but it has not drawn commensurate interest from researchers; no new therapies have been approved for bladder cancer in 25 years. CCR has recently set up a program to advance the treatment of bladder cancers, which is beginning to yield promising results. Andrea Apolo, M.D., and Piyush Agarwal, M.D., give a first account of this program in "Treating Bladder Cancer: From Primary Tumor to Metastasis." The story of patient Chris Hamilton that accompanies this article reflects the challenges and a sense of the possibilities for patients with this and other under-investigated cancers.

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

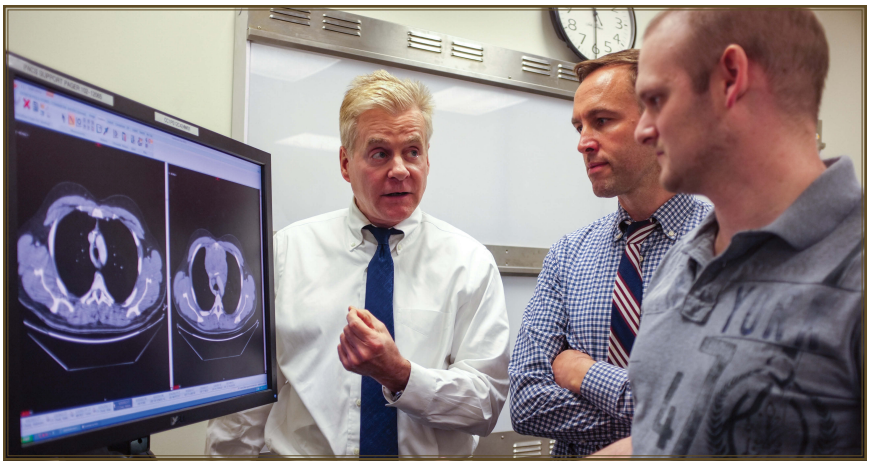
Radiating Good Health

A new study may eliminate the need for radiation in the treatment of a rare B-cell lymphoma.

Electromagnetic radiation caused Marie Curie's untimely death from cancer at the hands of her own Nobel Prize-winning research. Radiation is also, of course, a key part of the therapeutic arsenal against cancer, along with chemo-, immuno-, and targeted therapies. Curse or cure, radiation operates essentially through DNA damage. Cancerous cells are more vulnerable to treatment, but healthy cells also succumb, leading to known side effects. More recently, it has been recognized that therapeutic radiation can seed new tumors, even decades after its administration.

That is why a group of researchers led by Wyndham Wilson, M.D., Ph.D., and Kieron Dunleavy, M.D., of CCR's Lymphoid Malignancies Branch, has set out to do away with radiation in the treatment of primary mediastinal B-cell lymphoma. This rare subtype of B-cell lymphoma primarily affects people in their teens and twenties, making the potential long-term consequences of radiation exposure particularly concerning. The researchers felt that by focusing on this single subtype and taking advantage of newer, more targeted therapies, they could both improve outcomes and eliminate radiation from the treatment strategy.

Based on prior work on related cancers, Wilson and his colleagues conducted a single-arm study of a chemotherapy regimen consisting of dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine and prednisone, combined with the monoclonal antibody rituximab (DA-EPOCH-R) in 51 patients with primary mediastinal B-cell lymphoma over a 13-year period. The results of this work were published in the *New England Journal of Medicine* earlier this year.



(Photo: R. Folkers, NCI)

Wyndham Wilson, M.D., Ph.D., and Kieron Dunleavy, M.D., reviewing CT scan with 26-year-old male patient (far right). Scan on right side of screen shows 15 cm mass in mediastinum and scan on left shows complete remission 7 months later.

"The outcome of our study is the best that has ever been reported in primary mediastinal B-cell lymphoma," said Wilson. "And it is the only outcome that has been reported without radiation." Following patients for an average of five years, the clinicians found that 93 percent of patients experienced no recurrence of the cancer, and that the overall survival rate was 97 percent. Oncologists are often reluctant to talk about outright cures, but Wilson noted that after a couple of disease-free years, the chances that patients relapse with this type of lymphoma are virtually zero. "We follow patients for many, many years, and we simply do not see relapses after that time."

Part of their therapeutic regimen involved a strategy developed by Wilson for pharmacodynamically adjusting the dosages to match individual patients' responses. Because individuals clear drugs that enter the body at different rates, the same dose administered systemically will not result in the same concentration of drug at the tumor. Wilson's strategy ties the amount of drug administered to a cellular response to chemotherapy, namely, the number of white blood cells remaining in circulation.

Learning about early results from the study presented at a conference, clinicians at Stanford University Medical Center decided to begin treating their patients with DA-EPOCH-R. Their retrospective analysis of 16 patients treated from 2007 through 2012 provided independent confirmation of the effectiveness of this treatment strategy. Similarly, in 2010, the Non-Hodgkin's Lymphoma Berlin–Frankfurt–Münster (NHL-BFM) study adopted DA-EPOCH-R for children and adolescents with primary mediastinal large B-cell lymphoma and reported equally strong results. "If I were a patient or referring oncologist," concluded Wilson, "I'd be pretty hard pressed not to try a regimen that is producing these outcomes."

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

Dramatic Responses
in a Rare Type of Sarcoma

Positive results in an early trial for rare sarcoma have spurred a multicenter randomized registration trial.

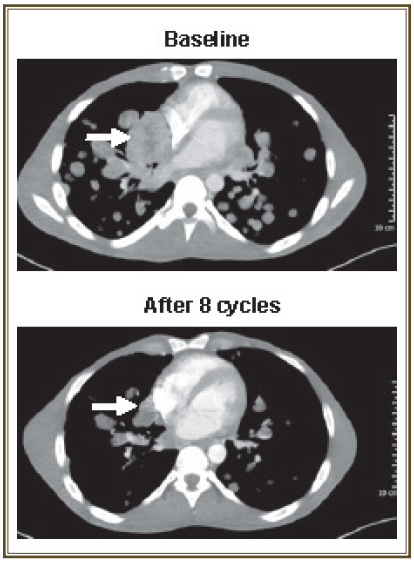
"Most oncologists have probably never seen a patient with alveolar soft part sarcoma (ASPS)," explained Shivaani Kummar, M.D., Head of the Developmental Therapeutics Clinic within CCR and NCI's Division of Cancer Treatment and Diagnosis. "So, it is treated like regular sarcoma, but it doesn't respond to the regimens." A rare and highly vascularized form of cancer for which radical surgery is the only known cure, ASPS has recently been the subject of a promising prospective phase 2 clinical trial conducted by Kummar and her colleagues. Their recent publication in the *Journal of Clinical Oncology* details the response of 46 patients to treatment with cediranib, an inhibitor of vascular endothelial growth factor receptors (VEGFRs).

Kummar was excited to test cediranib in this patient population after a phase 1 clinical trial in the U.K. gave the first signs of the drug's effectiveness in this patient population. She worked with NCI's Cancer Therapy Evaluation Program (CTEP), which had access to the drug through a Cooperative Research and Development Agreement (CRADA) with its maker AstraZeneca, to get the necessary approvals. They started judiciously, enrolling only nine patients at first, before bringing in additional patients as encouraging initial responses emerged. Eventually, 24 patients were enrolled in one cohort and an additional 22 in a replicate cohort, making this the largest prospective trial of a systemic drug to treat ASPS.

The paper reports results in which 15 of the first 43 patients saw at least a 30 percent reduction in the diameters of their tumors (measured along the longest dimension). For 26 patients, the cancer stabilized. At 24 weeks into the treatment, disease was under control in 84 percent of patients. "It was a very satisfying response," said Kummar. "Both the high response rate and the durability of the response. We now have patients three years out on this drug."

On the strength of these results, an open-label, multicenter randomized phase 2 trial has begun, comparing cediranib with sunitinib, a drug that acts to inhibit several receptor kinases including VEGFRs. The study will include M.D. Anderson Cancer Center, Dana-Farber Cancer Institute, Memorial Sloan-Kettering Cancer Center, and Santa Monica Oncology Center. As always, patient recruitment will be a challenge for a disease that accounts for less than one percent of cancers affecting supporting and connective tissues. "When we first began this work, it was hard to get the word out," said Kummar. Support groups like iCureASPS have made a large difference in educating patients and oncologists alike about this unusual sarcoma.

In addition to monitoring tumor shrinkage, the clinical researchers also took tumor biopsies from patients to study changes in gene expression. Consistent with VEGFR inhibition, they found downregulation of the angiogenesis-related gene *ANGPT2* and the gene encoding VEGFR-1. These gene expression analyses give insight into the mechanism of action of the drug, as well as suggest combination therapies that could be tried in the future.



(Image: S. Kummar, CCR)

CT scan from 25-year-old patient with newly diagnosed metastatic ASPS; top scan is cross-section of the chest at the start of the trial and bottom scan is post-treatment, with significant tumor shrinkage.

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

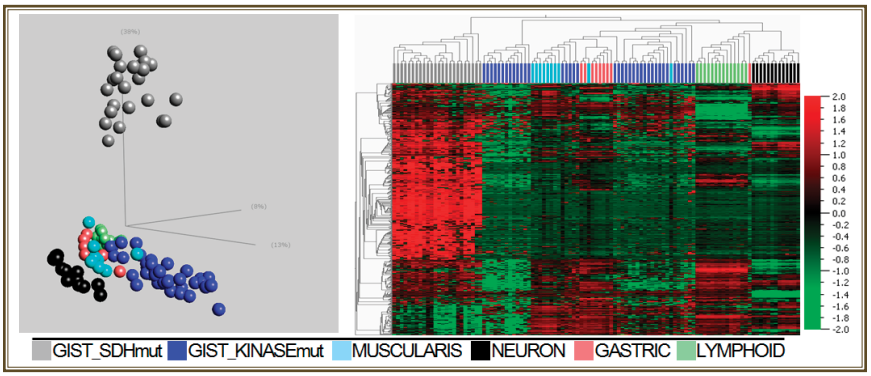
The GIST of One Cancer:
Two Distinct Molecular Diseases

New results from the pediatric GIST program find that radically different epigenetic patterns define rare disease subtypes.

Most adults with gastrointestinal stromal tumors (GIST) have mutations in one of the known cancer signaling pathways—usually in the gene for the receptor tyrosine kinase, KIT—that both drive the disease and render it vulnerable to targeted drugs. However, other patients—usually children and younger adults—have tumors that lack such obvious genetic causes and historically, were simply described as wildtype GIST. In a recent issue of *Cancer Discovery*, Keith Killian, M.D., Ph.D., Paul Meltzer, M.D., Ph.D., and their colleagues in CCR's Genetics Branch, describe the identification of two distinct GIST categories based on patterns of DNA modifications. Their findings have implications that go beyond GIST itself to other cancer types.

Several years ago, CCR's Scientific Director for Clinical Research, Lee Helman, M.D., set up the pediatric oncology program to study wildtype GIST by bringing together patients with this mysterious disease under one clinical roof. The program's multidisciplinary team of clinicians and pathologists discovered that a subset of GIST tumors harbored different mutations in the genes encoding subunits of succinate dehydrogenase (SDH), a key enzyme complex in the Krebs cycle, the backbone of aerobic energy production. SDH mutations affect mitochondrial metabolism, but their role in tumorigenesis is uncertain.

Looking beyond mutations in the primary sequence of tumor DNA, Killian and colleagues wondered whether epigenetic DNA modifications—key arbiters of gene expression that help to define cellular type—could be playing a critical role in the disease. By studying the patterns of one such modification—methylation—they found that GIST was clearly separable into two forms: one in which the pattern of DNA methylation resembled surrounding and related tissues, and the other in which there was a profound increase in methylation observed over hundreds of genetic loci accompanied by a more restricted but equally distinct hypomethylation. The latter pattern included tumors with all known SDH mutations.



(Image: K. Killian, CCR)

Unsupervised principal component analysis (left) and 2D hierarchical clustering (right) of genomic methylation profiles reveal that SDH-mutant GIST possess a characteristic, unifying epigenomic divergence from kinase-mutant GIST and normal tissues.

Mutations in SDH result in an accumulation of succinate, which itself acts as an inhibitor of several enzymatic reactions, some of which are involved in the maintenance of DNA methylation. With that in mind, Meltzer's team looked at the methylation patterns in tumors from other types of cancers with similar metabolic abnormalities, e.g., paragangliomas associated with SDH mutations and gliomas associated with mutations of the isocitrate dehydrogenase gene (IDH). They found the same striking pattern of predominant hypermethylation associated with these very different cancers.

The researchers neither know how these epigenetic changes are related to the cancer phenotype, nor do they know exactly how the metabolic alterations lead to changes in DNA methylation, although they are following evidence that suggests that inhibition of the cells' ability to remove methylation is at play.

"When we started the GIST clinical program, we didn't even know if we had a true subset of the disease," said Meltzer. "So our work represents some real progress in the classification of these patients, which should eventually lead to improved care. These patients have also led us unexpectedly to the fascinating intersection of cancer genetics, epigenetics, and metabolism."

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

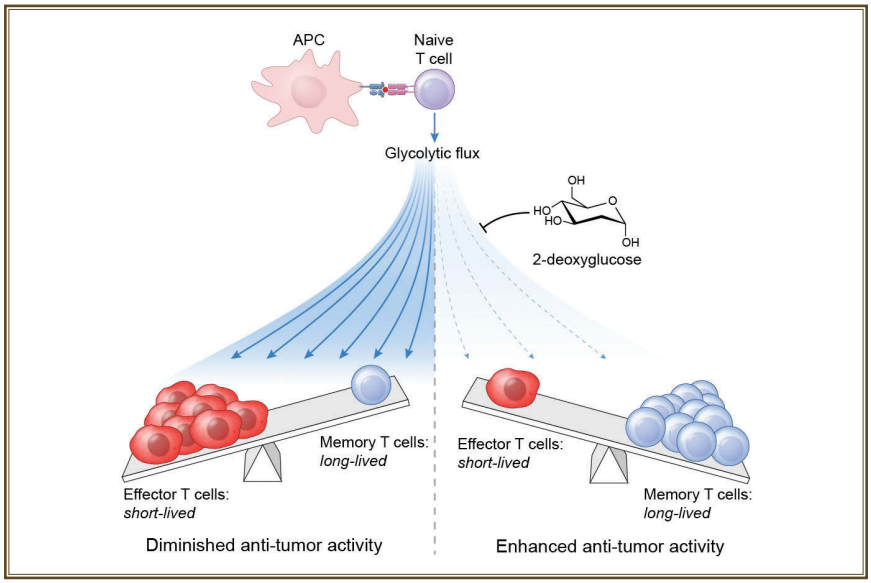
A Bias for Memory

Manipulating glucose metabolism predictably alters T-cell phenotypes.

Luca Gattinoni, M.D., Earl Stadtman Tenure-Track Investigator in CCR's Experimental Transplantation and Immunology Branch, has a rather straightforward goal for his research: to design better immunotherapies for cancer. In particular, he focuses on the adoptive transfer of T cells, naturally strong defenders against tumors that become exhausted and subverted as cancers progress. When primed with a tumor antigen, T cells are stimulated to produce two classes of cells: memory cells that live to fight another day and effector cells that immediately seek out and destroy their foes. The long-term success of adoptive transfer depends on the proportion of memory T cells. In the *Journal of Clinical Investigation*, Gattinoni, Nicholas Restifo, M.D., in CCR's Surgery Branch, and their colleagues show that they can bias this proportion by making systematic adjustments to cellular metabolism.

More than 50 years ago, Nobel laureate Otto Warburg first noted that, in contrast to normal cells, cancer cells employ glycolysis to generate the energy needed for cellular growth even in the presence of oxygen. Similar metabolic changes occur in T cells following activation. While quietly patrolling for specific miscreants, T cells rely on aerobic fatty acid oxidation to fulfill their energy requirements. However, when faced with the need to proliferate and differentiate, their metabolism is diverted towards glycolysis, which generates a number of biosynthetic intermediates that are required by growing cells.

Given this background, Gattinoni and his team asked whether the switch from fatty acid oxidation to glycolysis is an adjustment the cell makes once its fate is sealed or whether the switch might in fact contribute to the cell's fate *in vivo*. To address this question, first they monitored glucose metabolism in T cells with a fluorescent probe before injecting them into mice and challenging them with a pathogenic signal. They separated the T cells into low and high glucose metabolizers and found that the mice injected with low glucose metabolizers were much more effective in mounting an immune response when challenged several weeks later. In parallel, they saw that the low and high glucose metabolizers displayed gene expression signatures that aligned with memory and effector cells, respectively.



(Image: L. Gattinoni, CCR)

After encountering antigen, naïve CD8+ T cells undergo an extensive period of proliferation and expansion, and differentiate into effector cells and distinct memory T cell subsets. Increasing glycolytic flux pushes CD8+ T cells towards a terminally differentiated state that diminishes antitumor activity. In contrast, inhibiting glycolysis using 2-deoxyglucose (2DG) maintains the formation of long-lived memory CD8+ T cells and enhances antitumor activity.

The next logical question was whether altering glycolysis could influence T cell effectiveness. To manipulate the glycolytic pathway, Gattinoni and his colleagues used overexpression of the enzyme phosphoglycerate mutase-1 to show that genetically enforced glycolysis was sufficient to bias the population towards effector phenotypes and reduce the strength of the immune response over time. Inhibition of glycolysis with 2-deoxyglucose had the opposite effect, increasing the population of memory T cells and the concomitant response to immunologic challenge.

Taken together, this work suggests strategies to increase the therapeutic efficacy of adoptive T cell transfer by biasing the population towards a memory response. Because cancer cells also have a heightened glucose metabolism, clinical strategies are already under development to inhibit this pathway in cancer cells, which might be extrapolated to the immune system. "Modulation of the metabolism of the antitumor T cell response could be an important addition to the immunotherapist's armamentarium," concluded Gattinoni.

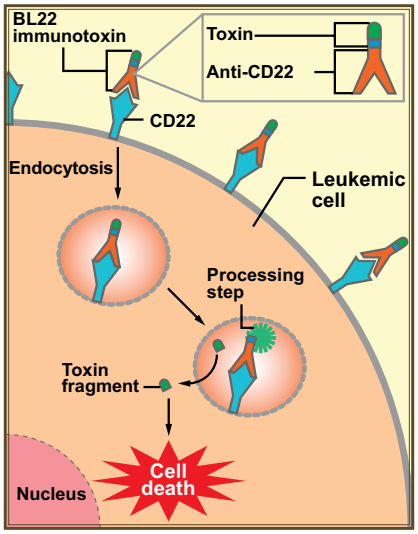
To learn more about Dr. Gattinoni's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=Gattinoni.

Fast Track to the Clinic

In 2008, Ira Pastan, M.D., Co-Chief of CCR's Laboratory of Molecular Biology (LMB), told *CCR connections*, "Since I was a physician trained to do research, I wanted to use what I knew to do something relevant to the treatment of cancer." [See "A Better Immunotoxin," *CCR connections* Vol. 2, No. 1]. That ambition received a major boost earlier this year when a drug developed in his laboratory—moxetumomab pasudotox—leap-frogged from a phase 1 dose-escalation trial directly into a fully fledged phase 3 multicenter trial to confirm efficacy.

Phase 1 trials are preliminary by design, and yet 49 patients with chemotherapy-resistant hairy cell leukemia (HCL) saw their tumors shrink with the drug treatment and half of those saw their tumors completely disappear. Based on the dramatic positive outcomes observed, the U.S. Food and Drug Administration agreed that the more foundational steps of phase 2 trials could be bypassed. The first patients have already been enrolled in the phase 3 trial, which is being led by Robert J. Kreitman, M.D., Head of the LMB's Clinical Immunotherapy Section, and others.

Moxetumomab pasudotox is an immunotoxin comprising one part targeting antibody and one part lethal toxin. The targeting antibody seeks out differentiated B cells, which then internalize the toxin. Pastan with his colleague David Fitzgerald, Ph.D., pioneered this technology by engineering derivatives of a naturally occurring bacterial toxin. AstraZeneca subsidiary MedImmune licensed the technology from NCI and has worked with NCI's Cancer Therapy Evaluation Program (CTEP) to further its clinical development.



(Image: I. Pastan, CCR)

Moxetumomab pasudotox binds CD22 receptors on the surface of cancerous B cells, where it is internalized and processed to release its toxic payload.

Dream Team Takes New Approach to Childhood Cancers

(Photo: R. Baer)

Crystal Mackall, M.D.

Crystal Mackall, M.D., Chief of CCR's Pediatric Oncology Branch, has been named co-leader of a Stand Up to Cancer (SU2C)—St. Baldrick's Foundation Pediatric Dream Team: Immunogenomics to Create New Therapies for High-Risk Childhood Cancers.

Mackall, along with team leader John Maris, M.D., Director of the Center for Childhood Cancer Research at The Children's Hospital of Philadelphia, helped bring together a group of researchers from seven institutions across North America with unparalleled expertise in the fields of genomics, immunotherapeutics, and pediatric oncology. Their goal is to find new treatments for the deadliest childhood cancers, including malignant brain tumors, high-risk leukemias, neuroblastomas, and sarcomas.

The Dream Team is addressing unique challenges associated with childhood cancers, which are less likely to arise from the types of recurring mutations that can be countered with small molecule drugs developed against cancer in adults.

First, they are using genomics to identify cell-surface molecules that are specifically expressed in high-risk pediatric cancers. Once validated, these molecules will serve as targets for the development of immunotherapeutic strategies, including specific antibodies, immunotoxins, and engineering immune cells. Included in the plan will be pivotal first-in-child trials of the most promising candidates.

"We are confident that this combined approach will lead to novel therapies that will improve outcomes for some of the most lethal childhood cancers," said Mackall.

A Vision for Prostate Cancer

Current methods for prostate cancer screening have drawbacks. Rectal exams only catch relatively large, posterior tumors. Levels of prostate-sensitive antigen (PSA) are a controversial tool—many men with high PSA levels never develop the disease; conversely, low levels of PSA do not mean a cancer-free guarantee. Needle biopsies provide the best evidence for cancer, but until now, doctors had no choice but to go in blindly with a needle, taking random samples and risking damage to sensitive nerves and ducts.

Over the last 10 years, a team of NIH scientists—including Peter Choyke, M.D., Chief of CCR's Molecular Imaging Program, Bradford Wood, M.D., Director of the NIH Center for Interventional Oncology, and Peter Pinto, M.D., Staff Clinician, CCR's Urologic Oncology Branch—have developed the technology to perform visually guided needle biopsies for prostate cancer. First they demonstrated that magnetic resonance imaging (MRI) has the power to detect prostate tumors. Then, they developed techniques to fuse images taken with conventional MRI with real-time ultrasound scans, enabling urologists to guide their ultrasound biopsies using the superior resolution of MRI. This technology has now been commercialized by Invivo (a subsidiary of Philips Medical Systems) and was unveiled as UroNav at the 2013 annual meeting of the American Urological Society.

The team that developed UroNav is not resting on their laurels. They are looking beyond diagnosis to improve treatment. "Prostate cancer has been treated for over a century by removing the whole prostate," said Pinto. "Image-guided focal therapy for prostate cancer can avoid the side effects of whole gland therapy, erectile dysfunction, and urinary incontinence." They have already conducted safety and feasibility studies, and are beginning a phase 2 trial for efficacy.

Natural Disaster Brings Lasting Scientific Exchange

(Photo: M. Gottesman, CCR)

Michael Gottesman, M.D., and Susumu Satomi, M.D., Ph.D., President, Tohoku University

Natural disasters make immediate and dramatic headlines, but repairing their damage—both physical and emotional—is a much longer process. On the second anniversary of the Great East Japan Earthquake, a delegation of researchers from the NIH, including CCR Investigators Michael Gottesman, M.D., Tom Misteli, Ph.D., and Shioko Kimura, Ph.D., traveled to Tohoku University in Sendai, Japan, to continue building the scientific bridges that were first formed after that tragic event.

Japanese researchers are a strong presence at the NIH, not only among the senior scientific ranks but particularly as part of a large postdoctoral program sponsored by the Japanese Society for the Promotion of Science (JSPS). In the wake of the tsunami and earthquake that devastated several Japanese cities including Sendai in March 2011, these links served as a gateway for scientists from Tohoku University to continue their work at the NIH while their labs and lives were being repaired.

To commemorate the event and to continue building the collaborations and exchanges that stemmed from it, the NIH, Tohoku University, and JSPS held a joint two-day symposium, followed by site visits to the affected areas, including a regional hospital built only a few years before the earthquake hit. "Our guide took us to the basement and asked us to kneel down to look below," said Misteli. "It turns out the entire hospital is built on springs and lifted several feet off the ground." This feat of engineering enabled the hospital to escape damage during the disaster and to serve as an effective emergency response center during the crisis.

A second symposium is planned for the fall of 2014, which will bring a delegation of Tohoku University scientists to the NIH.

Recent CCR Awards

National Academy of Sciences Fellow

Louis M. Staudt, M.D., Ph.D.
Deputy Chief, Lymphoid Malignancies Branch

Lifetime Achievement Award

Society for Melanoma Research

For major and impactful contributions to melanoma research

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

2013 Von Recklinghausen Award

Children's Tumor Foundation

For major contributions to neurofibromatosis research

Brigitte Widemann, M.D.
Pediatric Oncology Branch

2013 PhRMA Research & Hope Award for Academic or Public Research in Vaccine Development

For the discovery of the human papilloma virus (HPV) vaccine for the prevention of cervical cancer

Douglas R. Lowy, M.D.
Office of the Director, National Cancer Institute

John T. Schiller, Ph.D.
Laboratory of Cellular Oncology

2013 CSIRO Chairman's Medal

Commonwealth Scientific and Industrial Research Organization, Australia

For work on a Hendra virus vaccine with the Hendra Virus Research Team

Dimiter Dimitrov, Ph.D.
Laboratory of Experimental Immunology

Zhongyu Zhu, Ph.D.
Laboratory of Experimental Immunology

2013 Henry M. Stratton Medal

American Society of Hematology

For outstanding contributions to hematology

Elaine Jaffe, M.D.
Laboratory of Pathology

European Molecular Biology Organization Member

Andre Nussenzweig, Ph.D.
Chief, Laboratory of Genomic Diversity

American Crystallographic Association Fellow

For contributions in research and service to the crystallographic community

Alexander Wlodawer, Ph.D.
Chief, Macromolecular Crystallography Laboratory

American Psychological Association Fellow

For outstanding and unusual contributions to the science and profession of psychology

Lori Wiener, Ph.D.
Pediatric Oncology Branch

Center of Innovation Award

Waters Corporation

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

Honorary Doctor of Science

Amherst College

Robert Yarchoan, M.D.
Chief, HIV and AIDS Malignancy Branch

NICBR Collaboration Project Award

National Interagency Confederation for Biological Research (NICBR) Program

Barry O'Keefe, Ph.D.
Molecular Targets Laboratory

Sreejith Raran-Kurussi, Ph.D.
Macromolecular Crystallography Laboratory

Recently Tenured CCR Scientists

Julia P. Cooper, Ph.D.
Laboratory of Biochemistry and Molecular Biology

Esta Sterneck, Ph.D.
Laboratory of Cell and Developmental Signaling

Kylie Walters, Ph.D.
Structural Biophysics Laboratory

Katherine Warren, M.D.
Pediatric Oncology Branch

Staff News at CCR

Announcements

(Photo: B. Branson)

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

James Gulley has been named Director of CCR's Medical Oncology Service. He will continue his role as Chief of CCR's Genitourinary Malignancies Branch. Gulley received his M.D. and Ph.D. degrees from Loma Linda University in California. He completed his residency in internal medicine at Emory University and a medical oncology fellowship at NCI. Following his fellowship, he was retained as senior staff at NCI. Gulley has developed a productive translational program and has run multiple clinical trials in immunotherapy for prostate cancer. 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.

(Photo: B. Branson)

Melinda Merchant, M.D., Ph.D.

Melinda Merchant has been appointed as Clinical Director of CCR's Pediatric Oncology Branch (POB). She received her M.D. and Ph.D. degrees from the University of Miami and completed a residency in pediatrics at Children's National Medical Center in Washington, D.C. After completing a clinical fellowship in POB, she served on the faculty at Memorial Sloan-Kettering Cancer Center. She rejoined POB in 2009 to lead its clinical program in pediatric solid tumors. Her research focuses on the translation of immunotherapies and molecularly informed therapies in pediatric solid tumors, including bone and soft tissue tumors, melanoma, and neuroblastoma.

New Tenure-Track Scientists

(Photo: B .Branson)

Luca Gattinoni, M.D.

Luca Gattinoni is now a Tenure-Track Investigator in CCR's Experimental Transplantation and Immunology Branch. He received his M.D. from the Università degli Studi in Milan, Italy, and completed his residency in medical oncology at the Istituto Nazionale dei Tumori in Milan. He then joined NCI as a Visiting Fellow and in 2008, he became a Staff Scientist in CCR's Surgery Branch. His research focuses on T cell-based immunotherapies with an emphasis on T-cell differentiation and transcriptional regulation of T-cell self-renewal and memory formation.

(Photo: B .Branson)

James N. Kochenderfer, M.D.

James Kochenderfer is now a Tenure-Track Investigator in CCR's Experimental Transplantation and Immunology Branch. He received his M.D. from West Virginia University and completed his clinical training in internal medicine at Vanderbilt University. Following oncology and hematology fellowships at The University of Texas M.D. Anderson Cancer Center and at Baylor College of Medicine, he joined NCI in 2002 as a Clinical Fellow. He has also held the position of Assistant Clinical Investigator. His research focuses on the development of genetically engineered T cells aimed at fighting lymphoma and leukemia.

In Conversation:
Postdoctoral Fellow Alyson Freeman, Ph.D.

CCR: Alyson, we read your paper characterizing RAF dimerization in *Molecular Cell* earlier this year. What drew you to study signaling mechanisms in cancer?

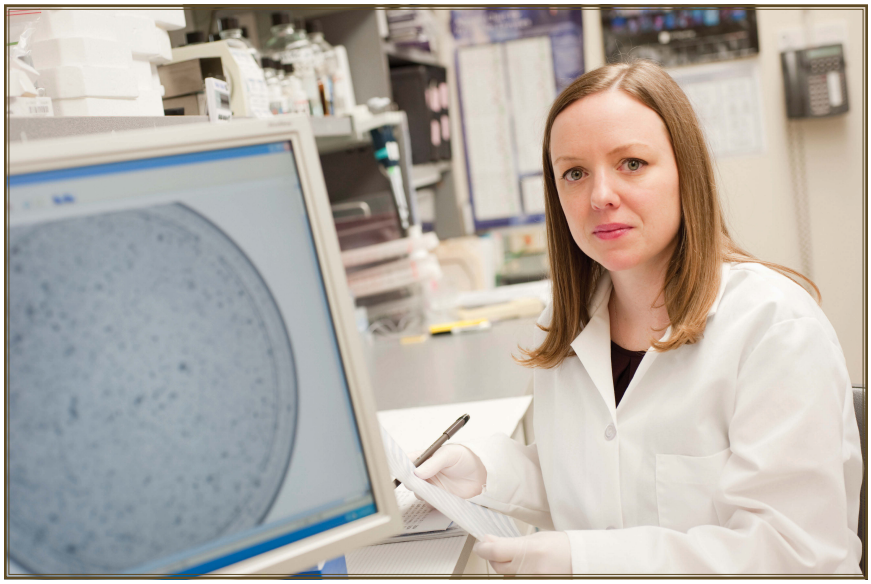
Alyson: I got interested in research as an undergraduate, and afterwards, I took two years as a technician at Boston College to really think about what I wanted to do next. I decided to focus on cancer. It's just one of those scientific problems that fascinated me. Cancer is so complex, involving almost every pathway in the cell. I wanted to explore those details and build them into a global picture. So, I decided to go the Moffitt Cancer Center for my Ph.D., principally because they have a joint program with the University of South Florida in integrative cancer biology for graduate students.

CCR: What led you to work with Deborah Morrison, Ph.D., in CCR's Laboratory of Cell and Developmental Signaling?

Alyson: As a grad student, I worked on the DNA damage response. I wanted to build on that knowledge, but also broaden my expertise. Debbie's laboratory is great at biochemical techniques—*in vitro* kinase assays, mass spectrometry—about which I knew enough to hit the ground running, while learning from the real pros.

CCR: What is most exciting to you about the work you have done so far?

Alyson: It's been known for a long time that RAFs dimerize, but now we really know which residues are critical, in which signaling contexts and how dimerization is affected by *RAF* mutations. This level of detail is going to be key to developing good therapeutics. It turns out that RAF dimerization plays an important role in the drug resistance that develops to first generation RAF inhibitors.



(Photo: R. Baer)

Alyson Freeman, Ph.D.

CCR: We understand that you are leaving soon on maternity leave. Congratulations! What are your plans going forward?

Alyson: I'm planning on returning to the lab after my leave. There's so much to do! The RAS-RAF-ERK-MEK pathway is heating up and NCI has just committed to the new RAS Initiative at the Frederick Laboratory for Cancer Research to translate our increasing knowledge into therapies. Understanding the signaling nuances is going to matter for developing the best drugs. Based on our work with peptide inhibitors of dimerization, we might be able to collaborate with a chemical laboratory to develop a small molecule inhibitor of RAF dimerization, and even take that further into a mouse model.

CCR: It sounds like you are succeeding in exactly the mission you embarked on before going to graduate school. Do you have any advice for budding cancer researchers?

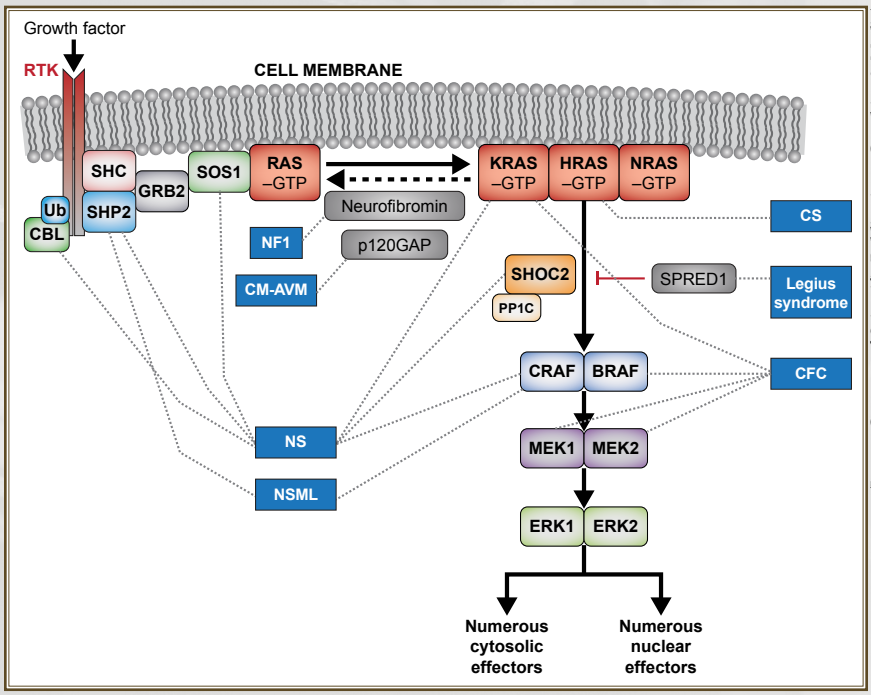
Alyson: When starting a postdoc or even deciding whether to go to grad school, it's so important to really think about your long-term goals and get advice from as many people as possible on how to achieve them. I'd also encourage other trainees to take full advantage of the opportunities we have at NCI. I first arrived in the lab right before the CCR Fellows and Young Investigators annual colloquium; one of the senior postdocs encouraged me to attend. It gave me great ideas for collaborations, expanding my science and thinking about my career. It really opened my eyes to everything that I could be a part of here.

RAS Takes Center Stage

Wreaking cellular havoc in approximately one-third of all cancers, oncogenic RAS signaling has been extensively studied in the 30 years since the gene first associated with rat sarcoma virus was identified in human tumors. But, devising anticancer drugs that target RAS proteins has remained frustratingly elusive. RAS molecular structures lack obvious pockets for small molecule disruption and early attempts to inhibit an enzymatically driven modification of RAS (farnesylation) thought to be necessary for its translocation to the cellular membrane led to disappointing failure in clinical trials. As scientists have continued to focus on the details of RAS signaling and the extensive molecular network under its control, however, their persistence is beginning to pay off: new therapeutic approaches are once again on the horizon.

Sitting in the office of Debbie Morrison, Ph.D., Chief of CCR's Laboratory of Cell and Developmental Signaling, it is hard not to notice the 3-D molecular structure etched in glass that is prominently displayed on her desk. "This is the first structure of the B-RAF catalytic domain," explained Morrison. U.K. scientists Richard Marais, Ph.D., and David Barford, D.Phil., first described the structure at a FASEB meeting on protein kinases and protein phosphorylation. "Dr. Marais gave me the structure as a fellow colleague who has spent her career studying the RAF kinases."

RAF kinases are key effectors of RAS signaling; RAF is the initiating kinase in the RAF-MEK-ERK cascade that regulates cellular growth in a variety of biological and pathological contexts. Using biochemical and proteomic approaches, Morrison has delved into the molecular mechanisms that regulate the RAF kinases and their response to RAS activation. Much of her work has been in the context of normal growth factor signaling



(Image: Courtesy of Scientific Publications, Graphics & Media)

RAS is activated by growth factor receptor signaling at the cellular membrane to initiate the RAF-MEK-ERK cascade.

which relies on these signaling pathways for healthy proliferation of cells in the developing organism.

Of the components in this cascade, RAF regulation is the most complex, including negative regulation and feedback loops. RAF also exists as multiple subtypes (A-, B-, and C-), with different properties and functional contexts. "For many years, we knew that RAFs interacted, but because there are so many other components and interactions involved in the

RAF activation process, it wasn't clear how important the RAF-RAF interaction was and whether it reflected direct dimerization of the RAFs," said Morrison.

Then, a series of new findings piqued Morrison's interest, and that of her colleague, Postdoctoral Fellow Alyson Freeman, Ph.D. [See "In Conversation," *CCR connections*, Vol. 7, No. 2]. First, the 3-D structure published by Marais and Barford revealed that the B-RAF catalytic domain formed side-to-side dimers and that the dimer interface was in close proximity to the ATP-binding pocket. "Then, there were a series of papers looking at the use of ATP-dependent RAF inhibitors in melanoma," explained Freeman. "The inhibitors hampered disease progression in melanomas expressing a mutant B-RAF kinase, but in cells that contained wild-type RAF, there was a paradoxical activation of the ERK pathway that apparently involved RAS-dependent RAF dimerization."

"We decided it was really important to look at the endogenous proteins, rather than overexpressed proteins, and by studying homo- and hetero-dimerization of the different RAF subtypes, we discovered not only that dimerization is critical to RAF activation but that the dimer interface might be a target for therapeutic intervention," said Morrison.

Freeman, Morrison, and their colleagues went on to show that using a peptide to block the dimer interface, they could effectively silence RAF signaling in many contexts, including when RAS is activated by a mutation.

(Photo: R. Baer)

Daniel Ritt, Debbie Morrison, Ph.D., and Alyson Freeman, Ph.D.

Interestingly, they also found that the most prevalent oncogenic mutation of RAF, V600E-B-RAF, rendered the kinase independent of dimerization and that the peptide was not effective when RAF activation was dimerization independent. "So if we can block RAF dimerization clinically, we will need to determine what the specific mutations are in a cancer to know if blocking RAF dimerization would be an effective treatment. Given the resistance that develops to current RAF inhibitors, a dimer blocking agent may also help as a combination therapy to prolong disease-free survival."

"Understanding the details of RAF activation explains a lot of what is seen in clinical treatment—why some therapies are working or not working," said Morrison. She pointed out that certain other anticancer drugs can also promote the paradoxical activation of RAF. "The ATP binding site is a very conserved region, and some ATP-competitive kinase inhibitors can have off-target effects on RAF, such as those directed against BCR-ABL and p38. Thus, while you're trying to suppress BCR-ABL or p38 signaling, your drug may actually be binding quite well to RAF and inducing RAF dimerization. You may be trying to inhibit one pathway and be successful, but at the same time, you might be upregulating ERK signaling."

In June 2013, NCI Director Harold Varmus announced a $10 Million initiative to develop new ways to block oncogenic RAS signaling. Morrison participated in a workshop in advance of the announcement and is enthusiastic that the time is right for a concerted attack on RAS and that a more nuanced view of therapeutic mechanisms is emerging.

"If an inhibitor of RAF dimerization came out, I'd feel thrilled that we had contributed to it," said Morrison.

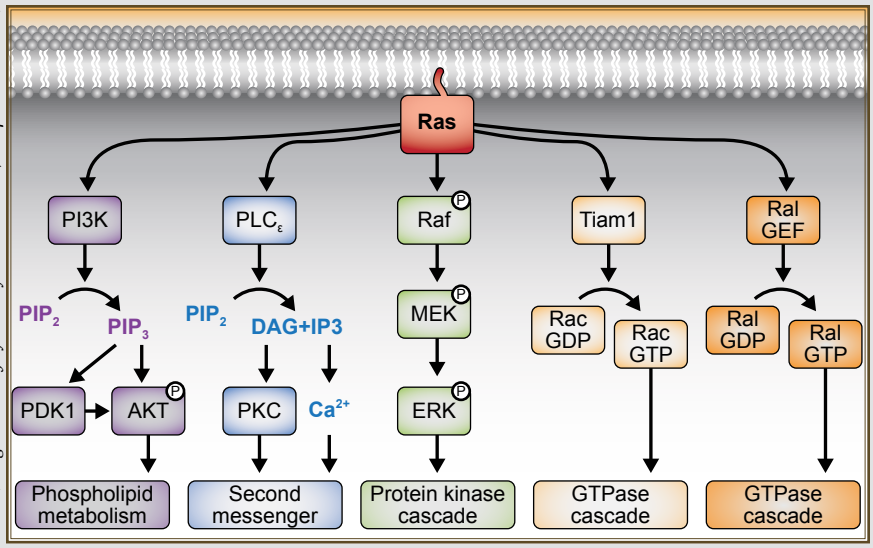
> "We discovered not only that dimerization is critical to RAF activation but that the dimer interface might be a target for therapeutic intervention."

Synthetic Lethality

Ji Luo, Ph.D., Tenure-Track Investigator in CCR's Laboratory of Cancer Biology and Genetics, agrees that the field is gaining critical momentum. "We know enough about the biology of RAS and have enough new molecular and genetic tools that we can revisit the issue of targeting RAS pathways."

Luo's laboratory is taking a multipronged approach to targeting

(Image: Courtesy of Scientific Publications, Graphics & Media)

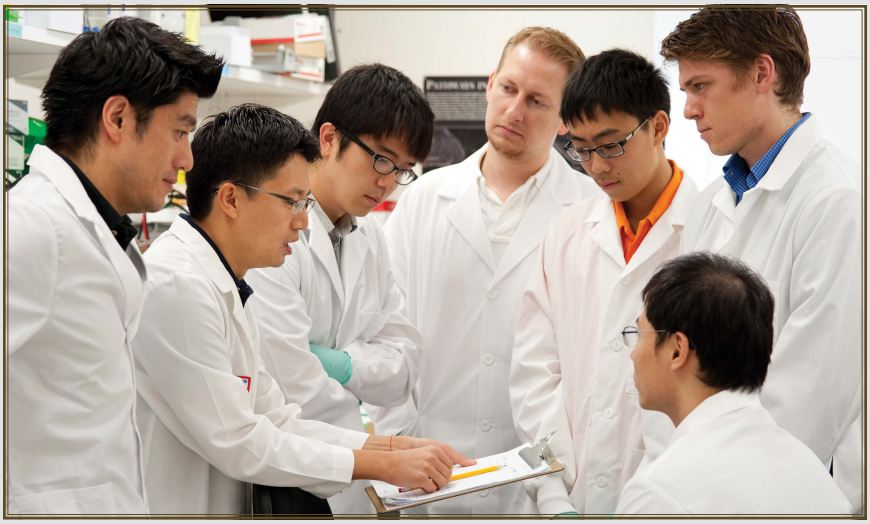


RAS signaling pathways in cancer

oncogenic KRAS, one of the three canonical RAS family members (H-, K-, and N-). At the heart of his approach is the concept of synthetic lethality, which comes from genetics, and refers to the impact of multiple genetic mutations on viability. Mutations in *KRAS* not only do not kill cells, they endow them with their invasive and proliferative advantages. But such oncogenic mutations achieve tumorigenesis at a cost: oncogenic stress. Cells experience increased apoptotic signals, metabolic stresses, and genomic instability, which must be ameliorated by the expression of supporting molecular factors.

Using RNA interference (RNAi) screens to inhibit expression of individual genes, Luo and his colleagues are searching for pathways that are required for the survival of cells which express mutant *KRAS*, but not wildtype *KRAS*. Among the genes that they have thus far explored, RNA splicing factors have come to the forefront. These factors are involved in editing mRNA transcripts to produce selected gene products and until recently, have not been strongly linked with cancer.

(Photo: R. Baer)



Chih-Shia Lee, Ph.D., Ji Luo, Ph.D., Changwoo Lee, Valentin Giroux, Ph.D., Hueyjong Shih, Joseph Carver, and Bing Yu, Ph.D.

"We think RNA splicing factors may be controlling key genes that maintain survival and growth; we have a number of candidates that we are investigating," said Luo.

Another pathway that has received scant attention from cancer researchers is a pathway that modifies proteins after they have been translated with the addition of small ubiquitin-related modifier (SUMO) proteins. A highly dynamic, regulated process, sumoylation affects diverse properties, including protein localization, activity, and stability. Luo's team has found that sumoylation is important for the ability of cells with KRAS mutations to thrive unanchored *in vitro* (the classic assay for oncogenic transformation). Furthermore, inhibition of protein sumoylation both *in vitro* and in xenograft mouse models suppresses the cancerous phenotype. "We have identified the E2 ligase UBC9 as central," said Luo. "Its enzymatic activity is important for *KRAS*-driven transformation." As a result, Luo is collaborating with Jay Schneekloth, Ph.D., a Tenure-Track Investigator in CCR's Chemical Biology Laboratory [See "Putting Peptides to Work," *CCR connections*, Vol. 7, No. 2], who uses structural approaches to design inhibitors against E2 ligases, including UBC9. "We have been going back and forth, combining my lab's expertise in genetics with his lab's chemical expertise to explore UBC9 as a druggable target," said Luo.

Cognizant of the failures to target *KRAS* with small molecules, Luo is excited about the possibilities of using small interfering RNAs (siRNAs) for targeted interference with gene translation. "We've developed very potent siRNAs to knock down *KRAS* at low nanomolar concentrations," said Luo, even while acknowledging that delivery is a major therapeutic challenge. Knowing of several nanoparticles to package siRNAs that are under development in academic

and industrial settings, Luo is optimistic. "*In vitro*, it's magnificent. The beauty of siRNAs as a therapeutic is that they all work the same way, you just have to change the sequence. So, it's easy to do combinations and you can target anything."

Luo also points out that RAS has over 50 downstream effectors, if you count all the gene isoforms. And as is increasingly the case for all cancers, RAS-driven cancers are likely to be defeated ultimately with combinatorial approaches, whether by small molecules, biologics, or RNAi. "So far, we don't have a drug against every RAS effector, but we do have potent siRNAs, so we can develop screens to address the combinatorial issues up front. It gives us a rational path for drug discovery."

All in the Family

RAS is a small GTPase, meaning it is active when bound to GTP and inactive when the GTP hydrolyzes to GDP. RAS GTPases are among the most well studied, but they are also just part of a superfamily which includes other well-known actors in cancer signaling including RHO and RAB.

"I work on the forgotten subfamily, in terms of cancer research," said Paul Randazzo, M.D., Ph.D., Senior Investigator in CCR's Laboratory of Cellular and Molecular Biology. "The Arf subfamily is known to regulate membrane trafficking and actin. We had the idea that it may be an important regulator of cell adhesions which are critical for survival, proliferation, migration… all things that are critical in cancer."

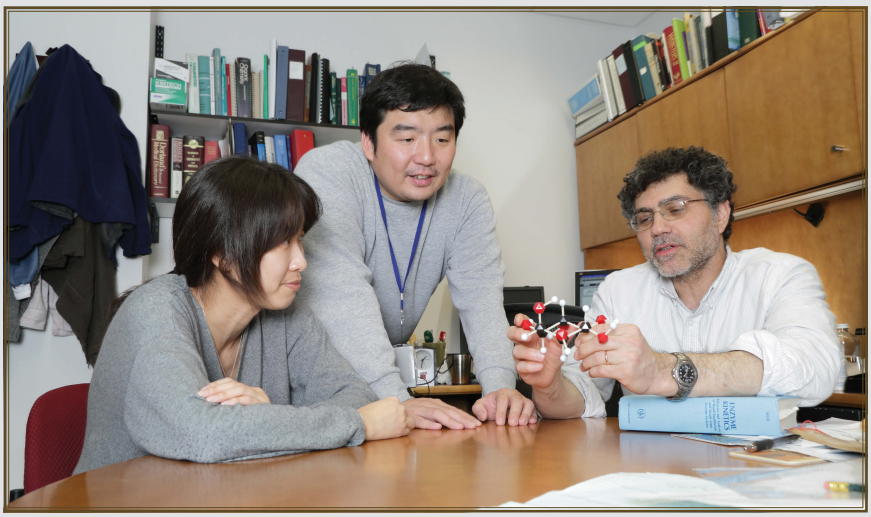
It has proved challenging to purify chemically useful amounts of native RAS and RHO because they have extensive lipid modifications. Arf, by comparison, has a simple lipid modification, which allowed Randazzo and his colleagues to prepare sufficient amounts of the native protein to study its catalytic and regulatory mechanisms.

Many proteins that regulate RAS superfamily members contain Pleckstrin Homology (PH) domains. The standard dogma is that PH domains recruit the regulatory proteins to membranes on which the RAS protein resides. Randazzo's data indicate that in fact, lipid binding exerts a conformational change that opens up the catalytic pocket for more efficient interaction with the RAS superfamily protein. Membrane attachment itself is not required. "If you want to disrupt the function of one of these proteins, the PH domain may be appropriate as a therapeutic target." As their research continues, Randazzo and his colleagues find that other laboratories are also beginning to question whether the membrane recruitment paradigm applies to all PH domains. "People pigeonholed protein domains based on the first discovered function—maybe the functions are a bit broader and more variable."

Randazzo has also purified ASAP1, an Arf GTPase activating protein (GAP), regulated by phosphatidyl inositols, Src, and focal adhesion kinase (FAK). They have found that ASAP1 regulates invadopodia, which, as their name implies, are invasive protrusions of the cellular membrane. The gene for ASAP1 is amplified in 50 percent of uveal melanomas, a very aggressive cancer that metastasizes to the liver. It is also amplified in 40 percent of ovarian carcinomas, as well as in 20 percent of breast and 20 percent of hepatocellular cancers. A group in Japan has recently shown with a mouse orthotopic xenograft model that elevated ASAP1 expression accelerates invasion and metastasis of breast cancer. "At this point it is part of the machinery that is necessary for malignancy, but I don't think it's a driver like RAS," said Randazzo. "My goal is to acquire solid data that can be used to understand these important processes that contribute to human disease."

"People pigeonholed protein domains based on the first discovered function—maybe the functions are a bit broader and more variable."

(Photo: B. Branson)



Peng Zhai, Ph.D., Pei-Wen Chen, Ph.D., and Paul Randazzo, M.D., Ph.D.

Paving Paths to Translation

Terry Van Dyke, Ph.D., Senior Investigator in CCR's Mouse Cancer Genetics Program came to CCR six years ago to create a program that would give researchers with therapeutic hypotheses the means to put them through rigorous preclinical testing. The resulting Center for Advanced Preclinical Research (CAPR) works in partnership with researchers around the world to conduct and analyze experiments in a variety of cancer models, with an emphasis on genetically engineered mouse models.

"We set up the center as a hybrid between a rigorous research institute and an industry infrastructure… it's an efficient way to have a completely integrated set of expertise," said Van Dyke.

Several of CAPR's collaborations are centered on RAS. For example, CAPR is working with Glenn Merlino, Ph.D., Chief of the Laboratory of Cancer Biology and Genetics, and a melanoma consortium from around the country, which will involve testing an immunotherapy approach in an NRAS-driven model, among other projects. Whereas almost all other work is in the primary tumor domain, Merlino is including a rare mouse model of metastasis.

Merlino and his colleagues devised a scheme for engineering tractable preclinical mouse models by transplanting rare metastatic tumors from genetically engineered cancer models into recipient immunocompetent mice. Together, the Merlino lab and CAPR have successfully turned that concept into a preclinical model of metastasis and have begun to evaluate treatment strategies. "Early results indicate the utility in such models," said Van Dyke. "For example, in one case the primary and metastatic tumors have had distinct responses to the same therapeutic. This panel will be a valuable resource for drug and biomarker development for what is now a deadly disease." The same scheme is currently being utilized to generate metastatic NRAS- and BRAF-driven melanoma models.

(Photo: R. Baer)

Terry Van Dyke, Ph.D.

> "CAPR has honed the ability to test combination therapies rapidly and efficiently."

A newly launched partnership between the Lustgarten Foundation and CAPR is focused on preclinical development of therapeutics for pancreatic cancers, 95 percent of which are driven by RAS. The mouse model at the heart of this collaboration is one that has been engineered to develop pancreatic cancer that is extremely similar to the human disease, both at the genetic level and at the biological level. Notably, the notorious difficulty of penetrating human pancreatic tumors with administered drugs is recapitulated in the mouse model. The so-called KPC model is derived from multiple genetic events: mutations in *KRAS* and *p53* are conditionally driven and tissue specific. "The mice are completely normal until you feed them tamoxifen," said Van Dyke, "And then the oncogenes are specifically activated in pancreatic cells." Disease modeling is, of course, not limited to animals. CAPR is working with a European partner on developing organoid cultures—living tissue slices—including a model of RAS-driven lung cancer to test potential therapeutics at a more preliminary, higher-throughput stage. CAPR also works with the National Center for Advancing Translational Sciences (NCATS) on screening drug combinations. "Combination therapies are key to treating smart tumors," said Van Dyke. "CAPR has honed the ability to test combination therapies rapidly and efficiently."

Given the complexities of RAS signaling networks and the "cleverness" of tumors in evading the impact of individual drugs, equally clever approaches to combinatorial therapeutics will likely play a crucial role in defeating RAS-driven tumors.

"Good translation always has to come from a very solid and detailed understanding of molecular mechanisms," said Luo. "Nothing is quick and painless, especially in the RAS field. Viral Ras was discovered around the time I was born, and human *RAS* genes were cloned when I was a kid. But we do have new technologies now and every time that happens, an 'impossible' problem becomes accessible to new therapeutic strategies."

Putting Peptides to Work

When people think about amino acids, they may worry about getting their daily nutritional requirement to build and maintain muscle. For the more biologically sophisticated thinker, amino acids are the building blocks of peptides and proteins, which are the primary effectors of our genetic code—the enzymes and transporters and regulators of cellular function in health and disease. When Joel Schneider, Ph.D., Chief of CCR's Chemical Biology Laboratory, thinks about amino acids, he sees them as the building blocks of new materials. From his research into the fundamental mechanisms of peptide folding, he explores novel ways to address challenging medical needs. Research in his laboratory has applications ranging from tissue repair to drug delivery.

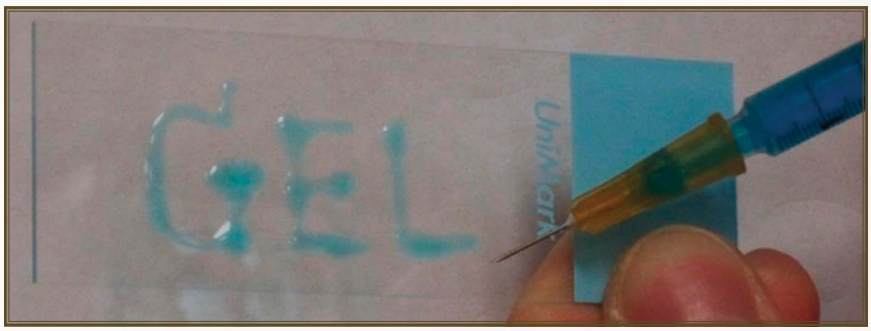
Lending a Hand

These days, cutting off a hand is not irreversible. Gerald Brandacher, M.D., Scientific Director of the Composite Tissue Allotransplantation Program at the Johns Hopkins Medical Institute, for example, specializes in reconstructive transplantation, such as whole-hand transplants. Donor hands are flown in and painstakingly attached, micrometer-wide blood vessel by blood vessel, to the recipient's forearm. "But there's a problem," explained Schneider, who is actively collaborating with Brandacher to improve this procedure. "The blood vessels are collapsed in the donor hand. It's like taking a hollow spaghetti noodle that has collapsed and trying to stitch it to another."

Dan Smith, a graduate student in Schneider's laboratory, earning his Ph.D. from the University of Delaware, is developing a gel that can be injected into the donor hand's vessels to effectively plump them up. The concept sounds simple, in principle, but the material has to be able to transition from a semi-solid gel to a viscous liquid that can be delivered smoothly to the lumen of the vessel through a syringe. However, once delivered, the material must transition back into the original gel that will fill out and support collapsed blood vessels once it is in place. (This sought-after quality—shear thinness—is the property that turns ketchup from a thick syrup into a free-flowing fluid with a squeeze of the bottle). Then, after the surgeon sutures the vessels together, the gel must undergo yet another phase transition forming a liquid so that the introduction of circulating blood at the end of the procedure can carry it away.

This anastomosis gel that Smith and Schneider are developing is made from peptides. "We use and design peptides that form fibrous molecular networks, in other words, gels," explained Schneider, "We have designed gels that are self-assembling, shear-thinning, and self-healing."

Like all good designers, Schneider and his team operate from a set of core principles that motivate a prototype and then they iteratively refine the material until it exactly suits their purpose. Schneider's academic career traces its roots to the study of protein folding and the prediction of that folding from amino acid sequences. "We work from our knowledge of protein structure—rules that have been established by ourselves and others—to design materials *de novo*. Often, we initially design something that's not quite what we are shooting for, but we learn from it and improve on it. It's an iterative process."



(Image: J. Schneider, CCR)

A syringe deliverable shear-thinning peptide gel encapsulating a blue dye for visualization

(Photo: R. Baer)

Cem Sonmez, Joel Schneider, Ph.D., Michael Giano, and Katelyn Nagy

Healing Deep Wounds

Proteins are ancient adhesives. According to Wikipedia, the oldest known bow for hunting was constructed some 10,000 years ago using glue boiled down from animal hoof protein. When a wound heals naturally, cells lay down a matrix of proteins, along with carbohydrates to form an extracellular matrix (ECM), a sort of adhesive that holds tissue together. Schneider's group has been developing novel peptide gels that mimic native ECM in efforts to enhance the wound-healing process. These gels are designed to provide the scaffolding for cells until they can remodel and rebuild the wound site.

In separate work that also involves the ECM, Postdoctoral Fellow Yuji Yamada, Ph.D., is developing a new bioadhesive that is made from two primary components: a carbohydrate and a therapeutic protein. When they are mixed together from a dual-barrel syringe (much the same technology as used for epoxy from a hardware store), they form an adhesive that chemically bonds with the ECM. The carbohydrate portion of the gel is responsible for the chemical bonding, while the protein acts as the crosslinker that defines the gel. As the material degrades, it is designed to slowly release the therapeutic protein locally to the tissue.

> As the material degrades, it is designed to slowly release the therapeutic protein locally to the tissue.

Mike Giano, a graduate student in the lab, has recently replaced the protein component of the gel with a polyamine polymer and the result is a bioadhesive polymer that is extremely antibacterial. At physiological pH, polyamines are positively charged—polycationic—which makes them toxic to microbes, including gram-positive and gram-negative bacteria, both of which Giano has tested *in vitro*. Mammalian cells, on the other hand, are unaffected by these polycationic surfaces. "The idea is to use these bioadhesive gels as wound fillers, for example, after tumor resection. The gel would not only help maintain structural integrity of the tissue as it heals, it would limit opportunistic infections," said Schneider.

Delivering and Releasing Drugs

Basic cancer biologists focus on identifying drivers of disease. But, the development of molecules that can effectively target those drivers is as great a scientific challenge [See "A Rich Legacy and a Bright Future"]. Over 30 percent of small-molecule drugs, over 90 percent of approved anticancer drugs, and nearly all protein therapeutics cannot be delivered orally. Instead, they are delivered parenterally, meaning via injection into the blood stream, into muscle, or under the skin. Schneider's laboratory is developing materials to facilitate those delivery modes in order to improve patient compliance by lowering dosing frequency, improving efficacy, and ameliorating toxicity.

One of the key attributes of many of Schneider's new materials is reversibility. Whether for blood vessel anastomosis or wound healing, the gel should not persist indefinitely. Reversibility also confers the possibility of controlled, slow-release drug delivery. With several classes of materials, it is possible to encapsulate even living cells and use the gel as a delivery vehicle to localize the therapy to the tissue before releasing it. "At NCI, of course, we are interested in delivery to tumors," said Schneider. "Imagine localizing a highly toxic small molecular therapeutic directly to a tumor while sparing healthy tissue."

Schneider's group is particularly interested in developing materials that can release interleukins at very slow, known rates. Interleukins are key modulators of the immune system, whose precise location and concentration are critical to their action. In collaboration

with Scott Durum, Ph.D., Deputy Chief of CCR's Laboratory of Molecular Immunoregulation, and Scott Walsh, Ph.D., University of Maryland Assistant Professor, Schneider's group is working on a material that can release minute amounts of IL-7 over time to stimulate T cell-mediated tumor clearance. Such a material could be introduced after a tumor resection to enhance immune surveillance and discourage recurrence.

Currently the project rests on Schneider's team developing a material that can release IL-7 with a consistent profile *in vitro*. Walsh, a protein biochemist, has previously developed methods to express IL-7 in large quantities, a prerequisite to developing the technology. And Durum, an immunologist with a longstanding interest in the mechanisms underlying IL-7's effects on T cells, has developed the animal models to test the material once it is refined.

"Honestly speaking, a lot of collaborations come about because people are friends," explained Schneider. "Scott Walsh and I were lab mates at the University of Pennsylvania. We got together for dinner one evening and just started talking about our work and hit upon the controlled release idea. Scott's ongoing collaboration with Durum, here at NCI, brought us full circle."

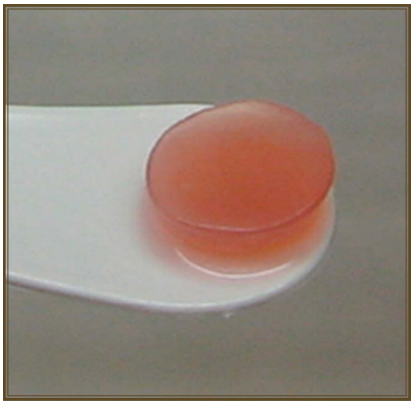
Developing the Basics

Prior to joining CCR, Schneider developed an interest in tissue engineering, with a focus on rebuilding cartilage. At a basic level, he and his team continue to study how different cell types interact with their peptide-based materials. They are exploring whether it is possible to design a material that is conducive to the growth of a particular cell type.

They have now developed a suite of materials that are more conducive to maintaining chondrocytes, the key cells that produce and maintain cartilage. By encapsulating cells within gels and then using the gels' shear thin capacity to conform the cells to molds, the team can study how the cells respond to the encapsulation, and how the nanostructure of the gel network affects the cells' ability to lay down new cartilage.

(Photo: R. Baer)

Dan Smith and Joel Schneider, Ph.D.



(Image: J. Schneider, CCR)

Chondrocytes embedded within a peptide gel produce cartilage

At an even more basic level, Schneider's group is furthering their abilities to predictively design new materials by studying the fibril network structures they produce, using a myriad of microscopy and spectroscopy techniques. Their laboratory is based at the NCI campus in Frederick, Md., which is NCI's hub for chemistry and physical sciences. In addition to chemical biology, the Frederick campus hosts high-throughput screening, structural biophysics, NMR spectroscopy, x-ray crystallography, and the world's largest, most diverse public natural product repository.

> "To be surrounded by biologists and clinicians is truly a gift that enables and inspires your own research program as a chemist."

Unique characteristics notwithstanding, the two campuses maintain strong ties. "To be surrounded by biologists and clinicians is truly a gift that enables and inspires your own research program as a chemist. When you are a faculty member of a traditional arts-and-sciences department at a university, you don't have the opportunity to rub elbows with physicians who have problems that need solving," said Schneider.

To learn more about Dr. Schneider's research, please visit his CCR Website at http://ccr.cancer.gov/staff/staff.asp?name=jschneider.

A Rich Legacy and a Bright Future

"NCI has a very old and rich history of chemistry-based research," explained Joel Schneider. "As far back as 1968, chemical research was an integral part of the Drug Development Branch. Later, chemistry was formalized with the creation of the Laboratory of Medicinal Chemistry, first headed by John Driscoll and later by a wonderful chemist named Victor Marquez."

When Marquez retired, the NCI leadership tapped Schneider, then a Professor at the University of Delaware, to extend CCR's reach beyond classical medical chemistry and forge a team of investigators focused on chemical biology. "Medicinal chemistry is necessary to bring a drug to the clinic. NCI was looking to maintain that ability but also to take advantage of the new tools and resources enabled by chemical biology to inform and hasten the discovery and development process," said Schneider.

After rising through the career ranks in a university setting, Schneider was ready for a new administrative and scientific challenge. He quickly hired three new investigators—Jay Schneekloth, Ph.D., Martin Schnermann, Ph.D., and Jordan Meier, Ph.D.—to run their own research programs in chemical genetics, organic synthesis, and chemical genomics, respectively. "The main criteria was to hire outstandingly smart people," said Schneider. "At the end of the day, research evolves, so I didn't want to hire folks based on their ability to fill a specific research niche that is hot now, but rather hire people that can identify opportunities, adapt to change, and make significant impact."

Schneider also established a synthetic core facility within the Chemical Biology Laboratory with full-time chemists who are charged with helping CCR investigators solve chemistry-based problems. "Our laboratory is surrounded by a sea of non-chemist investigators who need non-commercially available molecules to further their research. Before the core was established, they would ask us for help, but we didn't have the resources to divert away from our own research." Now, through the synthetic core facility, any CCR investigator can access the chemistry expertise he or she needs.

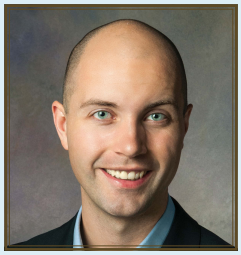
Susan Bates, M.D., a Senior Investigator in the Developmental Therapeutics Branch, recently took advantage of the facility to synthesize compounds for preclinical testing. Her team needed dual pathway inhibitors and discovered that they were not readily available. "We found two in the literature," recounted Bates. "In the case of one compound, the company that developed it did not have it on hand, but told us how to synthesize it. The other compound was not commercially available, but it was publicly reported so the core facility chemists were able to develop the synthesis. Dr. Schneider's group produced both compounds rapidly and of good quality. We already have some nice data with them. The core facility is a great resource for biologists and clinicians with chemistry-based needs."

(Photo: B. Branson)

Jay Schneekloth, Ph.D.

(Photo: B. Branson)

Martin Schnermann, Ph.D.



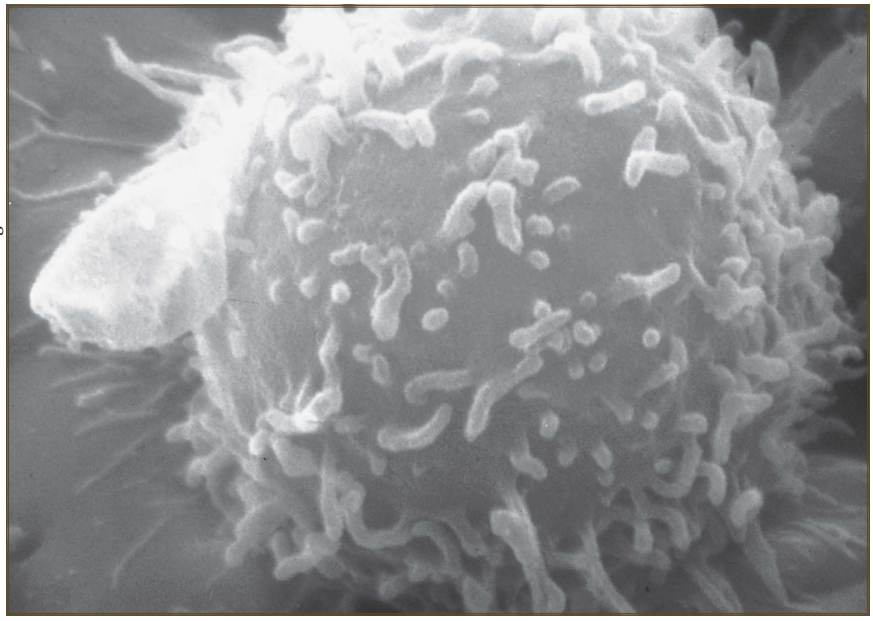
(Photo: B. Branson)

Jordan Meier, Ph.D.

Global Amplification:
A New Look at Transcriptional Regulation

Biologists often identify and describe cells based on the molecules they express. The expression of certain genes can be used to separate a stem cell from a neuron, a cancerous cell from a healthy one. However, as cells grow and adapt, they change not just the types of genes they express but the amount of that expression. David Levens, M.D., Ph.D., Senior Investigator in CCR's Laboratory of Pathology, and Rafael Casellas, Ph.D., Senior Investigator with the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and Adjunct Investigator in CCR's Laboratory of Cancer Biology and Genetics, came together to study genome-wide transcriptional regulation in B cells from two very different vantage points. Together, they are discovering that global transcriptional amplification is a uniquely regulated process.

(Image: National Cancer Institute)



Electron microscopic image of human lymphocyte, which consists of T cells and B cells

Over the last 20 years, David Levens and his colleagues have learned a lot about the regulation of a single gene: *myc*. But, *myc*'s well-known importance as a critical regulator of cellular growth, both during development and in cancer, was not Levens' chief concern. "I stayed out of studying *myc* function for a long time," said Levens. "I was more interested in the mechanisms of gene regulation. *Myc* seems to have one of the most complicated promoters around. And no one had a comprehensive model of how a cell decides how much *myc* to make and when."

As a result of their investigations, Levens' laboratory has put together a fascinating and unusual story of transcriptional regulation. Among the many regulators of *myc* expression, they found proteins that were not binding to classical double-stranded segments of DNA (dsDNA). Instead, they traced the action of these factors to a single-stranded DNA (ssDNA) element in the promoter region, the Far Upstream Sequence Element (FUSE).

Molecular forces predispose DNA to adopt the famous Watson-Crick double helix when its bases are appropriately paired: adenine (A) with thymine (T), guanine (G) with cytosine (C). But that seemingly static picture changes with transcription. As DNA is screwed through the active site of enzymes that travel along it—DNA polymerases, RNA polymerases, helicases—rotational forces are transmitted through the DNA. "We saw that at particular sites on the DNA, the dsDNA would essentially buckle, popping open like the threads of a rope unfraying at particular sites." This turned out to be important for *myc* regulation.

Levens hypothesized that FUSE regulation was occurring once other transcriptional initiation events had been precipitated. "The cell can sense how much *myc* is

being made—not how much has already been made," said Levens. "The element senses in real time how much Myc is being made and allows the cell to respond to that." This mechanism differs from feedback regulation, which relies on the actions of the end-product feeding back on the production process and which occurs widely in cellular signaling pathways, but suffers from inevitable delays. "For the FUSE mechanism to evolve, rapid fluctuations in Myc must be deleterious to the cell," said Levens.

Based on their work with the *myc* promoter, Levens began working on a way to assess how broadly such changes in DNA structure occur. "I don't really believe there are principles that apply to a single gene," said Levens. "When Nature develops a useful trick, she uses it over and over again, modifies it, plays with it, and finds new ways to exploit it."

Mechanism Meets Action

"One of our lab's main interests is to understand B-cell activation during the immune response. When I joined the NIH 10 years ago I decided to approach this problem from a nuclear standpoint," said Rafael Casellas. Casellas' laboratory uses genomics approaches to study mouse B-cell development.

B cells first arise in the bone marrow. They then migrate to the periphery, where they remain quiescent until encountering antigens, at which point they rapidly proliferate and differentiate into cells with more specialized immune functions to respond to the threat. Major changes in the quantity and quality of gene expression occur during these different phases.

"Rafael is a very courageous scientist. He is unafraid to take big steps into new areas," said Levens. "When he heard that we were developing a method to study DNA structure on a genome-wide level, he wanted to apply it."

The method they developed was based on potassium permanganate ($KMnO_4$), which oxidizes nucleotides if they are not base-paired, disrupting the DNA structure so it cannot refold. In theory, therefore, it could be used to signal the presence of alternative DNA structures. "The problem is that the average base pair in DNA is flipping in and out of a double helix about 100 times per second, for only a fraction of a microsecond. That's enough for permanganate to react with them," said Levens. So his team introduced an enzymatic step to the process in which only DNA that had multiple bases oxidized within a small region would be cut across both strands, reducing the impact of random events.

Getting this method to work required a thorough appreciation of the fundamentals of nucleic acid chemistry, biochemistry, and biophysics. "I've had some great teachers for nucleic acid structures and chemistry, and I've had a lot of experience—when I was in grad school, there were no kits," said Levens. He and his colleagues were confident in their success in developing the technique, but they needed to test it. "Because there had been so few studies of ssDNA conformations in living cells that had been both characterized and accepted, we needed an unimpeachable gold standard to test whether the new method was working. We picked transcription bubbles."

(Photo: NIH-IRP)

Rafael Casellas, Ph.D.

Transcriptional Amplification

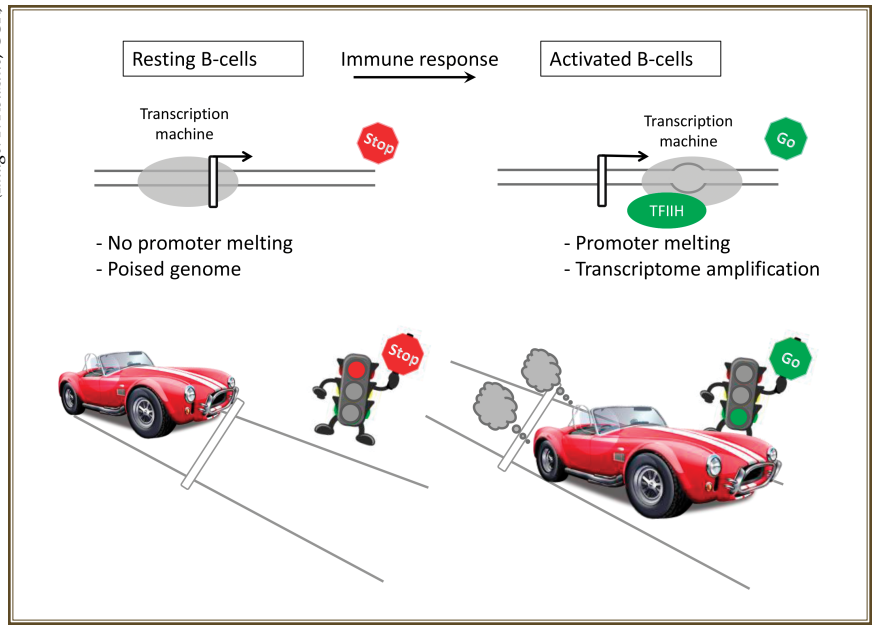
Transcription bubbles form when RNA synthesis is initiated. After initiation, RNA polymerase begins the process of elongation, traveling along the DNA and locally unwinding or "melting" it to allow focal RNA hybridization within the enzyme. Levens and his colleagues found that they could detect this melting in a Burkitt's lymphoma cell line with their assay.

"David's laboratory had put together a biochemical assay to measure promoter DNA melting in live cells," explained Casellas. "Conversely, our laboratory had used deep-sequencing protocols to create genome-wide maps of more than 40 chromatin modifications, polymerase recruitment, and RNA synthesis, so we decided to complement these datasets by mapping ssDNA in the entire genome."

Moving away from their initially encouraging results in Burkitt's lymphoma cells, Casellas wanted to test resting B cells. To their disappointment, they saw practically no promoter melting whatsoever.

"...we needed an unimpeachable gold standard to test whether the new method was working. We picked transcription bubbles."

(Image: F. Kouzine, CCR)



Unmelted promoters help to limit transcription in resting B cells (left), whereas in activated B cells promoters are melted and transcription has progressed further downstream to support higher levels of expression (right).

"That shouldn't happen," said Levens. "We thought it hadn't worked." But repetition confirmed the results, as did a comparison with activated B cells.

In a paper published in *Cell* earlier this year, Levens and Casellas described their overall findings. They found that resting B cells have very low basal gene expression, but are poised for a massive increase in gene expression, dependent on promoter melting. Approximately 90 percent of the promoters for genes that will be expressed once the B cell is activated are already loaded with RNA polymerase, but unmelted. Concurrently, these same promoters lack virtually all subunits of the transcription factor IIH (TFIIH) complex, which spurs promoter melting and transcriptional elongation. Basal gene expression levels therefore remain low until the cell is activated, at which point the TFIIH complex is recruited to the gene promoters and gene expression increases 10 to 15 fold.

"If you are a little cell and you want to become big rapidly, how do you do that?" asked Levens. "What's the switch you have to throw to make everything bigger? When you look at a resting cell, it expresses largely the same genes as fully active cells." TFIIH is part of that story, but only one part.

Back to *myc*

In a separate line of investigation, Levens' laboratory was venturing into functional studies of *myc*. "The literature made it sound as if *myc* was some kind of master decision maker, setting precise levels of gene expression. These studies weren't dealing with the integration of *myc* regulatory mechanisms with its function," said Levens. Most of the literature was based on work in which *myc* was overexpressed stably or induced at highly unphysiological levels for prolonged periods of time. Levens' team created knock-in mice in which normally regulated Myc was tagged with the enhanced green fluorescent protein (EGFP). Casellas offered his expertise in activating *myc* in specific cell populations.

"We thought it hadn't worked." But repetition confirmed the results, as did a comparison with activated B cells."

"The two projects advanced in parallel originally; we didn't realize they would reinforce the same point about transcriptome amplification," said Levens.

Given the vast literature on the topic, it may be surprising that *myc* targets are not well enumerated. Myc is a basic helix-loop-helix leucine zipper (bHLH-Zip) transcription factor, which conventionally means that it dimerizes with a partner Max to bind preferentially to DNA sequences known as E-box motifs.

Working with Keji Zhao, Ph.D., Senior Investigator in Laboratory of Epigenome Biology of the National Heart Lung and Blood Institute (NHLBI) and the inventor of the ChIP-Seq method for genome-wide analysis of chromatin modifications, Levens and his colleagues analyzed genome-wide binding of EGFP-tagged Myc. Their results were consistent with reports that Myc prefers specific to nonspecific binding sites by 200:1. "That sounds like it could be reasonably specific, until you realize that the lac repressor has up to a million-fold preference for specific versus nonspecific binding. 200x is enough to bias, but not to determine targeting," said Levens.

In activated and resting B cells, they compared the genome-wide distributions of *myc*, gene expression levels, RNA polymerase II binding, and chromatin modifications.

(Photo: R. Baer)

Fedor Kouzine, Ph.D., David Levens, M.D., Ph.D., and Zuquin Nie, Ph.D.

"Putting everything together, it seemed to me that you could make almost all the problems of the *myc* literature go away by positing that it is amplifying expression, not determining gene expression. When you turn *myc* on, everything goes up," said Levens.

"I think some scientists still have reservations about Myc playing an amplifying role in transcription. One would expect such a reaction when a long-established idea is displaced by a new finding," said Casellas. "At the same time, because the new model explains the data better, a large fraction of the community has accepted the idea."

One reason that gene expression amplification may have gone unnoticed in previous studies is the way experiments are conducted. Gene expression is usually normalized when comparing cell populations, meaning that the same amounts of mRNA are typically compared between, for example, resting and activated B cells.

Going Forward

"The most important unanswered questions in biology have not changed that much in the past 25 years," said Casellas. "What has changed is how we approach them and bioinformatics has definitely revolutionized the way we do it. At the same time, the greatest challenge now is to obtain a holistic view of the cell."

Bioinformatics has played a critical role in these studies, particularly in analyzing the ssDNA assay data. "In our first data sets, Fedor Kouzine spent three days looking at chromosome 22—the smallest chromosome—by hand. We could see interesting features in the data, but we didn't have dense enough sampling. It became a computational problem to identify DNA structures," said Levens.

Levens was co-chairing a trans-NIH search committee for the Tenure-Track Earl Stadtman Investigators program, when he met committee member, Teresa Przytycka, Ph.D., Senior Investigator in the National Center for Biotechnology Information. "One day I turned to her and asked if she knew anyone who might be interested in helping us and she said, 'How about me?' I was delighted," said Levens.

Having succeeded with transcription bubbles, the collaborators are now venturing further into uncharted waters to survey other alternative DNA structures. A vast biophysical literature provides numerous examples of structures that do not conform to the Watson-Crick double helix, including the left-handed double helix (Z DNA) and quadraplex structures, in which one DNA strand folds up on itself. Many are likely not to be biologically significant. "The best-understood examples have occurred in bacteria; the literature is less cohesive, although considerable in mammalian cells," said Levens.

"When we started this work, certainly back when we started working on alternative DNA structures—there was no funding agency in the world that would have not considered this too outside the box to fund. Here, we were able to get together and share resources without petitioning for money up front. The hardest part was the three of us coming together," said Levens.

> "...because the new model explains the data better, a large fraction of the community has accepted the idea."

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

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

COMMENTARY

Cross-Presentation: For Better or Worse

Anne Hosmalin, M.D., Ph.D., is a Research Director and Professor at the Cochin Institute in Paris, France. She obtained her M.D. from Descartes University in 1986 and her Ph.D. in Immunology from Pierre & Marie Curie University. Her three-year postdoctoral Fogarty fellowship with Jay Berzofsky, M.D., Ph.D., now Chief of CCR's Vaccine Branch, in the early days of HIV vaccine research, kindled interests that still form the basis of her current research agenda. Her work spans basic investigations into the roles of antigen-presenting cells in orchestrating immune responses to the quest for more effective vaccines. She also serves as President of the French Society of Immunology.

When the immune system encounters a new microbe or a nascent tumor, a quick and effective defense is critical to good health; when the same system encounters an allergen or a cell from a developing fetus, tolerance is equally essential. I study dendritic cells because they are at the center of the immune system's decision to mount a defense or promote tolerance. They process antigens from a variety of sources and present them as foe or friend to lymphocytes that carry out the immune response. My laboratory is particularly focused on cross-presentation, whereby dendritic cells present exogenous antigens to cytotoxic T cells (CTLs) to promote their widespread destruction. Understanding and coaxing effective cross-presentation is central to vaccine development. Dendritic cells are a natural immunological adjuvant, but we are still learning how to stimulate them optimally.

From Mice to Man

I first became interested in antigen presentation during my postdoctoral work on HIV. It was a very exciting time in Jay's laboratory, during which we found the first viral epitopes that elicited CTL responses, and that formed the basis of early vaccine attempts. When I went back to France, I decided to move from studying antigen presentation in mice to investigating the properties of human dendritic cells. At the time, very few people were studying them; one had to isolate them with great difficulty from lymphoid organs. Once methods were developed to culture them from monocytes, the dendritic cell field exploded. A great deal has been learned about the mouse dendritic cell system, and how different subpopulations function; however, it has been a challenge to translate much of the work that has been done in mice into humans.

We have learned that populations of dendritic cells in the blood can perform cross-presentation; my laboratory was the first to demonstrate this function for plasmacytoid dendritic cells, which are relatively small cells, known for producing α-interferons in response to viruses. People were initially quite resistant to the idea that these cells were also antigen-presenting cells, but now there are even some tumor vaccine trials in the Netherlands and France that take advantage of these dendritic cells. We were also one of the teams that published four back-to-back *Journal of Experimental Medicine* papers in 2010, which identified a population of CD141+ dendritic cells in human blood that are equivalent to the well-studied mouse CD8+ dendritic cells that specialize in cross-presentation.

> Understanding and coaxing effective cross-presentation is central to vaccine development.

(Photo: Courtesy of A. Hosmalin)

Anne Hosmalin, M.D., Ph.D.

HIV and Hyperactivation

In 1999 we showed that there was a deficiency in the circulating dendritic cells of chronic HIV patients. Recently, we found that there is a subpopulation of nonclassical monocytes—not dendritic cells—expressing M-DC8 that are more numerous and hyperactivated in patients with elevated levels of the HIV virus.

HIV is relatively well controlled in patients from wealthy countries, but patients still suffer from a counterintuitive hyperactivation of the immune system, causing the immune system to age faster than it should and also producing cardiovascular complications whether or not patients are being treated with antiretrovirals. Depletion of CD4+ T cells protecting the gut opens the way for bacterial lipopolysaccharides (LPS) to chronically stimulate an immune response, even in the absence of significant active HIV virus.

When M-DC8+ monocytes are stimulated with LPS, we found that they secrete TNFα, one of the main cytokines responsible for immune hyperactivation. So we hope if we can neutralize this specialized cell population, we can break the vicious cycle of immune hyperactivation that occurs even under antiretroviral therapy. If we succeed in calming the immune system, interruptions of antiretroviral administration might become possible, which would improve quality of life.

Whereas many other countries have stopped or slowed their research on HIV, the French AIDS Research Agency (ANRS), like the NIH, has sustained funding over time, making France one of the strongest contributors to the literature on HIV and retrovirology. The ANRS supports basic research on immunology and virology, as well as research in the social sciences. As industry became disenchanted with the early failures of HIV vaccine research, it has been up to agencies like the NIH and ANRS to solve some of the fundamental challenges limiting our success.

Life at the Cochin Institute

The Cochin is a large institute, jointly supported by Institut national de la santé et de la recherche médicale (INSERM), Centre national de la recherche scientifique (CNRS), and the University Paris Descartes. It comprises 650 people who are divided among 35 teams and technological platforms. In addition to running my own laboratory, I am also Director of the Department of Infection, Immunity, and Inflammation, one of three departments in the Institute. The department comprises 14 teams that work on immunology—from immune cells to diseases such as infections, cancer and autoimmune or inflammatory diseases—and on host interactions with bacteria, parasites, and retroviruses.

If we succeed in calming the immune system, interruptions of antiretroviral administration might become possible, which would improve quality of life.

In France, the Cochin Institute was the first to develop a model in which we could systematically share cutting-edge technological platforms, much as the NIH has core facilities with staff dedicated to maintaining and building on valuable technologies for the benefit of a variety of investigators. In addition, there are many different competencies among the teams, so, for example, we can easily find an expert on cell signaling to share techniques or reagents, even if they are in another department altogether. It is a rich environment for young researchers, which is important to me. On a day-to-day basis, one of the aims in my scientific life—besides those of achieving new treatments or uncovering basic mechanisms of antigen presentation—is to pass along to the next generation of researchers the scientific and ethical values, as well as the mentorship and opportunities that I have received.

Treating Bladder Cancer:

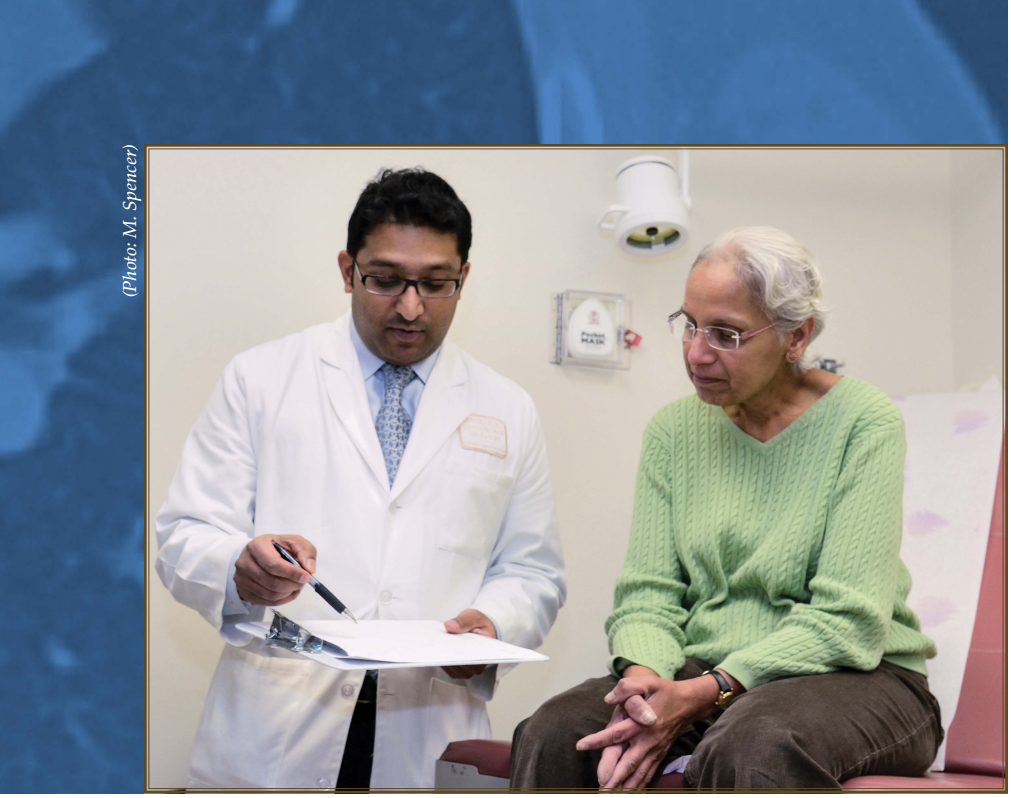
From Primary Tumor to Metastasis

As a Fellow at Memorial Sloan-Kettering Cancer Center (MSKCC), Andrea Apolo, M.D., gained valuable experience in the design and execution of clinical trials for bladder cancer, as well as a passion for this relatively neglected area of clinical research. As a result, she jumped at the opportunity to develop a new bladder cancer program at CCR, accepting a position as Assistant Clinical Investigator and Head of the Bladder Cancer Program in 2010. Less than two years later, Piyush Agarwal, M.D., was recruited from the Henry Ford Vattikuti Urology Institute in Detroit to become Head of the Bladder Cancer Section in CCR's Urologic Oncology Branch. His deep experience with robot-assisted minimally invasive surgeries to resect tumors and reconstruct bladders is combined with an equally strong ambition to make surgery for the treatment of bladder cancer obsolete through molecular therapeutic strategies. Apolo and Agarwal gave CCR connections *a first-hand account of their work in the lab and in the clinic to understand and treat progressive stages of the disease.*

Piyush Agarwal Starts from the Beginning

The treatment for most bladder (urothelial) tumors, if they are low grade, is surgical resection followed by surveillance. The high recurrence rate generally means routine lifelong surveillance. With disease progression and invasion of the surrounding muscle tissue, nonspecific immunotherapies and chemotherapies are the only approved options before more radical surgeries become a reluctant last hope.

Working with one of the pioneers of robotic surgery in bladder cancer, Mani Menon, M.D., I developed the expertise to do quite complicated reconstructive procedures with robotic assistance. Use of this technology translates to smaller incisions, less blood loss, and shorter stays in the hospital. In the most challenging cases of radical cystectomy, we remove the bladder and replace it with a bladder that we construct from bowel tissue. This is standard-of-care for advanced cases and when it works—and we cure someone—it's wonderful. But, even in the most expert hands, this surgery



(Photo: M. Spencer)

Piyush Agarwal, M.D., and Mangala Hari Prasad

has a 30–60 percent complication rate; and despite our best efforts, only about half of patients with muscle-invasive disease survive five years.

We haven't had a new FDA-approved drug for bladder cancer since the 1990s. The number of cases is increasing each year and there hasn't been much impact on mortality. But, I see a lot of untapped opportunity to combine advances in our understanding of the molecular drivers of bladder cancer with therapeutic progress that is emerging from other areas of cancer research.

Finding the Drivers

In building up our bladder cancer program at CCR, we have recruited patients with the dual goals of treating them with the best standard-of-care and studying their tumors for possible therapeutic targets. We have found that the epidermal growth factor receptor (EGFR) is over-expressed in many bladder cancers. EGFR overexpression is associated with several cancers; my own familiarity with it stems in part from my time as a Urologic Oncology Fellow at M.D. Anderson Cancer Center; the former President, John Mendelsohn, M.D., was instrumental in developing cetuximab (now known as Erbitux) to target EGFR for the treatment of lung cancer. But, as we have learned from that cancer and many others, focusing on a single target is seldom sufficient.

Bladder cancer is more prevalent in men than in women, even when you control for effects of smoking and environmental exposures. But, when women develop bladder cancer, it tends to be more aggressive. We think there is an interplay of steroid hormone receptors—androgen receptors (ARs) and estrogen receptors (ERs)—in bladder cancer. ARs are, of course, involved in prostate cancer and drugs like MDV3100 have been developed against them. So, we are analyzing AR and ER expression in bladder tumors, as well as the expression of other steroid hormone receptors, such as those for progesterone and glucocorticoids.

Illumination and Destruction

Meanwhile, Peter Choyke, M.D., and Hitsataka Kobayashi, M.D., Ph.D., in CCR's Molecular Imaging Program have done some beautiful work on a therapeutic approach that uses photoactivation of a molecularly targeted dye to induce tumor cell death. In a paper published in *Nature Medicine* in 2011, they used an infrared light-sensitive dye conjugated to an anti-EGFR antibody to target tumor cells. Application of near infrared light induced phototoxicity only in cells that had bound the dye-conjugated antibodies. The advantage of such a technique for bladder cancer is that the internal bladder surface is accessible through the urethra, making nonsurgical intravesical therapies possible.

We have been extending this work in bladder cancer cell lines, several of which I was able to bring with me from M.D. Anderson, and in other, newer lines which we have generated here at CCR. We've found that the cell lines that express EGFR respond to this photoimmunotherapy approach in a rather exquisite way: a minute or two of exposure to light kills 90 percent of the cells. The response appears to be dependent on the number of receptors expressed and the amount of energy delivered.

Our next step will be to deliver the drug directly into animal models. In parallel, we are also looking at other molecular targets to see if we can take a combinatorial approach to this therapy. If this approach continues to deliver promising results, I foresee a clinical trial in which we use standard techniques (flow cytometry, Western blots) to analyze the surface receptor profile of individual tumors and then develop a cocktail photoimmunotherapy approach to target the cancer cells and spare the healthy ones.

Insights from Tuberculosis?

The pioneering work of Burton Zbar, M.D., (formerly, Chief, Laboratory of Immunobiology, NCI) and Alvaro Morales, M.D., established the use of the tuberculosis vaccine—Bacillus Calmette-Guerin (BCG)—in the treatment of bladder cancer. In cases where the tumor burden is not too high and direct contact can be made with the urothelium surface of the bladder, BCG application appears to elicit an immune response that attacks the tumor as well as the attenuated virus. The use of BCG for the treatment of bladder cancer was approved by the U.S. Food and Drug Administration in the 1990s. However, we still don't completely understand the mechanism of action of BCG in eliciting an immune response.

We want to study this immune response, but in the context of a novel immunological tool. Our colleagues James Gulley, M.D., Ph.D., Chief of CCR's Genitourinary Malignancies Branch, and Jeffrey Schlom, Ph.D., in CCR's Laboratory of Tumor Immunology and Biology, have developed a tumor vaccine—PANVAC—that contains transgenes from two relatively common carcinoma markers, CEA and MUC-1. We will be opening a trial for patients who have elected against bladder removal and would, therefore, normally receive BCG alone. In our trial, half of these patients will receive BCG and the PANVAC vaccine. By studying the immune response before and after treatment, we will hopefully not only learn more about how BCG works but also learn whether we can augment the response.

> "One of the challenges with genitourinary cancers and metastatic disease in general is the difficulty of visualizing the cancer, both to discover its extent and to monitor the response to treatment."

I look at the bladder cancer program at CCR as a spectrum. I focus on primary urothelial disease, including cancers that invade the muscle tissue; my colleague, Andrea Apolo focuses on metastatic bladder cancer. We try to devise therapies coming from both directions along this continuum.

Andrea Apolo Continues the Fight

I initially became interested in bladder cancer while I was a Fellow at MSKCC, working with Dean Bajorin, M.D., a world-class expert on this disease. I really saw the need for more clinical research. In the Western world, bladder cancer is the fourth most common cancer in men and the ninth most common in women. The disease is very aggressive, but has not received the funding or research attention of other "higher profile" cancers. One primary reason is a lack of advocacy and disease awareness. It's a cancer that people don't really talk about. Especially in women, the symptoms are often treated as an infection and diagnosed very late.

I work with the Bladder Cancer Advisory Network (BCAN) to both raise awareness and help build a network of investigators across the country through our annual "Think Tank" meeting. At the Think Tank, researchers and clinicians interested in bladder cancer can brainstorm ideas for projects in bladder cancer research and can collaborate on clinical trials. I co-chaired this year's meeting in Colorado and will chair next year's meeting in San Diego. Bladder cancers are very smart tumors with multiple driver mutations that can change over time with treatment. The majority of my patients have metastatic disease, which is currently incurable. Chemotherapy is the only standard-of-care.

(Photo: R. Baer)

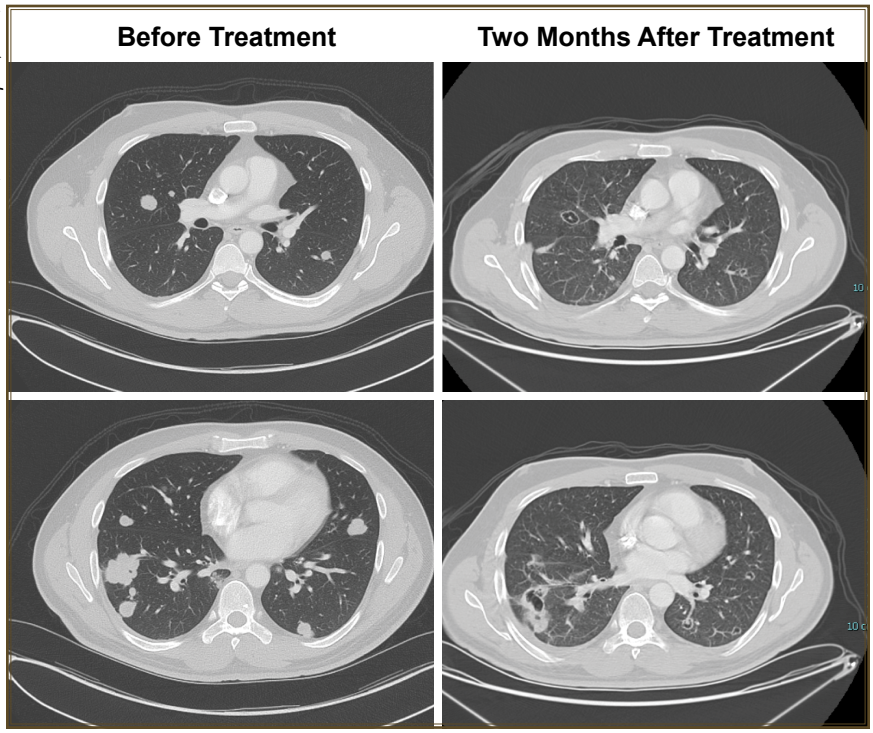
Andrea Apolo, M.D.

> "I look at the bladder cancer program at CCR as a spectrum. I focus on primary urothelial disease, including cancers that invade the muscle tissue; my colleague, Andrea Apolo focuses on metastatic bladder cancer."

Targeting Blood Vessels

However, we are seeing some very encouraging results in trials of more targeted agents. I am particularly interested in drugs that inhibit new blood vessel formation (angiogenesis) through inhibition of the vascular endothelial growth factor receptor (VEGFR) and their

(Photo: A. Apolo, CCR)



Computed tomography (CT) chest images of a urothelial cancer patient with lung metastases who received cabozantinib.

use in combination with agents that target tumor signaling pathways, as well as with chemotherapy.

As a Fellow at MSKCC, I was involved in and privy to early trials of two anti-angiogenic agents for the treatment of metastatic bladder cancer—sunitinib and bevacizumab—and the results were tantalizing, especially coupled to the fact that we see higher expression of VEGFR in the tissue, blood, and urine of patients.

In the last three years, I have opened five clinical trials for advanced bladder cancer. Currently, I have a phase 2 clinical trial underway of a single agent—cabozantinib—that primarily inhibits VEGF-R2 and the MET signaling pathway. I worked with the company that makes cabozantinib—Exelixis—and NCI's Cancer Therapy Evaluation Program (CTEP) to design the clinical trial. We have seen some dramatic results. In some cases, tumor shrinkage of over 30 percent, which we just haven't seen before. [See "Ongoing Trials"]

In collaboration with Donald Bottaro, Ph.D., Staff Scientist in CCR's Urologic Oncology Branch, we are trying to understand the role the MET pathway plays in the development and progression of bladder cancer. In addition, colleagues from the Laboratory of Pathology, Maria Merino, M.D., and Mark Raffeld, M.D., are looking at the expression of MET in the tissue of all the patients enrolled in the cabozantinib study.

As a result of the impressive responses we have seen, I am currently working on developing a multicenter randomized phase 3 trial, which would be the first such trial in the U.S. of a targeted agent for treatment of urothelial cancer.

Getting a Better Look

One of the challenges with genitourinary cancers and metastatic disease in general is the difficulty of visualizing the cancer, both to discover its extent and to monitor the response to treatment. I have several ongoing studies with Peter Choyke, M.D., Liza Lindenberg, M.D., and Karen Kurdziel, M.D., in the Molecular Imaging Program, and Les Folio, D.O., in NIH's Radiology and Imaging Sciences to explore the use of different tracers and imaging modalities.

We have found, for example, that sodium fluoride (NaF) is much more sensitive than the commonly used technetium-99 bone scans in detecting metastatic lesions in the bone. Bone disease in bladder cancer has simply not been assessed. This begs the question of whether the lesions we observe are actually real instances of disease. (We are working on tracers that illuminate particular molecular signatures of disease, e.g. MET expression, but these are still in preclinical development.) So, we do longitudinal studies to follow their progression over time; we hope to follow them for a year, but the reality is that most patients do not survive that long with metastatic disease.

The Program

When I arrived at NCI, there was no bladder cancer program. In the last three and a half years, we have seen over 200 patients come through the program. Particularly for metastatic disease, recruiting patients can be very challenging. The median age is about 70 and about half are former smokers so there are usually several comorbidities and their life expectancy is not long. We have a network of investigators outside NCI that send us patients; and I am also part of a Genitourinary Cancer Multidisciplinary DC Regional Oncology Project (GUMDROP)—a consortium of clinicians who specialize in genitourinary tumors across Maryland, Virginia, and the District of Columbia.

Within the program, we have medical, surgical, and radiation oncology specialists, supported by nurse practitioners and research nurses. We also have several scientists within NIH that are collaborating with us, including a geneticist—Ludmila Prokunina-Olsson, Ph.D., in NCI's Division of Cancer Epidemiology and Genetics—who is working on genome-wide association studies (GWAS) of bladder cancer. Together, I think we have the critical mass and momentum to make a real difference for this disease in the years to come.

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

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

Ongoing Trials

Chris Hamilton is participating in his third clinical trial at the NIH Clinical Center for metastatic bladder cancer.

(Photo: R. Baer)

Chris Hamilton and Andrea Apolo, M.D.

As a communications engineer, Chris Hamilton is used to facing complex challenges. In September 2011, while testing equipment in the deserts of White Sands, N.M., Chris was faced with a new and unanticipated challenge: the symptoms of metastatic bladder cancer. "I don't smoke, I don't really drink. I have mostly just worked and had a family. Probably the first outward sign was being tired and I didn't really recognize that until I started bleeding. Even then, I thought it was an infection." He flew back to his home in Baltimore, Md., where doctors at Johns Hopkins Medical Institute diagnosed him with stage 4 disease that had spread to his lungs.

"Right after the diagnosis, it was a mad rush to try and figure everything out," said Chris. He took 3 months' leave, during which he underwent the grueling standard-of-care chemotherapy regimen and researched his options. He quickly learned that the likelihood of significant remission was low and that he would need other options. He met with clinicians at the NIH, Memorial Sloan-Kettering Cancer Center, and Thomas Jefferson University's Kimmel Cancer Center. "Unfortunately, there was no clear answer," said Chris.

When the cancer did return, Chris opted to enter an NCI clinical trial. The first trial he entered was not a good match, but in the meantime, Andrea Apolo, M.D.'s trial of cabozantinib for metastatic cancer opened up. "I was really fortunate she was there to expedite the transition," recalled Chris. "Once it was clear that the first drug wasn't working, I was on another trial within two weeks."

With an expected survival time of only 3–6 months, Chris remained on cabozantinib for 11 months before his cancer progressed earlier this year. "Probably the best scan I had was around month eight—the cancer was clearing up, under control, and diminished to the extent that it wasn't really impacting my abilities." Chris continued working throughout the treatments, taking one or two days per week for clinical visits. "It was light years better than doing chemotherapy."

Now, Chris is embarking on a vaccine therapy trial conducted by Lauren Wood, M.D., Head of CCR's Vaccine Branch Clinical Trials Team. Grateful for all the federally funded care he has received and for the time these trials have given him, Chris is concerned that others around the country with lethal cancers are not as easily connected to the right trials. "For fast moving cancers, the process itself can kill you if it takes two months to get on a drug. Having a good advocate can make all the difference."

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

Web Sites with More Information about CCR

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

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

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

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

Patient Information on Cancer and Clinical Trials

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

How to Refer a Patient
https://bethesdatrials.cancer.gov/refer_a_patient

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

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