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CCR connections

CENTER FOR CANCER RESEARCH

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The Complex, Inner Life of T Cells

U.S. DEPARTMENT
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Center for Cancer Research

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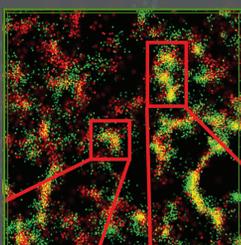
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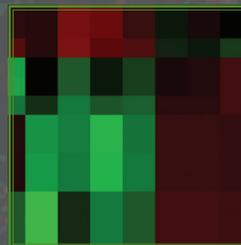
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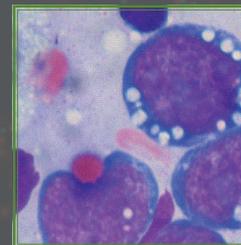
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Curing Lymphomas, One Subtype at a Time



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The mission of CCR is:

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

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

Do No Harm: Counteracting Side Effects of Cancer Therapies

Side effects from cancer therapies are a price that many patients have willingly paid for hope in the face of life-threatening disease. But the impact of aggressive therapies can be far from negligible, affecting quality of life and compromising health. As our ability to treat and even cure cancers has improved, we have the luxury of shifting some focus away from the front lines of cancer defense to include greater consideration of collateral damage. CCR investigators are actively pursuing strategies to improve not only antitumor efficacy, but also quality of life for patients treated with necessarily harsh chemotherapeutics, radiation therapies, and immunotherapies.

Ten years ago, CCR initiated a program to address chronic Graft versus Host Disease (cGvHD). As Steven Pavletic, M.D., M.S., explains in "Treating the Cure: Grappling with cGvHD after Hematopoietic Stem Cell Transplants," the fact that these transplants have been so successful has led to an increase in the number of patients who experience cGvHD, a multispectrum disorder around which the medical community found it difficult to organize systematic support. CCR created both a clinical program and convened a worldwide consensus project to establish guidelines and criteria for clinical research. This effort has helped to set the stage for testing drugs to counteract cGvHD.

CCR has played a pivotal role in the development of several

drugs approved by the U.S. Food and Drug Administration. Among them, the enzyme glucarpidase has recently been approved to treat rare cases of potentially lethal toxicity caused by accumulation of the first-line chemotherapeutic agent, methotrexate. As explained in "Epic Journeys from Bench to Bedside," Brigitte Widemann, M.D., has shown that the drug can prevent acute kidney dysfunction when administered to patients with high plasma concentrations of methotrexate.

As we learn in "Curing Lymphomas, One Subtype at a Time," Wyndham Wilson, M.D., Ph.D., and Kieron Dunleavy, M.D., have not only focused on efficacy, but also on minimizing toxicity from radiation and chemotherapeutic agents. They have recently been able to dramatically lower the aggressiveness of treatment for Burkitt lymphoma while achieving better therapeutic outcomes.

Over time, an additional hope for more effective therapies with fewer side effects lies in precision medicine—in which cancer cells are targeted at a molecular level, sparing even closely surrounding healthy tissues—and in immunotherapeutic approaches which target cancer antigens. As we learn in "The Complex, Inner Life of T cells," Larry Samelson, M.D., Ph.D., has been instrumental in laying the groundwork to understand T-cell receptor function, and is now bringing that depth of knowledge to



(Photo: R. Bauer)

Lee J. Helman, M.D.

the development of more effective immunotherapies.

Of course, the ultimate safeguard against therapeutic side effects is prevention. John Schiller, Ph.D., and Douglas Lowy, M.D., were recently recognized with the National Medal of Technology and Innovation for their work in creating vaccines against the human papilloma virus (HPV), which are now available for the prevention of HPV-associated cancers (see "CCR Researchers Awarded the National Medal of Technology and Innovation," *CCR connections* Vol. 8, No. 2). We are starting to not only defeat cancers, but defeat them on our own terms, without harm.

Lee J. Helman, M.D.

Scientific Director for Clinical Research
Center for Cancer Research

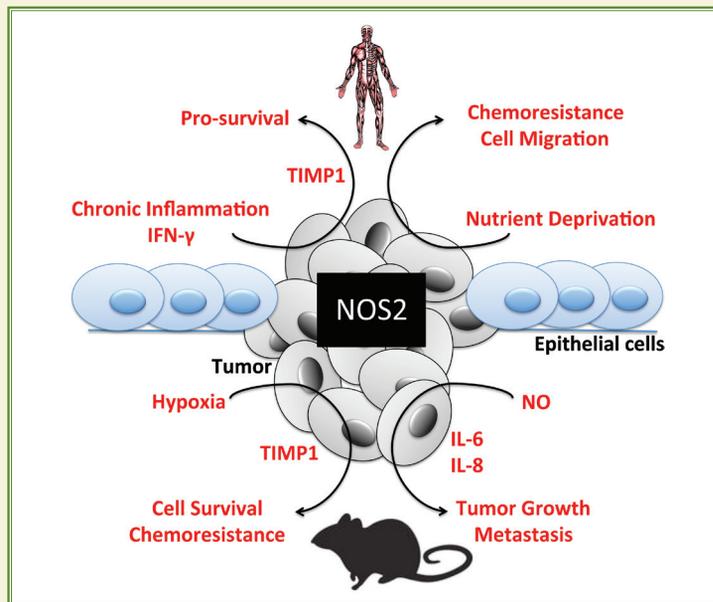
From Expression to Action: the Answer is NO

Nitric oxide plays an important role in aggressive breast cancers.

Among the most aggressive breast cancers are those that lack the estrogen receptor α (ER-), and have a “basal-like” gene expression signature. In 2010, CCR researchers led by Stefan Ambs, Ph.D., M.Ph., Senior Investigator in the Laboratory of Human Carcinogenesis, in collaboration with a research team led by David Wink, Jr., Ph.D., Senior Investigator in CCR’s Radiation Biology Branch, demonstrated that these ER- cancers frequently over-express inducible nitric oxide synthase 2 (NOS2) which induces a basal-like gene expression pattern and also associates with poor patient survival. The researchers hypothesized that NOS2 may play an important causal role in the progression of ER- cancers. A new study, published in *Proceedings of the National Academy of Sciences*, set out to further validate this observation, and to identify the likely mechanisms of its action.

Establishing causality in cancer is not the work of a single study, approach, or laboratory, so a multi-disciplinary team led by Wink including Ambs, as well as researchers from the Pediatric Oncology Branch and the Laboratory of Experimental Immunology, took up the challenge. Their strategy included two broad approaches: (1) manipulating nitric oxide (NO) levels in cell and animal models with the NOS2 inhibitor aminoguanidine (AG), and the NO-donor DETA/NO, and (2) simulating the tumor microenvironment by placing cells in the context of serum withdrawal, hypoxia, cytokines, and cancer therapeutics.

The team chose an aggressive mouse model of breast cancer: MDA-MB-231 human breast cancer cells implanted in the mammary tissue



(Image: J. Heinecke and L. Ridnour, CCR)

Nitric oxide synthase (NOS2) participates in an extensive network driving cancer progression and metastasis in ER- breast cancer.

of female mice with compromised immune systems. They tagged the cells with green fluorescent protein (GFP) to make them easy to identify. After 40 days, the reduction in tumor volume and metastases to the brain was dramatic in those mice treated with the NOS2 inhibitor. Furthermore, expression of genes associated with the ER- basal-like signature, *IL-8*, *IL-6*, *S100A8*, *CD44*, and *TLR4*, were all reduced in the treated mice.

To simulate the tumor microenvironment, in which cells become packed together, without proper exposure to oxygen and other circulating factors, the researchers grew breast cancer cells in culture and deprived them of serum. Serum withdrawal normally induces cell migration, but NOS2 inhibition detained the cells, an effect that could be overcome with an NO donor. NOS2 inhibitors also prevented the development of resistance to the chemotherapeutic taxol in this model.

If NOS2 plays a substantive role

in tumor growth, migration, and chemoresistance, what is inducing it? As expected, they found that both hypoxia and serum withdrawal caused a strong increase in NOS2 mRNA expression in breast cancer cells, as did a mix of cytokines, interferon- γ , and inhibition of NOS2 itself, suggesting the potential for a positive feedback mechanism that furthers the aggressiveness of the cancer.

“Understanding the factors within the tumor microenvironment that regulate NOS2 and NO flux-driven tumor progression could lead to more personalized therapeutic options for women whose breast tumors express high NOS2,” said Wink. “The use of NOS2 inhibitors combined with inhibition of upstream and downstream targets could improve clinical outcomes.”

To learn more about Dr. Wink’s research, please visit his CCR website at <https://ccr.cancer.gov/david-a-wink>.

HIV Integration and the Persistence of HIV Infection

During antiretroviral therapy, propagation and persistence of cells harboring HIV can depend on the DNA site of viral integration.

Combination antiretroviral therapy (cART), which can completely block viral replication, is now used to control HIV in millions of patients worldwide. However, during infection, a DNA copy of the HIV genome is inserted into the cells it infects, which persists as long as the host cell lives, and can initiate an active infection if therapy is discontinued. This makes curing HIV a daunting task.

In untreated patients infected for a few years, the viruses in the blood differ from one another. By contrast, in many of the patients treated with cART for long periods, identical strains emerge in the blood. In a recent paper in *Science*, Stephen Hughes, Ph.D., Director of CCR's HIV Drug Resistance Program, and his colleagues proposed that the identical viruses are the result of clonal expansion of infected host cells, and that, in some cases, this expansion is driven or facilitated by HIV DNA

integration in genes that promote the growth of the infected cell.

Hughes and colleagues sequenced the HIV DNA integration sites in peripheral blood mononuclear cells (PBMCs) or CD4+ T cells from the blood of five patients treated with long-term cART. Of the 2,410 integration sites they identified, approximately 40 percent were found multiple times, showing that these sites came from cells that had clonally expanded after infection. In one striking example, more than 50 percent of the infected cells in a patient were from a single clone. Moreover, some of the clones of HIV-infected cells were shown to persist in patients for more than a decade.

Among the patients studied, there were three or more independent integration sites in 29 different genes. Most of these genes (21/29) are known to be directly involved in cell growth. In two of the genes, *MKL2*

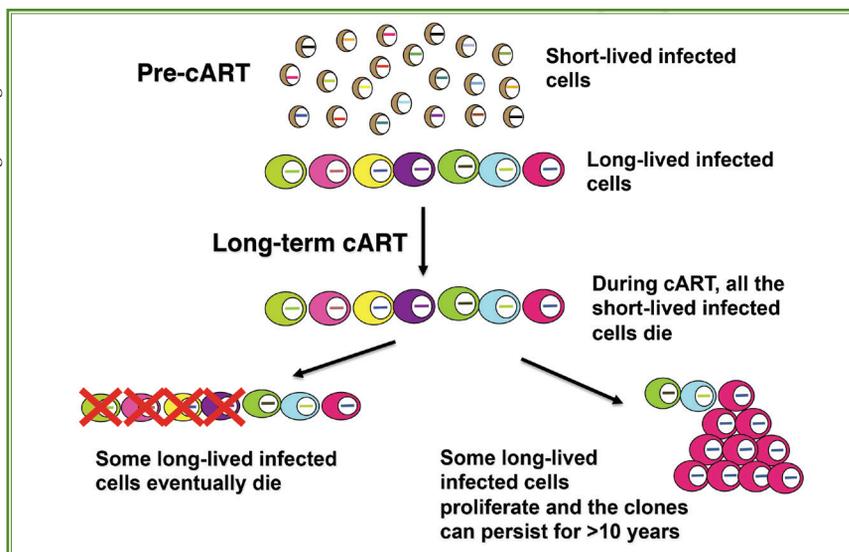
and *BACH2*, there were, respectively, 16 and 17 independent integrations. The sites and orientations of the integrations in *MKL2* and *BACH2* suggested that cells carrying these integrations were selected because they altered the expression or the protein products produced by these two genes.

From these data, the researchers proposed that HIV persists, in part, because infected cells can divide and grow clonally. In some cases, the clonal growth of the infected cells depends on where HIV integrates into the human genome. More research is needed to determine what fraction of the clonally expanded cells carry intact copies of the HIV genome that can give rise to infectious virus. However, the researchers did identify one expanded clone that produced the majority of the virus in the blood of a patient.

"If we are going to achieve a cure for HIV, we will need not only to suppress the replication of the virus, but also to block the expansion of infected cells," said Hughes. "Our research also suggests that gene-therapy patients who are treated with HIV-based vectors should be carefully monitored for the development of malignancies. We may also need to reexamine the question of whether HIV DNA integration may play a role in the development of some HIV-related malignancies."

To learn more about Dr. Hughes's research, please visit his CCR website at <https://ccr.cancer.gov/stephen-h-hughes>.

Image: S. Hughes, CCR



Some long-lived HIV-infected cells persist in patients who are treated with combination antiretroviral therapy (cART), preventing them from being cured. Some infected cells can grow and divide, and these expanded clones can persist for more than 10 years in patients.

Journey to the Center of the Synapse

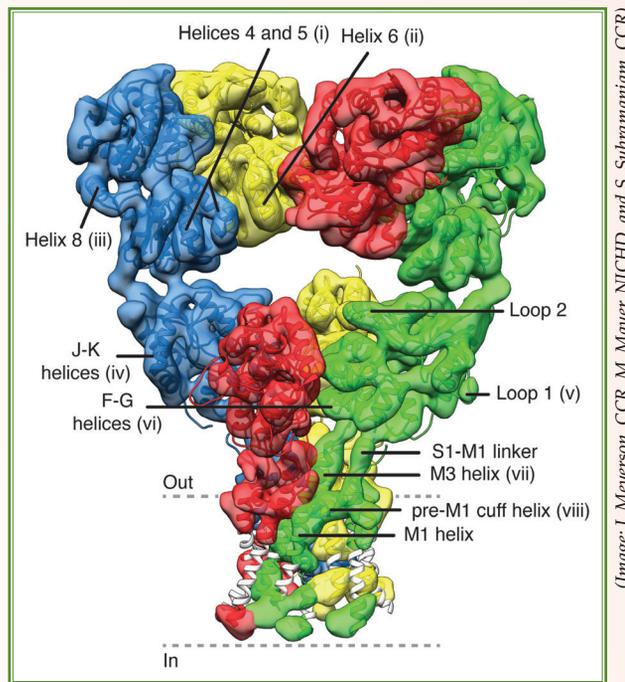
A new study reveals exquisite details of important cellular membrane proteins.

When it comes to understanding disease, a frustrating truth is that most of the critical mechanisms operate in regimes that are smaller than the eye can see. From the discovery of the cell to the structure of DNA, many of the greatest insights arise when biologists are able to surpass the limits of human vision through technology.

Sriram Subramaniam, Ph.D., Senior Investigator in CCR's Laboratory of Cell Biology, has been steadily pushing the limits of microscopy to visualize the changes in individual molecules that underlie their functions. In the August 3 issue of *Nature*, Subramaniam and a graduate student in his laboratory, Joel Meyerson, collaborated with Mark Mayer, Ph.D., Senior Investigator in the Eunice Kennedy Shriver National Institute of Child Health and Human Development, to examine the workings of one of the most important families of ion channels in the human brain, ionotropic glutamate receptors (iGluRs).

A million times smaller than a human hair, iGluRs sit in the cellular membrane, concentrated at synapses between brain cells, where they act as gates for excitatory electrical activity. Their amino-acid sequences are known, but this is not enough to determine how they open and close, instantaneously changing their conformations in response to chemical signals.

Because of their small size, scientists typically study such cellular components with X-rays, but even then, they cannot directly "see" individual proteins. The particles must first be trapped and packed into regular crystals to form a 3-D



3-D structure of a glutamate receptor in a desensitized state

(Image: J. Meyerson, CCR, M. Mayer, NICHD, and S. Subramaniam, CCR)

array, which can scatter the X-rays in ways that provide information about the component structures.

To examine structural alterations as they relate to function, Subramaniam and his colleagues chose single particle cryo-electron microscopy (cryo-EM). Given recent advances in electron detectors, it is now possible to take high-definition images of individual particles—that have been trapped in a frozen solution—with a transmission electron microscope. Because electron beams are damaging over time, each particle is only viewed very briefly, leaving a dark and grainy image. However, by taking images of tens of thousands of particles, it is possible to computationally average together similar images to create a picture of the particle in its different conformations.

Subramaniam, Mayer, and their colleagues were able to see iGluRs

at a resolution of approximately 8 Angstroms (the wavelength of visible light is a thousand times greater) and discovered that the four component subunits turn and twist around each other like a corkscrew to open and close.

“Not in a million years would I have dreamed that a cell receptor would work this way to open and close the gate for ion flow,” said Subramaniam. “We are now poised to analyze structures of a wide variety of biologically and medically relevant multiprotein complexes and membrane protein assemblies, which have historically represented the most challenging frontier in structural biology.”

To learn more about Dr. Subramaniam's research, please visit his CCR webpage at <http://electron.nci.nih.gov>.

Blood Vessel Blockade

A VEGF-independent route to tumor angiogenesis suggests a new therapeutic strategy.

Any student of military campaigns knows that cutting off supply routes can devastate an invading army. As cancers spread and grow, they spur angiogenesis, i.e., the development of new blood vessels, to supply their increasing numbers of cells. These new routes are also pathways to metastasis. Vascular endothelial growth factor (VEGF) is a key signaling molecule in angiogenesis and anti-VEGF therapies like Avastin (bevacizumab) have enjoyed significant therapeutic success, for example in certain lung and colon cancers.

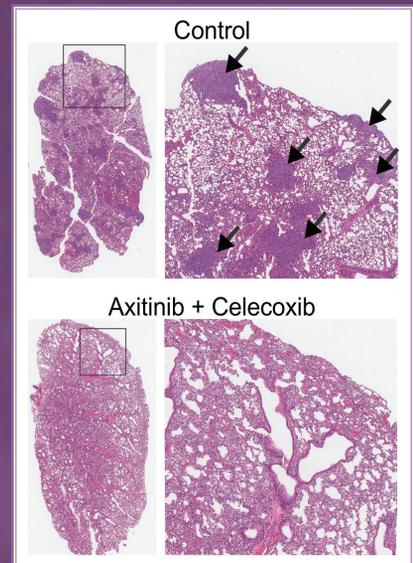
Unfortunately, in many cases, it is increasingly clear that VEGF blockade is not only insufficient to stop tumor growth, it may even promote the development of more aggressive tumors. Several other biological factors have been proposed as the source of this refractoriness, but none have been definitively established. In a recent *Science Translational Medicine* article, Brad St. Croix, Ph.D., Investigator in CCR's Mouse Cancer Genetics Program, and his colleagues identified prostaglandin E2 (PGE2) as a VEGF-independent promoter of angiogenesis in cancer, production of which may be blocked as a complementary therapeutic strategy.

St. Croix's laboratory has long been focused on identifying and blocking the factors that underlie tumor angiogenesis. In the course of their research, his team came across a human colon cancer cell

line, which formed tumors resistant to VEGF inhibitors when implanted into mice. Individual clonal populations from that cell line had variable success in establishing such xenograft tumors; and the researchers found that the highly tumorigenic clones were secreting a pro-angiogenic factor, which could be detected in the cell culture medium. So they took almost three gallons of this cellular broth and painstakingly separated out the active component, which turned out to be PGE2, a biological molecule with known angiogenic activity.

PGE2 is produced in cells through a series of biochemical reactions, one of which is controlled by the rate-limiting enzyme cyclooxygenase 2 (COX-2). COX-2 is known to be overexpressed in many tumors and the researchers were able to show that overexpression of COX-2 could convert the poorly tumorigenic clones into aggressively angiogenic cancers in xenograft models. They also showed that COX-2 inhibition could slow tumor growth and that this therapeutic effect was augmented by inhibition of VEGF. The cooperative actions of COX-2 and VEGF on angiogenesis and metastasis were confirmed in additional preclinical models of metastatic colon and breast cancer.

"Our hope is that cocktails of anti-angiogenic inhibitors will ultimately prove more effective than the current VEGF therapies," said



(Image: B. St. Croix, CCR)

Dual therapy with axitinib and celecoxib prevented the outgrowth of spontaneous breast cancer metastasis in the lung. Metastasis appeared as dense tumor cell clusters (arrows) in tissue sections.

St. Croix. "If our preclinical studies in mice translate into the clinical setting, then combining VEGF and COX-2 inhibitors could potentially prolong the survival of a subset of patients who have tumors with high levels of COX-2. However, not all tumors overexpress COX-2, so it will be important to screen patients to identify those that are most likely to benefit."

To learn more about Dr. St. Croix's research, please visit his CCR website at <https://ccr.cancer.gov/brad-st-croix>.

Treating the Cure:

Grappling with cGvHD after Hematopoietic Stem Cell Transplants

The second phase of a consensus project on chronic Graft versus Host Disease showcases a decade of progress.

Hematology/oncology specialists have been doing allogeneic transplants of hematopoietic stem cells to cure cancers of the blood like leukemia and lymphoma for 45 years; and, it works. The number of transplants is increasing worldwide, availability of donors is increasing through the unrelated donor registry, patients can be treated at an older age and with comorbid conditions, and the safety with respect to early complications and mortality is greatly improved.

But, because there are more long-term survivors, later complications from the therapy have risen to prominence. One in particular, chronic Graft versus Host Disease (cGvHD), develops on average about one year after the transplant and can take five years to resolve. cGvHD is a multisystem disorder, resembling systemic autoimmune disorders like lupus and scleroderma, involving many organs including the skin, joints, eyes, mouth, and lungs.

cGvHD has a tremendous impact on quality of life, and can even be life-threatening. Like autoimmune diseases, the causes are poorly understood.

“It has been very difficult for the oncology field to address the complicated needs of these patients,” explained Steven Pavletic, M.D., M.S., Head of the GVHD and Autoimmunity Unit in CCR’s Experimental Transplantation and Immunology Branch. “Oncologists are equipped to treat cancer, but these patients no longer have cancer. Primary internists don’t know what to do.”

To further complicate the issue of cGvHD, it appears that this disease is finely balanced with curing the original cancer. Bone marrow transplants are given to cure very aggressive cancers; most are done for patients that will likely otherwise die within a year. This form of immunotherapy relies in part on graft-derived cells eradicating the remaining cancer of the host. “Mild GVHD is actually desirable in some patients; with a little bit, they have better cure rates. Our goal is to find ways to effectively treat or prevent GVHD without harming the beneficial immunological effect. It’s a challenge,” said Pavletic.

Research into the disease has been severely hampered by the difficulties of organizing studies around outpatients and the need for multidisciplinary teams to analyze the multisystem causes and effects. To address this issue, in 2004, NCI decided to create a program within CCR to study cGvHD. Pavletic came

to CCR to establish the program, which brings together clinical investigators from eight institutes and five departments of the NIH Clinical Center.

“We started reaching out to people for referrals to the clinic, and soon we found that 85 percent of our patients were coming from the extramural community,” said Pavletic. “Because we have access to the NIH Clinical Center, we had a unique opportunity to mobilize researchers to create a model that could hopefully be exported.”

Having established the NIH program, the next step was to engage a broader community to advance research and improve treatments. There were no standard criteria for diagnosis, disease staging, or therapeutic response. There were no developed pathways to do clinical trials, nor recommendations for biomarker development. “We needed to create a whole medical discipline to study a disease that had been unstudyable,” said Pavletic.

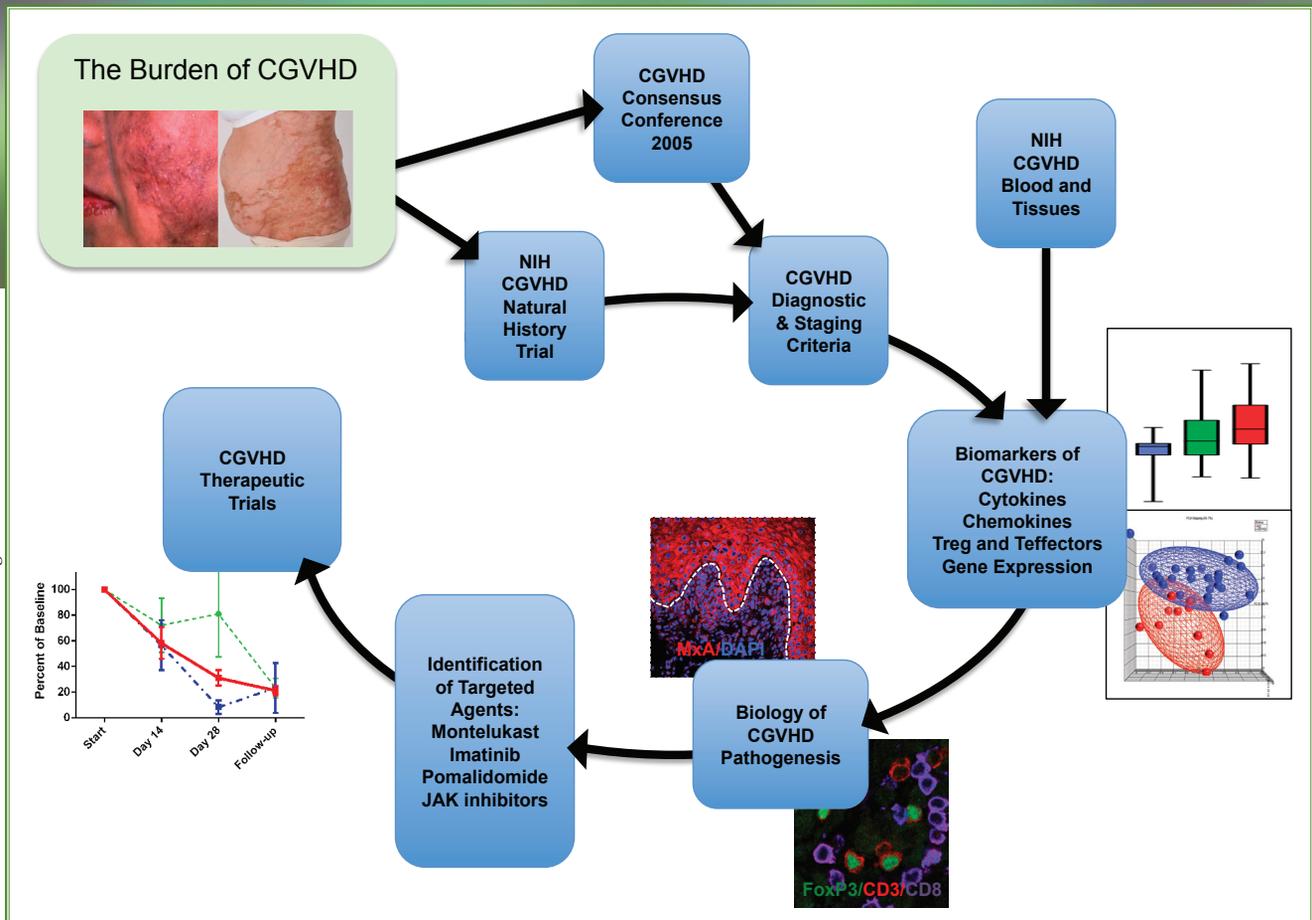
Thus, the first cGvHD consensus project was formed to provide expert-driven recommendations for the conduct of cGvHD clinical trials. From 2004–2006, it led to the publication of six papers that covered key areas to define the field. “Today, there have been 1,600 citations in peer-reviewed literature to those papers—a high number, especially for a relatively small field—showing the impact of this effort,” said Pavletic. “More importantly, the field became better organized. Collaborations improved nationally and internationally. Extramural



(Photo: B. Brantson)

Steven Pavletic, M.D., M.S.

(Image: S. Proletic and F. Hakim, CCR)



Generating new understanding of GVHD and developing new treatments per the NIH consensus project algorithm

investigators began submitting grant proposals to NCI. We were able to pursue multicenter prospective studies to look at the course of disease and initiate phase 1/2 trials here at the NIH.”

The first consensus project had very little hard data to work with. Ten years later, with the accumulation of research spurred by the project, the leadership felt the time was right to re-examine the guidelines and recommendations for cGvHD. On June 17, 2014, NCI hosted the second meeting of the cGvHD consensus project on criteria for clinical trials.

“Thanks to the support of CCR, we were able to invite panelists to come here for a conference. We reconvened 200 stakeholders from across the world: clinicians, scientists, as well as representatives from the pharmaceutical industry, patient advocacy, and key associations,

to refine and clarify the original recommendations,” said Pavletic.

Once again, the project examined six key areas: diagnosis and staging, measuring therapeutic response, histopathology, biomarkers, ancillary therapy and supportive care, and design of clinical trials. “This time we added a special focus on the biology of cGvHD. What are we missing that we still can’t decipher this whole complex pathophysiology?” said Pavletic. The findings of the second project will be published in the *Biology of Blood and Marrow Transplantation* in 2015.

“We have a much better understanding of the disease manifestations. We have new tools to measure the disease and conduct clinical trials. We have common language. None of this existed when we met for the first time,” said Pavletic.

“In parallel, certainly, everybody is asking can we cure or prevent this? The short answer is no; we don’t have any magic drugs yet. But the amount of information about potential points of intervention has dramatically increased, the number of molecules available to be tested has increased. So now it’s our job and task to overcome the main bottleneck in drug development: pursuing clinical trials effectively to apply emerging knowledge to benefit these patients,” concluded Pavletic.

To learn more about the NIH 2014 Chronic GVHD Consensus Project on Criteria for Clinical Trials, please visit <http://ncifrederick.cancer.gov/events/GoHD/resources.asp>.

Recent CCR Awards

Robert Feulgen Prize

Society of Histochemistry

For outstanding achievement in the field of histochemistry

Tom Misteli, Ph.D.

Laboratory of Receptor Biology and Gene Expression

Arthur S. Flemming Award for Exceptional Service

For her achievements in the field of directed cell migration

Carole Parent, Ph.D.

Deputy Chief, Laboratory of Cellular and Molecular Biology

Philip Levine Award for Outstanding Research

American Society of Clinical Pathology

For contributions to molecular pathology, immunohematology, and/or immunopathology

Elaine Jaffe, M.D.

Laboratory of Pathology

Massry Prize

Meira and Shaul G. Massry Foundation

For research on T cells, paving the way for innovative new immunotherapies for cancer patients

Steven Rosenberg, M.D., Ph.D.

Chief, Surgery Branch

Transatlantic Medal

Society for Endocrinology

For outstanding contributions to the discipline of endocrinology

Gordon Hager, Ph.D.

Laboratory of Receptor Biology and Gene Expression

Wagner Medal for Excellence in Mesothelioma Research

International Mesothelioma Interest Group

For his research in mesothelioma

Raffit Hassan, M.D.

Co-Chief, Thoracic and Gastrointestinal Oncology Branch

Elected to the American Academy of Arts and Sciences

Shiv Grewal, Ph.D.

Chief, Laboratory of Biochemistry and Molecular Biology

Donald Court, Ph.D.

Gene Regulation and Chromosome Biology Laboratory

Elected as Distinguished Fellows of the Collegium of Eminent Scientists

The Kosciuszko Foundation

Zbigniew Dauter, Ph.D.

Macromolecular Crystallography Laboratory

Alexander Wlodawer, Ph.D.

Chief, Macromolecular Crystallography Laboratory

Elected to European Molecular Biology Organization Membership

Susan Gottesman, Ph.D.

Co-Chief, Laboratory of Molecular Biology

CCR Researchers Awarded the National Medal of Technology and Innovation

Douglas Lowy, M.D., Chief, and John Schiller, Ph.D., Deputy Chief of CCR's Laboratory of Cellular Oncology, are recipients of the National Medal of Technology and Innovation. The award is America's highest honor for technological advancement bestowed by the President on the nation's leading innovators.

This Medal recognizes those who have made lasting contributions to America's competitiveness and quality of life and who have helped strengthen the nation's technological workforce. For nearly three decades, Lowy and Schiller have devoted their careers to understanding and preventing human papillomavirus (HPV) infections, the most common sexually transmitted infections in the United States and the cause of about five percent of worldwide cancers. Their breakthrough discoveries led to the development of the commercial vaccines Gardasil

and Cervarix, which are now available globally and recommended for adolescents to prevent HPV infections and the cancers and other neoplastic diseases that they cause. (See "Epic Journeys from Bench to Bedside," *CCR connections* Vol. 8, No. 2, and "A Victory against Cancer," *CCR connections* Vol. 1, No. 1.)

The National Medal of Technology and Innovation was created by statute in 1980 and is administered for the White House by the U.S. Department of Commerce's Patent and Trademark Office. By highlighting the national importance of technological innovation, the Medal is also meant to inspire future generations of Americans to prepare for and pursue technical careers to keep America at the forefront of global technology and economic leadership.

"These scholars and innovators have expanded our understanding of the world, made invaluable contributions to their fields, and helped improve countless lives."

—President Barack Obama

Staff News at CCR

Announcement

(Photo: R. Frederickson)



Mark Gilbert, M.D.

Mark Gilbert joins CCR as Chief of the Neuro-Oncology Branch (NOB). His appointment is in partnership with the National Institute of Neurological Disorders and Stroke (NINDS) where he will have an adjunct appointment to facilitate close collaborations. Gilbert received his M.D. from The Johns Hopkins University, where he also completed residencies in internal medicine and neurology, and fellowship training in both neurology and neuro-oncology. He has served on the faculties of the University of Pittsburgh, Emory University in Atlanta, Georgia, and the University of Texas M.D. Anderson Cancer Center. In 2005, he was appointed as Director of the Brain Tumor Trials Collaborative, a multicenter clinical trials consortium. Recently, Gilbert was named the Co-Chair of the Brain Tumor Committee in the Radiation Therapy Oncology Group. He is also the founder and leader of the Collaborative Ependymoma Research Network, a consortium studying ependymoma, a rare central nervous system cancer, by supporting basic research, clinical trials, patient outcomes research, and educational efforts in North America and Europe. Gilbert arrived at the

NIH Intramural Program with a vision to build a highly collaborative, robust translational research program centered on finding treatments for central nervous system tumors where basic research observations will be rapidly translated into preclinical testing and then hypothesis-based clinical research trials, including important correlative studies. His clinical trials will focus on early-stage testing of novel agents and evaluation of novel clinical trial designs and imaging paradigms, as well as examination of the impact of disease and treatment on cognitive function, symptom burden, and quality of life.

New Tenure-Track Scientists

(Photo: R. Baer)



Andrea Apolo, M.D.

Andrea Apolo is now a Tenure-Track Investigator in CCR's Genitourinary Malignancies Branch. She is also a Lasker Clinical Research Scholar. She received her M.D. from the Albert Einstein College of Medicine in New York and completed clinical training in internal medicine at New York-Presbyterian Hospital/Weill Cornell Medical Center. Following her residency, she completed a medical oncology fellowship at Memorial Sloan-Kettering Cancer Center. Apolo then joined CCR as an Assistant Clinical Investigator. Her research focuses on developing and designing clinical trials to test novel agents for the treatment of urologic cancers, primarily bladder cancer and prostate cancer. Other research focuses on improving tumor detection by using new imaging modalities in bladder and prostate cancer and identifying molecular alterations in bladder tumors that will serve as targets for individualized treatment strategies in patients with this disease.

(Photo: Courtesy of C. Nagao)



Keisuke (Chris) Nagao, M.D., Ph.D.

Chris Nagao joins CCR's Dermatology Branch as an NIH Earl Stadtman Tenure-Track Investigator. He obtained his M.D. from Keio University School of Medicine in Japan and joined the Dermatology Department. After completing his residency, he worked at two Keio-affiliated hospitals known for performing intensive care. He then returned to Keio University and started his research career in the field of medical mycology. After obtaining a Ph.D. in 2005, he conducted his postdoctoral research on skin dendritic cells in the laboratory of Mark Udey, M.D., Chief of CCR's Dermatology Branch. He then started his independent research at Keio University, where he served as a faculty member. His research focuses on how immune cells are regulated in skin, particularly by the hair follicles, and on how dysbiosis drives eczematous inflammation in mice.

Newly Tenured CCR Scientists

Avinash Bhandoola, Ph.D.

Laboratory of Genome Integrity

Christopher Buck, Ph.D.

Laboratory of Cellular Oncology

In Conversation:

Postdoctoral Fellow John Simmons, Ph.D.

CCR: You've been training in CCR labs for several years—as an undergraduate and graduate student and now as a Postdoc. Congratulations on completing your Ph.D. in April, through the NIH Graduate Partnership Program with Georgetown University. Could you tell us about your thesis?

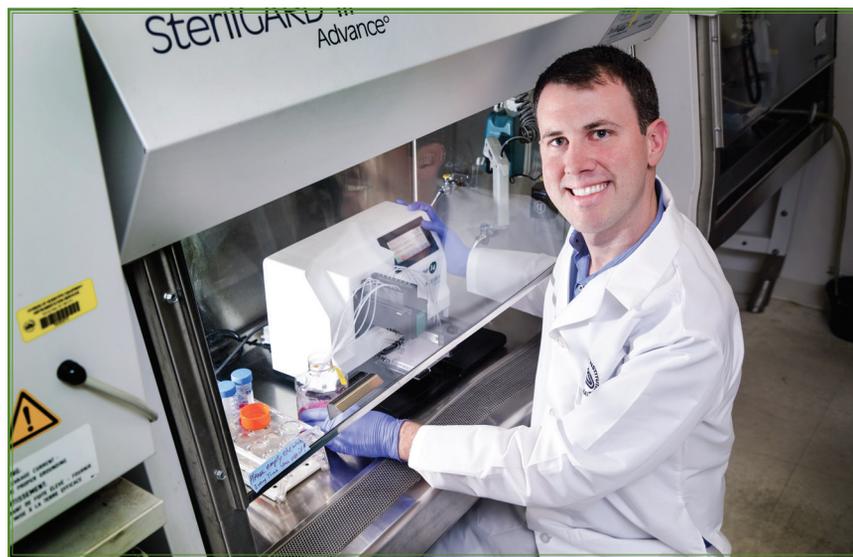
John: I focused on drug combinations to treat multiple myeloma. In 2009, I started working with Beverly Mock, Ph.D., Deputy Chief of CCR's Laboratory of Cancer Biology and Genetics. Her laboratory was trying to understand the synergistic interactions between inhibitors of two anticancer targets: mTOR and HDACs. We discovered that the drug combination actually had a synergistic effect on destabilizing the MYC protein.

CCR: How did you figure out that MYC was involved?

John: Working with a bioinformatician, Staff Scientist Aleksandra Michalowski, Ph.D., we evaluated drug synergy at the molecular level using transcriptomics. Instead of looking at changes in gene expression one gene at a time, we looked for correlations between gene expression changes. We found a network of genes that were affected by the drug combination and were correlated with improved prognosis in patient data. We discovered the relationship of this network to MYC protein regulation through data mining tools and then verified this finding *in vitro* and in mouse models.

CCR: That sounds like a lot of serious bioinformatics. Was that challenging?

John: I didn't have a lot of training in statistics and no training in any of



John Simmons, Ph.D.

the programming languages like R, but early on, I made a commitment to at least understand the principles behind all the different types of analyses. At this point, though, I mainly focus on the biology. These data sets are increasingly so large that it's not a place to really get your feet wet, so to speak.

CCR: Are you continuing this work as a Postdoctoral Fellow in Dr. Mock's laboratory?

John: Yes, in part, but I have expanded my focus beyond the particular drug combination that I focused on in my thesis. While still a grad student, I participated in a translational science training program run by the NIH Office of Intramural Training and Education (OITE). I wrote a proposal for how my thesis could benefit from the high-throughput screening capabilities at National Center for Advancing Translational Science (NCATS). In a collaboration with their Chemical Genomics team, we started looking at drug sensitivities in 70 genetically defined multiple myeloma cell lines. We incorporated

a subset of compounds to evaluate the cell lines in combination with a panel of 2,000 drugs. Along the way, I submitted a career development grant to the Multiple Myeloma Research Foundation, which was recently funded. We are particularly interested in drug combinations that are active in cell lines resistant to standard of care.

CCR: It sounds as though your focus as a cancer researcher is firmly rooted in translation.

John: Well, I've always had an interest in science, even as a child. But my interest in cancer came when an aunt was diagnosed with multiple myeloma when I was in middle school. She went on a clinical trial at Duke and lived about three years after diagnosis. It was the first time I'd ever heard of clinical research. I grew up in rural southern Virginia, so being interested in science, the obvious career option was medicine. It wasn't until I started interning at CCR as an undergraduate that I got a great appreciation of translational research and its potential impact.

(Photo: B. Branson)

Epic Journeys from Bench to Bedside

In a world that demands increasingly rapid returns on investment from all sectors of society, basic research can sometimes seem a luxury. Yet, time and again, the value of research that was initiated years or even decades ago is proven in new gains for human health. CCR scientists play a role in all stages of discovery, from basic mechanisms to clinical trials; here are four examples of work pioneered within CCR that has led to currently available drugs for the prevention and treatment of cancer.

An Ounce of Prevention

In the early 1980s, Professor Harald zur Hausen, Ph.D., made the remarkable, Nobel-prize winning discovery that a type of human papilloma virus (HPV)—HPV16—was found in over 50 percent of cervical cancers. This research led to the current understanding that approximately five percent of all cancers are caused by HPV infection. It also led, via CCR, to commercially available vaccines to prevent HPV-induced cancers.

In 1983, zur Hausen gave a lecture on his findings at the NIH in Bethesda. John Schiller, Ph.D., had coincidentally just joined NCI as a Postdoctoral Fellow. “The second lecture I went to at NCI was Harald saying ‘Eureka!’”

Schiller had joined the laboratory of Douglas Lowy, M.D., to study the basic mechanisms of viral oncogenesis, thereby beginning a 30-plus year collaboration that now finds them co-Principal Investigators in CCR’s Laboratory of Cellular Oncology. They began by studying the individual proteins of an HPV-related virus from cows, bovine papilloma virus type 1 (BPV-1), to understand the viral mechanisms of cellular transformation. After nearly a decade of research on the functions



(Photo: R. Baer)

Douglas Lowy, M.D., and John Schiller, Ph.D.

of these proteins, Schiller, Lowy, and their team began a new line of investigation and made a critical discovery about the virus’ outer coat protein, L1.

“Previously, people had been making L1 in *E. coli* expression systems, which meant that they were isolating the protein in a denatured state that did not induce antibodies able to prevent virus infection,” said Schiller. “When we expressed it in baculovirus-infected insect cells

instead, we not only got the native protein but amazingly, 360 copies of this one protein self-assembled into the outer coat of the virus, just as in the authentic virus.” Moreover, when these self-assembled virus-like particles (VLPs) were injected into rabbits, they were able to produce very high levels of antibodies that prevented infection by the authentic virus. These were the class of antibodies that are often the cornerstone of preventative vaccines.

The scientists had to overcome a further hurdle in translating this result from bovine to human papilloma virus and setting the stage for a vaccine. At first, they could not extend their findings to HPV16, because its L1 self-assembled very poorly. “That meant that either these two related viruses were acting very differently, or it meant that the HPV16 we were working with was a mutant,” said Schiller. The latter possibility made sense because the widely used clone of HPV16 had been isolated from a human cancer, and cancers usually have extensive genomic changes. So the team found a source of HPV16 genomes from normal productive HPV16 lesions—not cancers—and showed that its L1 efficiently self-assembled. A single amino acid change was found to be responsible for the difference in self-assembling properties.

“That really set the stage for bringing companies in,” said Schiller. “But, when we first approached the major vaccine manufacturers, there was extreme skepticism that a vaccine against sexually transmitted infections could work at all. There were no examples, despite considerable efforts. Two companies took a leap of faith: Merck and a then small local company, MedImmune.”

MedImmune sold its interest in the VLP strategy to GlaxoSmithKline Biologicals (GSK) after successful phase 1 trials. Today, GSK manufactures Cervarix, which contains the L1 VLPs of HPV16 and HPV18, and Merck manufactures Gardasil, which additionally contains L1 VLPs from HPV6 and HPV11, thereby targeting both cancer and



(Photo: B. Branson)

Susan Bates, M.D.

genital warts. The U.S. Centers for Disease Control and Prevention recommends that all preteens and previously unvaccinated teens and young adults be administered the vaccine.

NCI’s involvement did not end with a handoff to big companies for the clinical trials necessary to obtain regulatory approval. Simultaneously, NCI decided to sponsor its own trial, led by Allan Hildesheim, Ph.D., Chief of the Division of Cancer Epidemiology and Genetics’s Infections and Immunoepidemiology Branch at NCI.

“We have preliminary evidence that even a single dose of the vaccine gives very strong protection over four years,” said Schiller. “Now we are deciding whether to test, in a dose randomized trial, the efficacy of a single dose, which is unheard of for a vaccine based on only one protein.”

“I came to the lab to find out how viruses replicate and transform

cells,” concluded Schiller. “It was only years later that Doug and I decided to take a crack at this vaccine. It was a real departure for us, but it paid off.” (See “CCR Researchers Awarded the National Medal of Technology and Innovation,” *CCR connections* Vol. 8, No. 2)

A Pound of Cure

Susan Bates, M.D., Senior Investigator in CCR’s Developmental Therapeutics Branch, started out studying the compound that would become Istodax (romidepsin) because she was interested in multi-drug resistance mediated by the cellular pump, P-glycoprotein (PGP), in the 1990s.

Working with the NCI-60 Drug Screen’s cell line panel, a highly characterized set of cancer cells whose differential response to drugs is used as a signature of their mechanisms of action, Bates and her colleagues discovered that cytotoxic compounds could be identified on the basis of their vulnerability to extrusion by PGP. One of the identified compounds was a naturally occurring compound from *Chromobacterium violaceum*, which the Fujisawa Corporation (now Astellas Pharma) had identified as a potential cytotoxic agent, and had

“We have preliminary evidence that even a single dose of the vaccine gives very strong protection over four years.”

deposited with the Drug Screen. Bates and her colleagues at the Drug Screen and at NCI's Cancer Therapy Evaluation Program (CTEP) were intrigued enough by romidepsin's properties to initiate two phase 1 studies with the compound—one at NCI led by Bates.

In the lab, Bates and her NIH colleagues Dan Sackett, Ph.D., and April Robbins, Ph.D., found that the drug, romidepsin, promoted poor attachment of chromosomes to the kinetochore because of hyperacetylation in dividing cells, ultimately causing cell cycle arrest prior to mitosis, a mechanism which resembles the activity of the widely used chemotherapeutics, taxanes. Further preclinical work went on to show that romidepsin inhibits histone deacetylase (HDAC), thereby accounting for hyperacetylation and cell cycle arrest.

"We were trying it in solid tumors, because that is what I worked on, but we also enrolled a patient with T-cell lymphoma for this initial exploratory study. And his tumors just melted away," explained Bates. "When we saw the response in this patient, we quickly amended the study to add 10 more patients with T-cell lymphoma."

A phase 2 study soon followed, focused on both forms of T-cell lymphoma, which became a multi-institutional trial in 2001. In 2003, Bates presented their data to Richard Klausner, M.D., who was the NCI Director at that time, and to Klausner's visitor from Harvard, Greg Verdine, Ph.D. Verdine returned to Boston and, shortly thereafter, formed a company called Gloucester Pharmaceuticals to license the drug.

With exciting results mounting, CTEP was opening multiple clinical trials of romidepsin. And then, suddenly, several patients died. "We had two patients die in their sleep



(Photo: R. Fratrickson)

Michael Dean, Ph.D.

a few days after drug delivery," said Bates. Rather than stop the trial, Bates, Richard Piekarz, M.D., Ph.D., and other investigators at CTEP realized that the patients who had died had been at high risk for cardiac problems. Given the NIH Clinical Center's resources, they were able to augment the trial with close monitoring of EKG, cardiac tests, and supplemental potassium and magnesium.

"Then, we just kept accruing patients to the study," said Bates. Based on the phase 2 results from the studies performed by NCI and by Gloucester Pharmaceuticals, the U.S. Food and Drug Administration (FDA) approved romidepsin for the treatment of cutaneous T-cell lymphoma in 2009 and gave an accelerated approval for peripheral T-cell lymphoma in 2011. "I don't think it could have happened without CCR, because of the intensive safety data collection involved," said Bates.

"It's a very potent drug. We see prolonged responses, lasting years and years. One patient has been out of the trial for more than 10 years without relapse," said Bates. The overall response rate is a little over one third in the relapsed refractory

setting for both indications. Ongoing and planned clinical trials are testing combinations of romidepsin with other targeted agents as well as radiation therapy.

A New Target

"When I first came to NCI in the 1980s, for a postdoctoral fellowship with George Vande Woude, his laboratory was characterizing a newly discovered gene that could transform cells in culture," said Michael Dean, Ph.D., Deputy Program Director of CCR's Cancer and Inflammation Program. The gene—*c-MET*—was isolated from human cell lines that had been

"We see prolonged responses, lasting years and years. One patient has been out of the trial for more than 10 years without relapse."

Gleevec has since been found to affect more than just its target protein; cabozantinib seems likewise to affect multiple signaling pathways.

chemically transformed *in vitro*. At the time, finding oncogenes meant transfecting mouse cells with pieces of DNA and assessing their transformation in culture and their ability to grow as implanted tumors.

"I generated some of the first DNA sequences for the gene and showed that it was homologous to other tyrosine kinases, some of which were known to be oncogenes." Dean and his colleagues mapped the gene to its chromosome and characterized the exact mutation that gave MET its transforming potential.

Following up on the role of *c-MET* in human cancers, the laboratory showed in the 1990s with Bert Zbar, M.D., some inherited kidney cancers were associated with germline mutations in the *MET* gene, and that tumors in these patients had increased copies of the mutant allele.

Mutations in *c-MET* are now found in a variety of tumors, and its mutations usually signal a poor disease prognosis. The gene encodes a receptor for hepatic growth factor/scattering factor (HGF/SF), which normally promotes cellular growth and proliferation during organ development, regeneration, and wound healing.

As a result, many cancer therapeutics companies have been interested in developing MET inhibitors. In 2012, the first of these inhibitors, Exelixis' Cometriq (cabozantinib), was approved for the treatment of metastatic medullary thyroid cancer.

"When we first started working on MET, we had no idea how long it would take to develop a drug. The development of Gleevec (imatinib) to treat chronic myelogenous leukemia

in the late 1990s showed that it was possible to target oncogenic tyrosine kinases by targeting the ATP-binding domain in such a way that it shuts down the protein and isn't toxic." Once the principle was established, several companies joined the search for tyrosine kinase inhibitors as anticancer agents.

Gleevec has since been found to affect more than just its target protein; cabozantinib seems likewise to affect multiple signaling pathways. "There's a lot we don't understand about the biology of tumors and the importance of a single mutation," said Dean. "Once a drug is approved by the FDA, new uses will be discovered for that drug that we can't even envision."

Contingency Plan

Methotrexate, an inhibitor of folic acid metabolism, is a powerful chemotherapeutic agent, used for decades in the treatment of a broad spectrum of cancers. For a doubly unlucky 1.5–2 percent of patients treated with the drug, high-dose methotrexate administration can also lead to life-threatening toxicity. Usually given safely in very high doses, it can occasionally precipitate and lead to acute kidney dysfunction, which results from sustained exposure to very high drug concentrations.

When Brigitte Widemann, M.D., now a Senior Investigator in CCR's Pediatric Oncology Branch, began working as a fellow with David Poplack, M.D., and Peter Adamson, M.D., at NCI, they had shown in non-human primates that the bacterial enzyme carboxypeptidase-G2 (now glucarpidase) very rapidly reduced plasma concentrations of metho-

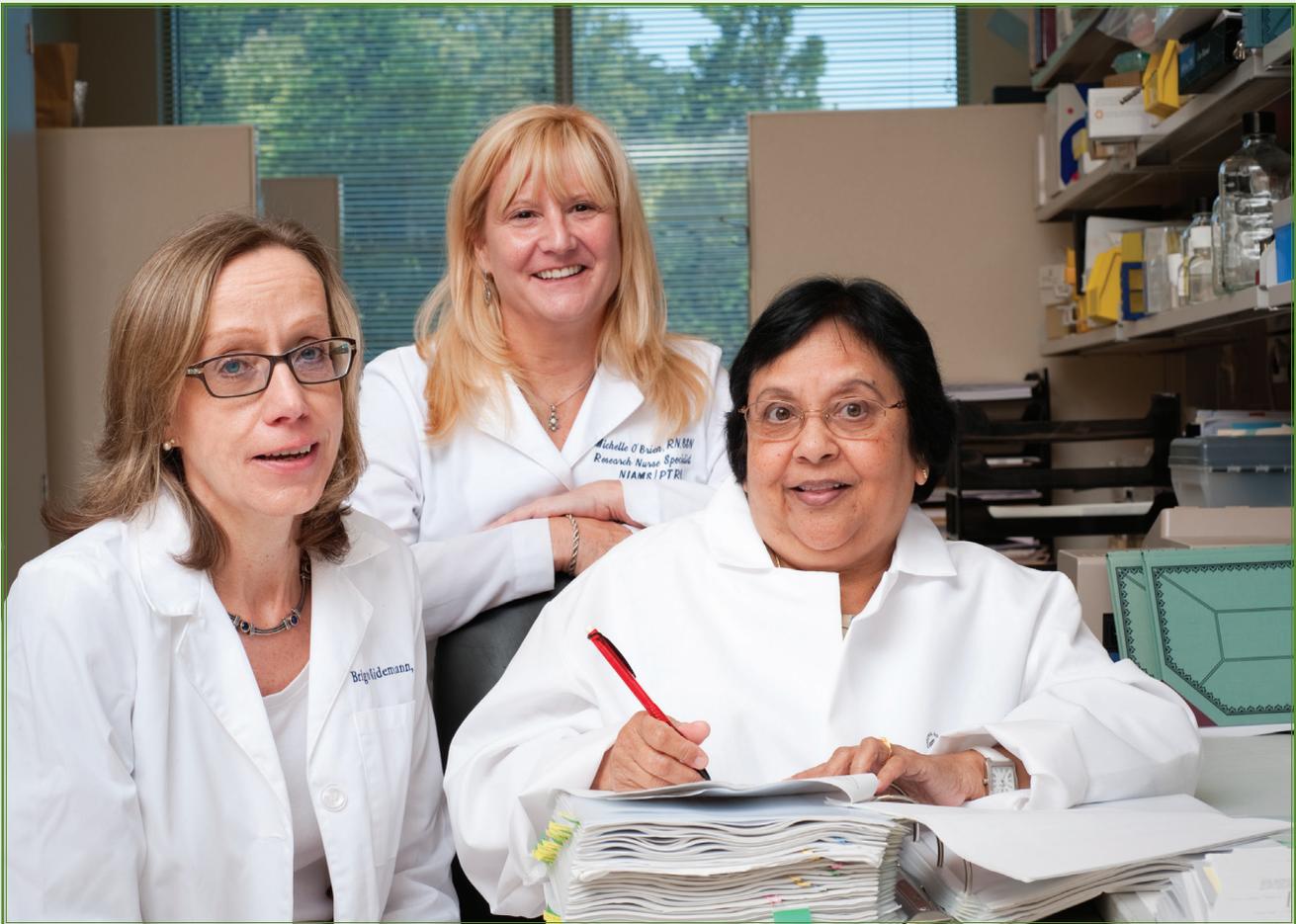
trexate. "With the data from monkeys, we developed a clinical trial to give the enzyme to patients who had high-dose methotrexate-induced kidney dysfunction," explained Widemann. The trial began with just a few patients in 1992; by the end, more than 300 patients were enrolled from across the U.S. and Europe.

"We were on call 24/7, as were the CTEP pharmacists," said Widemann. "We'd assess the patient information, talk to the physicians, and ship the drug to wherever it was needed. Blood samples were sent back to us for evaluation in real time. Within 15 minutes of administration of the enzyme, we would see an almost two-log decrease in methotrexate concentrations in every single patient."

Critically, by working with the FDA, the researchers were able to successfully make the case that plasma methotrexate levels were a good surrogate for the development of toxicity. "Ideally, you want to give this to patients with very high plasma methotrexate concentrations before they developed any clinical toxicities." Of the patients that

"Within 15 minutes of administration of the enzyme, we would see an almost two-log decrease in methotrexate concentrations in every single patient."

(Photo: R. Baer)



Brigitte Widemann, M.D., Michelle O'Brien, R.N., a Research Nurse who worked on the clinical trials for glucarpidase, and Nalini Jayaprakash, M.S.

received the enzyme within 48 hours of starting their methotrexate infusion, no fatalities were reported. But patients who received the enzyme at later time points after the drug was already distributed into the cells were often not so lucky.

However, by the time the healthcare company BTG sought approval for the drug, the availability of new FDA guidelines required additional work prior to seeking approval. "The drug was initially not manufactured according to current FDA requirements and the high performance liquid chromatography (HPLC) assays for methotrexate concentration had to be revalidated using the now available FDA guidance." Fortunately, Nalini Jayaprakash, M.S., who began working with David Pollack's team in 1991, and had come to NCI from the pharmaceutical industry was

able to revalidate the assay using the new FDA guidelines and compared old and new lots of the enzyme to satisfy regulators; meanwhile, the team ran a 30-patient prospective trial to confirm their earlier findings. Glucarpidase is now approved under the trade name Voraxaze, for the treatment of toxic plasma methotrexate concentrations.

"The FDA came to do an audit on our data and we learned how important meticulous documentation and standard operating procedures are for regulatory approval," said Widemann. "If you want to develop a drug, you have to be very patient and persistent. Ours was a real team effort within the laboratory, CTEP, and across agencies, and I am very happy to be part of bringing a life-saving drug to the clinic."

To learn more about Dr. Bates's research, please visit her CCR website at <https://ccr.cancer.gov/susan-bates>.

To learn more about Dr. Dean's research, please visit his CCR website at <https://ccr.cancer.gov/michael-dean>.

To learn more about Dr. Lowy's research, please visit his CCR website at <https://ccr.cancer.gov/douglas-r-lowy>.

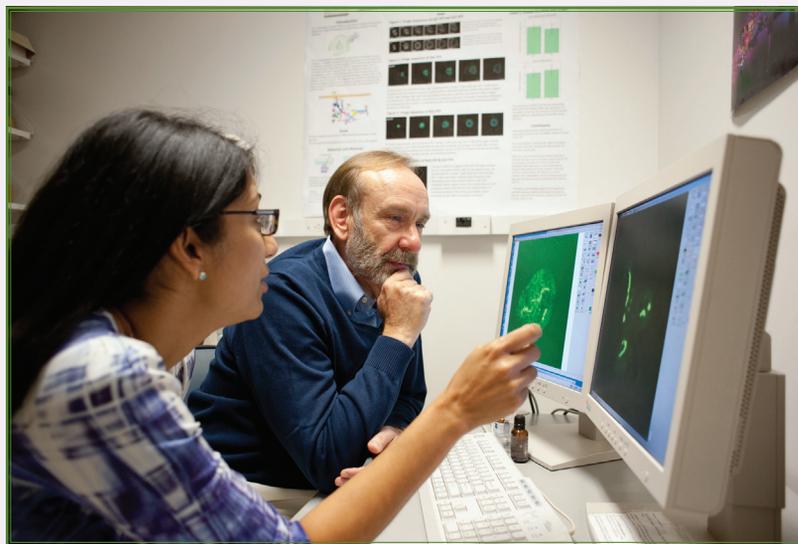
To learn more about Dr. Schiller's research, please visit his CCR website at <https://ccr.cancer.gov/john-t-schiller>.

To learn more about Dr. Widemann's research, please visit her CCR website at <https://ccr.cancer.gov/brigitte-c-widemann>.

The Complex, Inner Life of T Cells

Among their unique immunological capabilities, T lymphocytes distinguish self from nonself as they patrol the body for invaders. Their acute sensitivity—even a few properly identified molecular intruders can incite them to action—means that their behavior is tightly regulated. The molecular recognition and signaling processes at the heart of the T-cell response have fascinated Lawrence Samelson, M.D., Chief of CCR’s Laboratory of Cellular and Molecular Biology, since he chose to do his Yale medical school thesis on immunology in the mid-1970s. Today, his laboratory combines classical biochemistry, genetics, and state-of-the-art imaging to dissect the signaling events that underlie the diversity of T-cell responses at an increasingly fine spatial and temporal resolution. The knowledge they are accruing has contributed fundamental insight into and continues to inform the development of cancer immunotherapies.

(Photo: R. Baer)



Lakshmi Balagopalan, Ph.D., and Lawrence Samelson, M.D.

As a student in Elizabeth Simpson’s immunology laboratory in London in 1975, Samelson first learned that T cells simultaneously recognized antigens and the major histocompatibility complex (MHC) marker of “self” through the newly published and ultimately Nobel-prize winning discoveries of Rolf Zinkernagel and Peter Doherty. Seeing the same puzzling result in his own data, Samelson became convinced that he wanted to continue T-cell research after his medical training was completed.

“The T-cell receptor was still a fascinating, mythical creature,” explained Samelson.

Samelson joined the laboratory of Ron Schwartz, Ph.D., at the National Institute of Allergy and Infectious Diseases as a Postdoctoral Fellow, trying to generate monoclonal antibodies to a T-cell antigen receptor. “We were one of the early ones to succeed. At that point, I realized I was never going back to medicine. The big question for me was how does the receptor function: how does it recognize an antigen

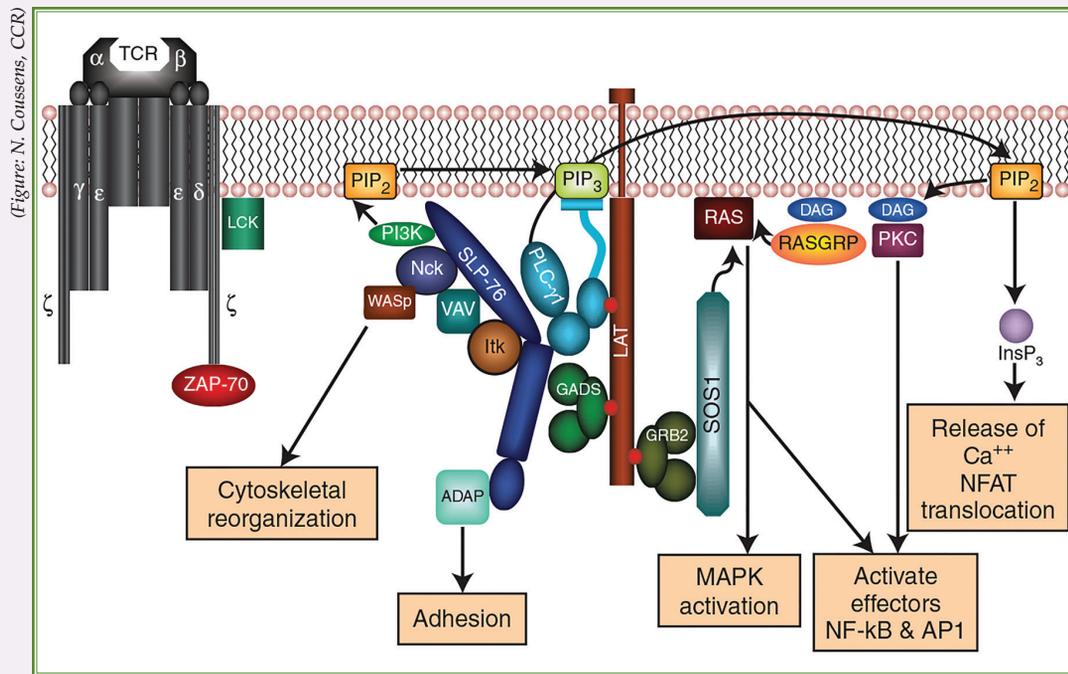
and transmit that recognition inside the cell. And that’s what I’ve been studying ever since.”

The Basics of T-Cell Receptors

T lymphocytes recognize antigens that are processed and presented to them in combination with MHC molecules by specialized antigen-presenting cells. During their development, individual T cells are selected for survival based on the ability of their receptors to recognize MHC molecules and are selected for destruction if they recognize antigens that are normally present in the body. Mature T cells are specialized to cooperatively mount a complex defense when presented with foreign antigens.

“T lymphocytes do a lot of things. As a cell differentiates in and later outside of the thymus, decisions are imposed by the environment that determine what kind of T cell it is going to be. What kind of cytokines does it make? Where does it go? Many of these complex differentiating events have to do with signaling through the antigen receptor,” said Samelson.

T-cell receptors comprise multiple



Ligation of the TCR induces tyrosine phosphorylation of numerous adapter and effector proteins leading to the activation of multiple signaling pathways important for gene transcription, cytoskeletal reorganization, and cell adhesion. LAT is central to this process by nucleating multiprotein signaling complexes that are important for enzyme activation and signal propagation.

subunits, which together span the outer membrane to transduce molecular recognition on the cell surface into intracellular action. During a multiyear collaboration with Richard Klausner, M.D., Samelson defined many of these subunits, including the critical ζ subunit.

Like many such transduction events, phosphorylation by tyrosine kinases is a key initiating step. Samelson became interested in the substrates of this phosphorylation and focused on one heavily phosphorylated molecule, which his laboratory finally cloned in 1998 and named Linker for Activation of T cells (LAT).

“LAT is heavily phosphorylated, after which many molecules are recruited to bind it from the cytosol and plasma membrane to create signaling complexes—probably not just one type, but hundreds of them. My underlying assumption is that understanding the complexity of signaling complexes helps you to understand the complexity of

T-cell function,” said Samelson. His laboratory has done extensive biochemistry to tease apart the binding events and interactions of LAT within a molecular alphabet soup that includes Grb2, Grap, PLC-γ1, and phosphatidylinositol 3-kinase (PI3K). From such studies and analyses of molecular mutations, the field has gradually built a picture linking LAT phosphorylation to pathways for cytoskeletal reorganization, adhesion, calcium influx, and gene expression (see Figure).

Clusters within Clusters

“Another approach that has proven very interesting is to do signal transduction studies through imaging,” said Samelson. “Instead of grinding up 10 million cells, you can look at one cell and a few thousand molecules. You can study signaling in time and space.”

The Samelson laboratory has employed a variety of imaging capabilities to get the spatial and temporal resolution they need to

study T-cell receptor activation. Using confocal imaging and total internal reflection fluorescence (TIRF) microscopy, they have looked at individual T-cell activation events. “Within the first few seconds, as a T cell is activating, a lot of rapid events important for later signaling occur,” explained Staff Scientist Lakshmi Balagopalan, Ph.D. “LAT is probably not binding all its associated molecules at once. So speed is important to understand the heterogeneity of responses.”

Spatial resolution is also critical. “The big discovery is that these signaling molecules are in microclusters, which are the basic signaling units,” said Samelson. Microcluster formation precedes the formation of immunological synapses, which mediate signaling between immune cells.

Initially working with Jennifer Lippincott-Swartz, Ph.D., at the Eunice Kennedy Shriver National Institute of Child Health and Human Development, to establish the technique, Samelson’s laboratory

has moved into super-resolution microscopy in order to see into the microclusters. The specific technique, photoactivated localization microscopy (PALM), uses stochastic activation and imaging of rare fluorescent molecules as a means to bypass the normal diffraction limits of light microscopy. PALM has given the team new insights into T-cell receptor organization, and new questions.

“You don’t see a homogenous distribution of molecules. Nano-clusters make up the microclusters you can see with confocal microscopy,” explained Samelson.

Balagopalan is particularly enthusiastic about a recent collaboration to do focused ion beam scanning electron microscopy (FIB-SEM) with Sriram Subramaniam, Ph.D., Senior Investigator in CCR’s Laboratory of Cell Biology. With this technology, they will be able to correlate the confocal images with structural information from electron microscopy. “We are actually going to look at how what we are seeing by fluorescence microscopy correlates with the ultrastructure of clusters.”

Linking to Function

Gathering biochemical, biophysical, and imaging data, Samelson and his colleagues are able to make predictions about the contributions of signaling molecules to T-cell function, which they test *in vivo*. Recently, in a paper in *Science Signaling*, they were able to elegantly separate two distinct functions of the interactions between LAT and its partner, Sos1.

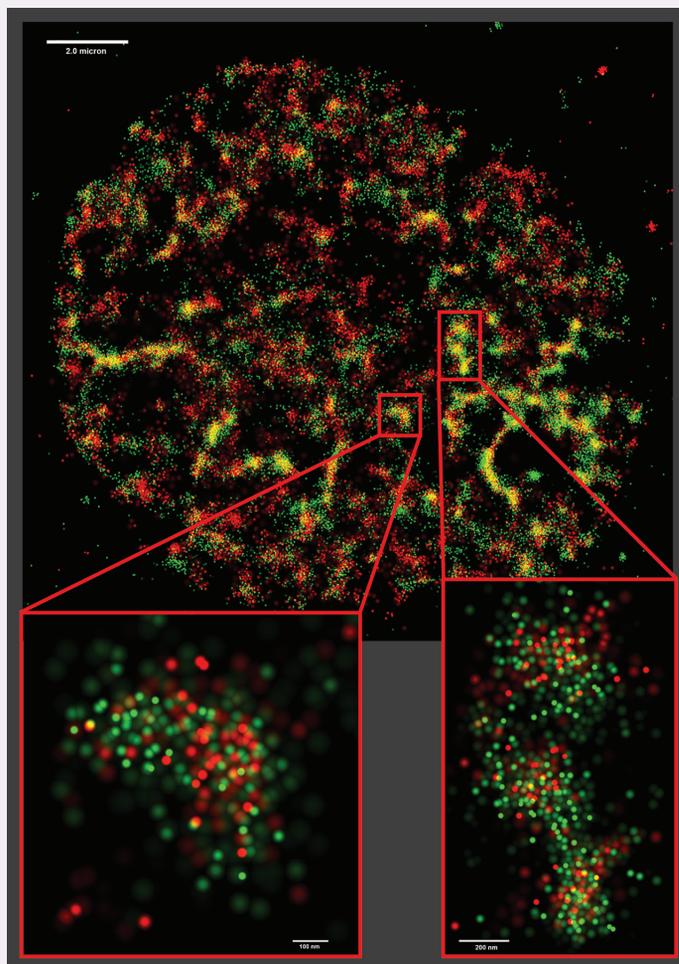
Sos1 is a prototypical guanine nucleotide exchange factor (GEF), which activates the small GTPase Ras by replacing GDP with GTP, thereby initiating a signaling cascade important for cell proliferation. By introducing a point mutation in Sos1, inhibiting its GEF catalytic activity, they showed that Sos1 has an independent ability to act as a

scaffold for multiple LAT molecules. This oligomerization—and not the GEF activity—was necessary for optimal T-cell receptor-dependent phosphorylation and activation of calcium signaling in thymocytes. But, both activities were required for normal T-cell development.

“That work is an excellent example of the multiple approaches employed in Larry’s laboratory coming together to answer an important question. It started out with a prediction of a former Postdoctoral Fellow, Jon Houtman, Ph.D., now at the University of Iowa, based on isothermal titration calorimetry with little pieces of protein in a test tube, that Sos1 wasn’t just

acting as a guanine nucleotide exchange factor for Ras in this system,” said Balagopalan. “And it ended with a talented postdoc, Rob Kortum, making a series of mutant mice to successfully test this hypothesis.”

“Making the transgenic mice took about five years,” said Samelson. “Sos1 is one of the granddaddies of the field, but no one had made a conditional knockout. The NCI has this tremendous transgenic mouse facility in Frederick, which made it possible to go through many different transgenes to get sufficiently low levels of expression to minimize artifacts and do all the necessary crosses.”



(Image: J. C. Yi, E. Sherman, and L. Samelson, CCR)

PhotoActivation Localization Microscopy (PALM) was used to image Jurkat T cells activated by contact with a stimulatory surface. This method is able to show the location of proteins with a resolution of 20 nm. Two proteins needed for signal transduction are shown; the green dots show molecules of the adapter LAT, while the red dots show molecules of Grb2. This image shows that these two proteins mix randomly within large microclusters of signaling proteins. Eric Betzig, Ph.D., was awarded the Nobel Prize for Chemistry in 2014 for developing this technique.

Toward a Better T Cell

The immune system has a special challenge in identifying cancer cells which, after all, are derived from “self”. Moreover, cancers evolve rapidly and take active steps to camouflage themselves and subvert immune signaling. Recently, the use of designer T cells, whose receptors have been engineered to recognize cancers, has made some promising advances in the clinic. These chimeric antigen receptors (CAR) are made by coupling an antibody that recognizes a tumor antigen to the T-cell receptor ζ chain, thereby coupling it to the entire T-cell signaling machinery.

“If you focus on the CAR story, you find that bypassing the normal antigen receptor isn’t enough. You need to add signaling elements from costimulatory pathways,”

said Samelson. “Understanding and manipulating the signaling pathways is how immunotherapy will continue to improve.”

Progress will no doubt be accompanied by unexpected complexity. Making another point mutation in LAT, Samelson’s laboratory found that they were able to make T cells hyperactive and more potent as measured in a number of assays. Early signaling events like calcium influx were greater. A mouse expressing the mutant T cells had increased cytokine production. In another assay, the mutant T cells were able to destroy targets more effectively. “We thought we had made a better T cell,” said Samelson.

Working with Nicholas Restifo, M.D., Senior Investigator in CCR’s Surgery Branch, Samelson discovered that the story was not

so straightforward. “We did a lot of studies to see whether the T cells in our mouse would clear tumors better. And they didn’t. The cells were too good; they got over-activated and exhausted.”

However, the collaboration with the Restifo lab continues, focused on another knockout mouse model that has improved tumor clearance. Samelson and his colleagues have recently identified the mechanism of action accounting for the superior T-cell response, a prerequisite for developing superior therapies.

To learn more about Dr. Samelson’s research, please visit his CCR website at <https://ccr.cancer.gov/lawrence-e-samelson>.

Scientists without Borders

The Laboratory of Cellular and Molecular Biology (LCMB), of which Lawrence Samelson is Chief, comprises six research groups working on diverse questions in signal transduction, ranging from receptor activation to nuclear transport. When the laboratory moved to its current facilities, Samelson ordered that the doors between the labs be taken down.

“Some people were skeptical, but it has really been transformative in the free flow of traffic between labs. He has literally taken down the barriers between us!” said Staff Scientist Lakshmi Balagopalan, Ph.D. “The Samelson group and LCMB are by far the most open and congenial environments I have ever been in; and, a large part of the credit for this goes to Larry.”

Balagopalan joined Samelson’s laboratory as a Postdoctoral Fellow

in 2003. Trained as a fly geneticist, she was looking to switch into immunology. She did some homework, interviewing current and former members of the lab, who gave strong positive recommendations. “Larry is a fantastic mentor, who creates a great environment conducive to creativity and research. No politics, just focus on the work.” Senior lab members like Balagopalan and fellow Staff Scientist Connie Sommers, Ph.D., are encouraged to take senior authorship on manuscripts. “That is a big motivator for us to drive and take ownership of projects.”

The strength of the immunology field in CCR and the NIH Intramural Program is tremendous including multiple institutes, like the National Institute of Allergy and Infectious Diseases, and the National Heart, Lung, and Blood Institute. “It’s a

tight community,” said Balagopalan. “When I entered the field, it was like learning a new language. I took an immunology course that Larry encourages incoming trainees to take run by the Foundation for Advanced Education in the Sciences. It’s an amazing introduction, taught by leaders in the field like Ron Germain, Bill Paul, and of course, Larry.”

“The kind of materials we use cannot be bought on the street. Experts here can make phosphorylated 80-amino acid peptides. Experts here are world leaders in analytic techniques and high-resolution microscopy. And I can’t think of a place in the world where there are more outstanding immunologists,” said Samelson. “The NIH Intramural Research Program makes it possible to do the work we do.”

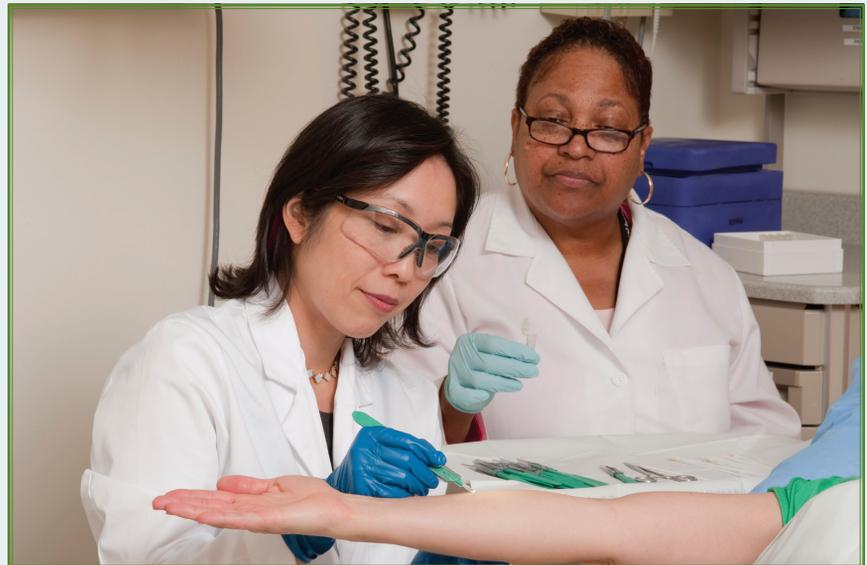
On Being Human: Life in the Microbiome

When leaders of the Human Genome Project promised new insight into what it means to be human, they did not have microorganisms in mind. With massive advances in tools to rapidly sequence and assemble billions of DNA base pairs—which have seen sequencing costs drop by orders of magnitude—clinical researchers were understandably focused on decoding human genes. But the beauty of scientific inquiry is that a journey toward one destination often leads to unanticipated treasure troves. The new sequencing technologies have quickly given scientists an unprecedented view not only of the human genome, but of the ecosystems of microbial life forms that share the human epithelium. CCR scientists are embracing the microbiome as a heretofore unrecognized and potentially important player in human health and disease.

Skin Deep in Microbes

“One of our approaches to understanding disease is to step back and determine what is considered healthy or normal,” said Heidi Kong, M.D., M.H.Sc., Investigator in CCR’s Dermatology Branch. Her interest in diseases of the skin, made its microbial landscape a natural research subject. Kong and her intramural collaborator, Julie Segre, Ph.D., Chief of the National Human Genome Research Institute’s Translational and Functional Genomics Branch, have been working together for several years to map the microbial ecosystem of the skin.

The presence and diversity of microbes have been vastly underestimated in many contexts, primarily because these organisms have been so difficult to study. Typically, researchers have needed to grow a critical mass of a given bacterium or fungus in a dish. “Culturing techniques depend on certain conditions, so there are many microbes that can be difficult to isolate,” explained



(Photo: R. Baer)

Heidi Kong, M.D., M.H.Sc., collects skin samples with the assistance of Research Nurse Shelia Phang.

Kong. “Genomic methodologies are somewhat less biased in their assessment of diversity.”

In one of their first papers, which appeared in *Science* in 2009, Kong, Segre, and their colleagues looked at bacterial diversity on multiple

skin sites on the human body. All prokaryotes have distinct sequences of rRNA that comprises the 16S small-subunit of the ribosome. Part of the sequence is variable among species, which makes it suitable for bacterial identification; part is highly

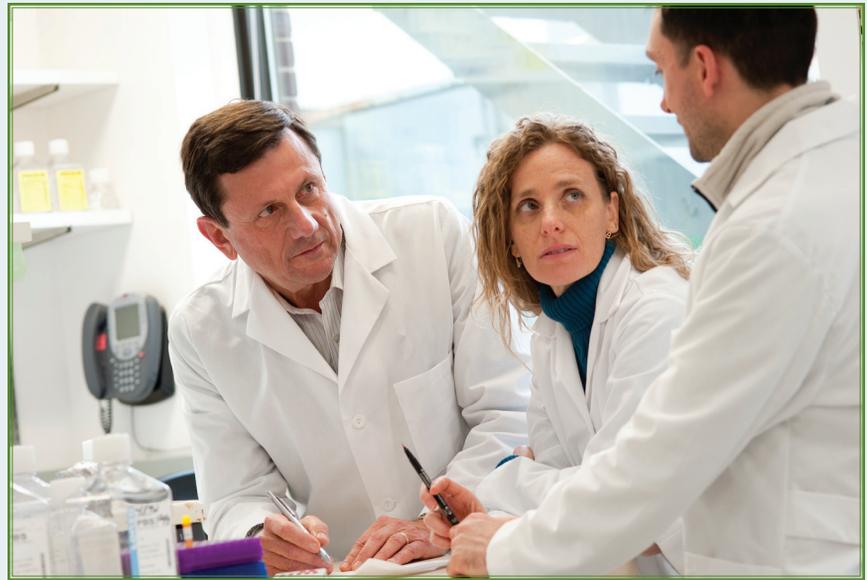
“We showed that two skin sites a few inches apart might have quite different microbial communities.”

conserved which means it can be easily targeted for sequencing. By sequencing the 16S ribosomal RNA from 20 distinct skin sites, Kong and Segre found patterns of microbial species related to skin type: oily, dry, or moist.

“We showed that two skin sites a few inches apart might have quite different microbial communities,” said Kong. This result not only spoke to the diversity of the skin microbiome, but the need for very careful sampling in ongoing studies. “Human microbial load on the skin is very low, as compared to stool samples, for example,” said Kong. “We’ve had to optimize clinical protocols and methods in the laboratory to obtain robust results.”

Kong and her team went on to examine bacterial communities in skin diseases such as atopic dermatitis, a common chronic inflammatory skin condition also known as eczema, that has been on the rise among children in the industrialized world. The disease has been associated with *Staphylococcus aureus* and is commonly treated with antibiotics and corticosteroids. By looking at patients over multiple time points, during and between flare-ups of the disease, they found that inflammation was associated with an increase of both *S. aureus* and *S. epidermis*, and a decrease in overall bacterial diversity.

More recently, the researchers have modified their techniques to study fungi as well as bacteria. In addition to targeting a different ribosomal RNA gene, they also had to adapt their laboratory handling protocols to capture these distinct species. “The body sites with much more fungal diversity were on the feet, which is strikingly different from bacterial distributions,” said Kong. They also looked at patients with primary immunodeficiencies, who often suffer from recurrent skin



(Photo: R. Bauer)

Giorgio Trinchieri, M.D., Romina Goldszmid, Ph.D., and Marco Cardone, Ph.D., in the lab

infections and found patterns of bacteria and fungi that aren’t found in healthy individuals. “It suggests that the defect in their immune systems allows their skin to be more permissive for fungi and bacteria,” said Kong.

In October 2014, Kong, Segre, and their colleagues published a paper in *Nature* describing the results of shotgun metagenomics sequencing, a technique that allowed them to capture the entire genomes of microbes present on the skin. “Instead of only looking at one group of microbes, now we look at all microbes *in toto*. We get a better sense of how the bacteria relate to the fungi and viruses in one sample. And with the whole genome, we potentially obtain some insight into the functional capacity of these microbes.”

Getting beyond correlations is a key challenge for microbiome researchers. “With sequencing data, you are inundated with the millions of sequences, so trying to tease apart what all these data mean is a challenge. The key point is that these studies are helpful for identifying associations that can be further studied in a more

targeted fashion, including a host immunological perspective.”

A Cancer Ecosystem

Giorgio Trinchieri, M.D., Program Director of CCR’s Cancer and Inflammation Program and Chief of the Laboratory of Experimental Immunology, has long focused his research on the interplay between innate and adaptive immune systems, and their role in cancer progression and therapy. The innate immune system is the evolutionarily older, first line of defense against a variety of threats, with cells ready to mobilize at a moment’s notice. Trinchieri sees innate immunity as an adaptation of signaling mechanisms that evolved as eukaryotes emerged.

“As soon as these multicellular organisms formed, they were living in a world that was full of microbial bacteria, viruses, fungi, and so on. Multicellular organisms are symbionts of host cells with commensal microorganisms and cannot live without these interactions. What we use today for natural immunity—and even adaptive immunity—are the mechanisms that originally allowed the components of the symbionts to

“We want to understand how the gut signals to these cells and what happens at the genetic and epigenetic level to prime them differently.”

communicate with each other, maintain equilibrium, and avoid pathogens,” explained Trinchieri.

This point of view led Trinchieri to consider how differences in an individual’s microbiome might affect cancer and its treatment. Trinchieri and Staff Scientist Romina Goldszmid, Ph.D., led their colleagues in a study, published in *Science* in 2013, of three xenograft tumor models, based on lymphoma, carcinoma, and melanoma cell lines chosen for their sensitivity to specific therapies (see “Interspecies Cooperation to Fight Cancer,” *CCR connections* Vol. 8, No 1). To deplete their microbiomes, mice were either pre-treated with a cocktail of antibiotics or raised from birth in a germ-free environment. Then, once the tumors reached a certain size, the mice were challenged with the appropriate immunotherapy or a conventional platinum-based chemotherapy.

Regardless of the tumor type or treatment examined, mice depleted of their gut microbes responded less well to therapy than controls, as measured by reduction in tumor volume. Moreover, the number of myeloid-derived cells infiltrating the tumors was reduced in the absence of microbiota and was associated with reduced tumor necrosis factor (TNF) production by myeloid cells in the case of immunotherapy and reduced reactive oxygen species (ROS) production after chemotherapy. Administration of bacterial products could restore TNF production by tumor myeloid cells in animals depleted of their gut microbiota. Allowing microbial colonies to

reestablish after antibiotic treatment, they further determined that bacterial composition—and not just abundance—was critical.

“One big question that emerges is how changes in the gut microbiome (and possibly in other anatomical barrier sites), affect the way the inflammatory cells inside the tumors respond to different types of therapy,” said Trinchieri. “In germ-free animals, we were quite surprised that there were only very minor differences in the numbers and gene expression parameters of inflammatory cells. But, when you treat the tumors, the response is completely different. We want to understand how the gut signals to these cells and what happens at the genetic and epigenetic level to prime them differently.”

In addition to further work in animal models, Trinchieri and his colleagues are beginning to investigate the human microbiome, for example, by taking advantage of blood and microbiota samples from existing protocols in which volunteers are treated with antibiotics. “But we also want to look at cancer patients—characterizing their microbiota and their responses to treatments—and look epidemiologically at patients treated with antibiotics for different reasons.”

“When you have any type of disease, but especially cancer, you must consider that it is growing in a metaorganism, which plays a role in creating the microenvironment in which the tumors grow and regulates the immune response. Even tumor therapy is going to be affected.”

Metabolic Reduction

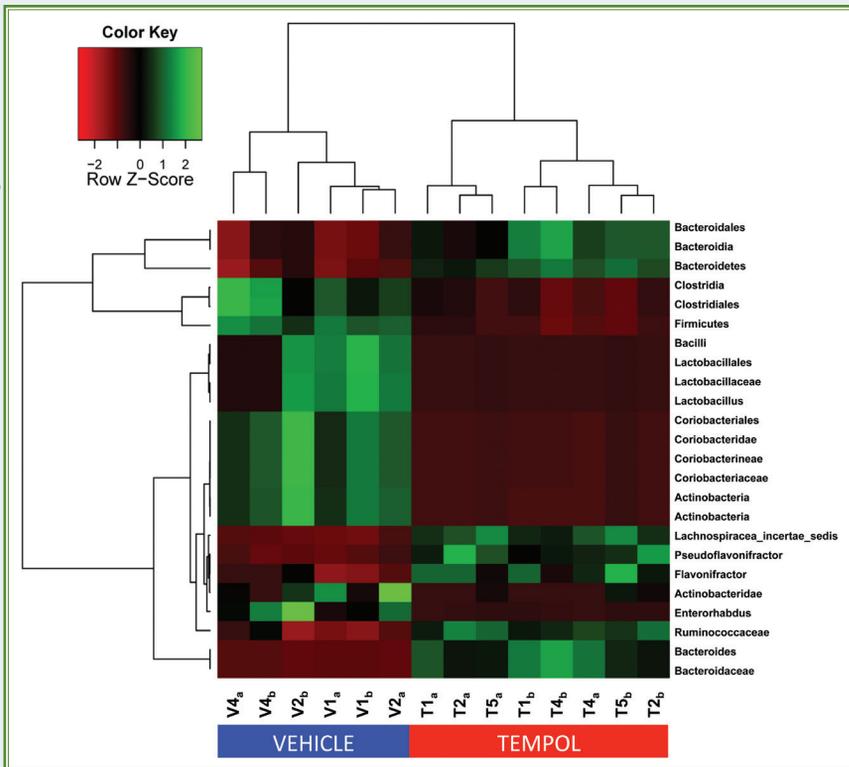
Frank Gonzalez, Ph.D., Chief of CCR’s Laboratory of Metabolism, specializes in the use of liquid-chromatography mass spectrometry (LCMS) as a tool to explore the mechanisms of cancer progression and therapy. His colleague, James Mitchell, Ph.D., Chief of CCR’s Radiation Biology Branch, asked him to take a look at the metabolomics profile of tempol, a nitroxide compound identified by Mitchell’s group as a protectant against radiation damage and independently associated with obesity prevention and protection from insulin resistance in mice.

“We found altered levels of suspected gut microbe-generated metabolites indicating that tempol either changes the composition of the microbiome or alters the metabolic potential of gut bacteria,” said Gonzalez.

Using 16S rRNA sequencing alongside metabolomics analysis, Gonzalez and his colleague Andrew Patterson, Ph.D., at Penn State University, found that tempol did indeed alter the gut microbiome, dramatically reducing both the genus *Lactobacillus* and levels of its enzyme bile salt hydrolase (BSH) in the feces. BSH enzymatically hydrolyzes conjugated bile acids produced in the liver; and the team found that the decreased BSH levels translated to an accumulation of one particular bile acid, tauro- β -muricholic acid, (T- β -MCA), an antagonist of the nuclear receptor farnesoid X receptor (FXR).

Modulation of the gut microbiota populations with either tempol or antibiotics decreases obesity in high-fat diet-fed mice and in genetically obese mice through inhibition of FXR signaling. It also improves insulin resistance (type 2 diabetes) and fatty liver disease, all of which are risk factors for cancer. Tempol would

(Figure: F. Gonzalez, CCR)



Gonzalez and colleagues found that tempol induces changes in the gut microbiome, including decreases in the family *Lactobacillaceae*. The heatmap of 16S rRNA gene sequence analysis depicts the microbial population found in caecum content after 5 days of treatment with tempol. The relative values for microbes are depicted by color: green colors indicate high values and red colors indicate low values.

not be a good therapeutic candidate because of the high doses required; the same beneficial results can be obtained by use of an FXR antagonist. Gonzalez and his colleagues have identified a derivative of T-β-MCA that exhibits the same efficacy toward metabolic diseases as modulation of the microbiota.

“We’ve finished the preclinical mouse studies filed a patent on this compound and its derivatives and are launching a study to treat diet-induced obese monkeys,” said Gonzalez. “It’s a promising compound because it is not absorbed into the bloodstream;

it stays in the intestine and there’s no indication of any intestinal toxicity in our studies. This is the first direct demonstration that metabolism by bacteria alters nuclear receptor signaling in the intestinal epithelium that effects obesity, diabetes, and fatty liver disease.”

Being in the Minority

“Within CCR, there are many separate lines of investigation on the microbiome that were initiated for very different reasons, but that I think will merge very quickly,” said Trinchieri.

“This is the first direct demonstration that metabolism by bacteria alters nuclear receptor signaling in the intestinal epithelium that effects obesity, diabetes, and fatty liver disease.”

Trinchieri points to two new initiatives that should aid in investigations of the microbiome across CCR: a germ-free gnotobiotic facility at the NCI campus in Frederick and a CCR core facility for molecular and bioinformatics studies of the microbiome in Bethesda. Moreover, CCR and the National Institute of Allergy and Infectious Diseases intramural programs are actively engaged in efforts to provide integrated support as scientists with diverse research interests consider how to assess the impact of the microbiome on their findings. As recently reported by Michael Gottesman, M.D., NIH Deputy Director for Intramural Research, in *The NIH Catalyst*, research on the human microbiome and drug resistance will be included in “The Future of IRP” document as one of the areas of scientific opportunity in which the NIH Intramural Program is best poised to succeed.

“When we look at an organism like a human being, we should not just focus on its own cells. The organism is really a symbiote, a metaorganism,” said Trinchieri. “In terms of numbers, we are up to 90 percent microbial cells.”

To learn more about Dr. Gonzalez’s research, please visit his CCR website at <https://ccr.cancer.gov/frank-j-gonzalez>.

To learn more about Dr. Kong’s research, please visit her CCR website at <https://ccr.cancer.gov/heidi-h-kong>.

To learn more about Dr. Trinchieri’s research, please visit his CCR website at <https://ccr.cancer.gov/giorgio-trinchieri>.

The Art of Endocrine Surgery

Martha Zeiger, M.D., is Professor of Surgery, Oncology, Cellular and Molecular Medicine, Associate Vice Chair for Faculty Development in the Department of Surgery, and Associate Dean for Postdoctoral Affairs at The Johns Hopkins University School of Medicine. In addition to an active endocrine surgery practice, she maintains a basic research laboratory focused on the identification of molecular markers associated with the diagnosis and prognosis of thyroid cancers. Committed to guiding the next generation of endocrine surgeons, she also directs the endocrine surgery fellowship program at Johns Hopkins and founded Endocrine Surgery University, a two-day course that brings together endocrine surgery fellows from across North America. From 1990–1993, Zeiger was a Surgical Oncology Fellow at NCI, under the mentorship of Steven Rosenberg, M.D., Ph.D., Chief of CCR's Surgery Branch, and Jeffrey Norton, M.D., now Professor of Surgery at Stanford University School of Medicine.

I am fascinated by endocrine tumors. While I was a fellow at NCI, I was exposed to a variety of clinical syndromes and clinical problems associated with them; it was one of the highlights of my academic career. Because these cancers arise from relatively small organs, which secrete hormones with wide-ranging effects on the body, they cause physiologically unique syndromes. Moreover, they are almost always curable; you can take out a tumor and dramatically improve a patient's life.

I devote about 60 percent of my time to my surgical practice, and perform approximately 250 endocrine surgeries per year. The surgeries themselves are aesthetically pleasing; there is a real delicacy associated with the procedure, whether for adrenal or parathyroid tumors, thyroidectomies, or resection of islet cell tumors of the pancreas. Endocrine surgery also has a fascinating history, which my co-editors and I have

recently highlighted in an edited collection of surgical stories, *The Supreme Triumph of the Surgeon's Art: A Narrative History of Endocrine Surgery*. For example, the tiny parathyroid gland was first discovered (and then forgotten) through the meticulous surgical necropsy of an Indian rhinoceros. The rhinoceros was most likely killed by an elephant at the London Zoo and later examined by the prominent anatomist and Darwin-opponent, Sir Richard Owen in the mid-1800s.

The other 40 percent of my time is devoted to basic and translational research. In addition to our surgical team, we have a large molecular biology research group, comprised of scientists, postdoctoral fellows, and students, who use a variety of genomic platforms to study molecular markers associated with the diagnosis and prognosis of thyroid cancer. It is truly exhilarating to be a part of the search, discovery, and challenging statistical analyses associated with this line of research.



(Photo: Courtesy of M. Zeiger)

Martha A. Zeiger, M.D.

Thyroid cancers
are the most
commonly
occurring
endocrine tumor.

Separating the Benign from the Malignant Thyroid Tumors

The thyroid resides at the base of the neck; it regulates cardiovascular function, temperature, and body weight. Thyroid cancers are the most commonly occurring endocrine tumor. For unknown reasons, their incidence has been steadily increasing over the past several decades; over 60,000 new cases were diagnosed in 2014. And, thyroid cancers range from one of the most indolent human cancers (papillary thyroid cancer) to one of the deadliest (anaplastic thyroid cancer).

We can tell a great deal about the diagnosis from clinical and histological features based on fine needle aspiration biopsies, but still many tumors remain indeterminate on biopsy without clear features of a benign or malignant lesion. Therefore, we and others are searching for molecular biomarkers that could add further precision to our ability to distinguish these tumors and allow us to tailor surgical operations appropriately.

There are a number of promising biomarkers under development. We have recently published encouraging results with DNA copy number variation for the diagnosis of follicular cancers (indistinguishable from benign adenomas by needle biopsy), and we are in the process of gathering data on prognostic markers for thyroid cancer as well. We are also studying microRNAs and mutations in *BRAF* in the prognostic evaluation of papillary thyroid cancer. Additional platforms

BRAF is an interesting example of the challenges that academic research faces in establishing the true clinical value of individual biomarkers with regard to predicting prognosis.

that we study include methylation arrays and alternative splice variant analyses.

BRAF is an interesting example of the challenges that academic research faces in establishing the true clinical value of individual biomarkers with regard to predicting prognosis. Recently, we analyzed more than 200 patients from four academic centers and found that there was no association of *BRAF* with lymph node metastases. While studies from other investigators have suggested a link between *BRAF* mutations and papillary thyroid cancer outcomes, many studies have not. Many variables account for the differing conclusions and only large-scale, well-designed, prospective studies will ultimately validate prognostic utility. We are currently conducting this very research with three other academic centers.

Recently, I was part of the NCI Cancer Genome Atlas (TCGA) program to genotype 400 papillary thyroid cancers. The exciting data resulting from this comprehensive

analysis suggests that we will ultimately need to redefine thyroid cancer subtypes. That will be the first step towards developing new treatment options.

Industrial Strength Cooperation

Our group has been involved with several companies in the development of molecular panels used for better thyroid nodule diagnosis. Many have very promising results and provide good negative and positive predictive information. Their use and impact in the context of a clinical setting has yet to be carefully evaluated and, many groups, including ours, are in the process of doing so. Researchers, clinicians, and biomedical industry ultimately seek the same thing—better diagnosis and better treatment for cancers. In order to tackle these clinical questions we need to work very closely together, carefully designing and evaluating molecular panels that are introduced into the market for clinical efficacy.

There are a number of promising biomarkers under development.

Curing Lymphomas, One Subtype at a Time

Now a Senior Investigator in CCR's Lymphoid Malignancies Branch, Wyndham Wilson, M.D., Ph.D., came to the NIH as a Clinical Fellow in 1984. After completing fellowships in oncology and infectious disease, Wilson joined NCI's Division of Cancer Treatment, where he began working on improving chemotherapy treatments for aggressive lymphomas. Wilson recruited Kieron Dunleavy, M.D., as a Clinical Fellow in 2002; Dunleavy has gone on to become a Staff Clinician. Working closely with their CCR collaborator, Louis M. Staudt, M.D., Ph.D., Wilson and Dunleavy have continued to optimize therapeutic strategies through clinical trials, which include targeted agents against molecularly differentiated subtypes of diffuse large B-cell lymphomas. Two of their recent trials, published in the New England Journal of Medicine, had cure rates above 95 percent.

Wyndham Wilson Throws Down the Gauntlet

My father was an academic physician who did medical research, so I knew I wanted to be an academic doctor since the age of six. My focus on clinical translation—integrating both laboratory and clinical work to make a real difference for patients—evolved with my training.

The backbone chemotherapy for the work we do today really started in 1989. We were trying to understand why people were failing chemotherapy. For the most common type of lymphoma, diffuse large B-cell lymphoma (DLBCL), only 30 percent of patients were being cured. So in collaboration with Tito Fojo, M.D., Ph.D., I developed the DA-EPOCH regimen right after my fellowship as a strategy to overcome the multidrug resistance and to optimize chemotherapy efficacy using continuous infusion schedules.



(Photo: R. Baer)

A patient, Kieron Dunleavy, M.D., Wyndham Wilson, M.D., Ph.D., and members of Wilson's clinical research group, Catherine Lai, M.D., and Mark Roschewski, M.D., having a conference

Building a Backbone

EPOCH consists of multiple chemotherapeutic agents—doxorubicin, vincristine, and etoposide—administered with prednisone and cyclophosphamide. Rather than simply specifying dosages based on amount

and dosing schedule, I brought in the concept of pharmacodynamically adjusting the dose (DA-EPOCH), I hypothesized that we could biologically assess drug clearance by monitoring the number of neutrophils in the blood and

developed a strategy to increase the chemotherapy doses until the neutrophils went below 500 cells. Depending on factors like age and genetics, different dose escalations are necessary to reach that same goal in different patients.

Following the development of rituximab, a monoclonal antibody therapeutic that targets a protein found prominently on the surface of B cells, we incorporated it into DA-EPOCH (DA-EPOCH-R). Based on multiple clinical trials, DA-EPOCH-R appeared to be more effective than the standard of care, R-CHOP (a combination of rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone).

Unfortunately, the history of the field made it difficult to convince the community to adopt DA-EPOCH-R until relatively recently. CHOP was developed in 1975, leading to a rash of published studies from single institutions that tested multiple variants of CHOP that incorporated more drugs, and appeared to be superior to CHOP. However, a randomized study ultimately showed that all these variations were equivalent to the original. Thus, there was a strong burden of proof required to renew optimism in the field.

To further confound the field, Lou Staudt elegantly showed that DLBCLs are not a single tumor type and, moreover, that different subtypes had different outcomes with R-CHOP. Based on this work, we showed that DA-EPOCH-R was particularly effective in specific molecular subtypes of DLBCL and have just completed a large randomized study comparing DA-EPOCH-R and R-CHOP within the molecular subtypes of DLBCL.

We have not only worked to improve efficacy, but also to limit the toxic side effects of treatment. We published two papers in the *New England Journal of Medicine*, testing

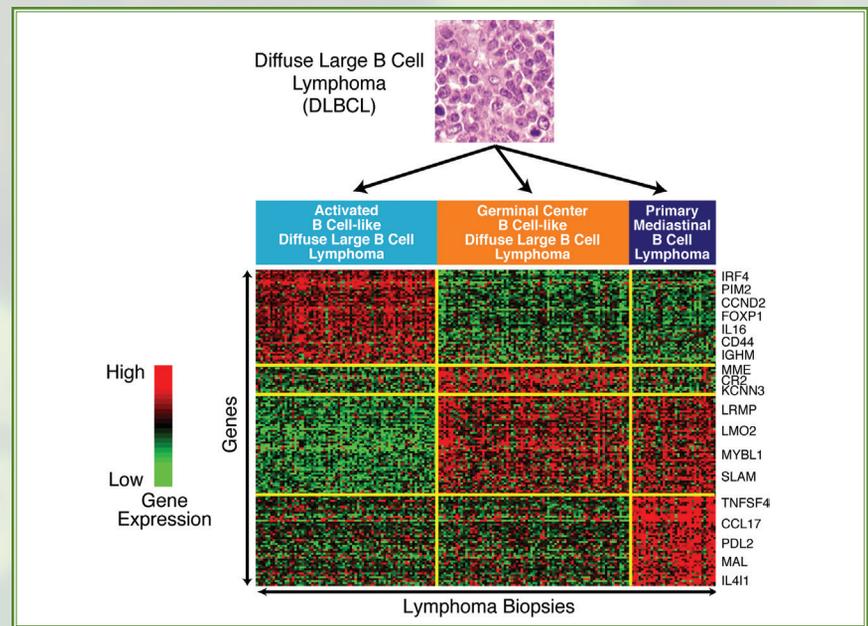


Figure: L. Staudt, CCR

Biopsies of diffuse large B cell lymphoma (DLBCL) reveal varying gene expression levels in activated B cell-like DLBCL, germinal center B cell-like DLBCL, and primary mediastinal B cell lymphoma.

DA-EPOCH-R in two subtypes of DLBCL: primary mediastinal B-cell lymphoma and Burkitt lymphoma. In the first, we were able to eliminate radiation therapy and in the second, we were able to substantially lower the amount and duration of chemotherapy while achieving long-term remissions and likely cure rates in approximately 95 percent of patients.

ABCs of Lymphoma

Lou and I met around the late 1990s, here at NCI. Interestingly, we were both in the M.D./Ph.D. Medical Science Training Program, which is sponsored by the Federal Government to train medical scientists—I was at Stanford and he was at the University of Pennsylvania. While I focused on clinical research, Lou pursued basic laboratory research, but we were both working on lymphoid tumors so it was a very logical thing to work together. He wanted to see his discoveries translated into therapeutic advances for patients, and I wanted to understand and advance the fundamental science

underpinning our work. It really was a synergistic situation and today, our groups work closely together.

One of the subtypes of DLBCL identified by Lou's laboratory—activated B cell-like (ABC)—has notably poor outcomes. Lou has identified a number of the molecular abnormalities and affected signaling pathways in ABC, so we have been working on a series of studies using drugs to block activation of these pathways.

In 2009, we published a paper in *Blood*, in which we showed that bortezomib increases the sensitivity of the ABC subtype to chemotherapy. Bortezomib is an inhibitor of the proteasome, which normally degrades cellular proteins. In particular, we were interested in stopping the degradation of I κ B α , an inhibitor of the NF- κ B pathway, which Lou's laboratory has found is constitutively active in ABC DLBCL. Based on our results, Millennium Pharmaceuticals, which produces bortezomib under the trade name Velcade, has started a large clinical trial looking at this drug in ABC DLBCL.

...we can now
cure most high
grade, B-cell
lymphomas.

We also did a study with the drug ibrutinib (Imbruvica), a potent inhibitor of Burton's tyrosine kinase (BTK), in DLBCL. BTK is a prominent signaling molecule in the NF- κ B pathway. Based on our findings, Pharmacyclic's partner Janssen, is sponsoring a large international randomized clinical trial with 800

patients to develop this drug in a combination therapy for patients with newly diagnosed ABC DLBCL.

As we dig deeper, we see that there are specific constellations of mutations that probably require different therapeutic strategies. Ultimately, for precision medicine to be successful, we need to develop the molecular tests to identify targets and combine those with the treatments in clinical trials. Lou and I have done that twice now and it's quite challenging. As we get into smaller molecular subsets, it becomes labor intensive and ultimately, a drug company needs to

step in and sponsor a large trial with a companion diagnostic.

With the strategies that we are pursuing here at NCI and the work that is ongoing worldwide, we can now cure most high grade, B-cell lymphomas. When we started, we were lucky if we could cure one in three. So, that's exciting and gratifying. But, other cancers have not fared so well. One of the most exciting things we are working on currently is a new program to treat primary central nervous system lymphomas (PCNSL), which is being led by my colleague, Kieron Dunleavy.

Kieron Dunleavy Takes Up the Challenge

After finishing my medical oncology training in Dublin, Ireland, I was interested in coming to NCI for a medical oncology fellowship because I wanted to focus on innovative clinical and translational research. Working with Wyndham Wilson and collaborating with Lou Staudt, much of the work that I have been involved with focuses on developing novel rational strategies for the treatment of various subtypes of aggressive lymphoma, as for example, in our work with modulators of the NF- κ B pathway.

When I first joined CCR, different subtypes of large cell lymphomas had been described, but developing therapies within molecular subtypes had not been done. With Wyndham and Lou, we performed a study in relapsed and refractory DLBCL using bortezomib with chemotherapy and incorporated gene expression profiling. Our hypothesis was that bortezomib would inhibit NF- κ B and we would see preferential activity in the ABC subtype (where NF- κ B is constitutively activated)

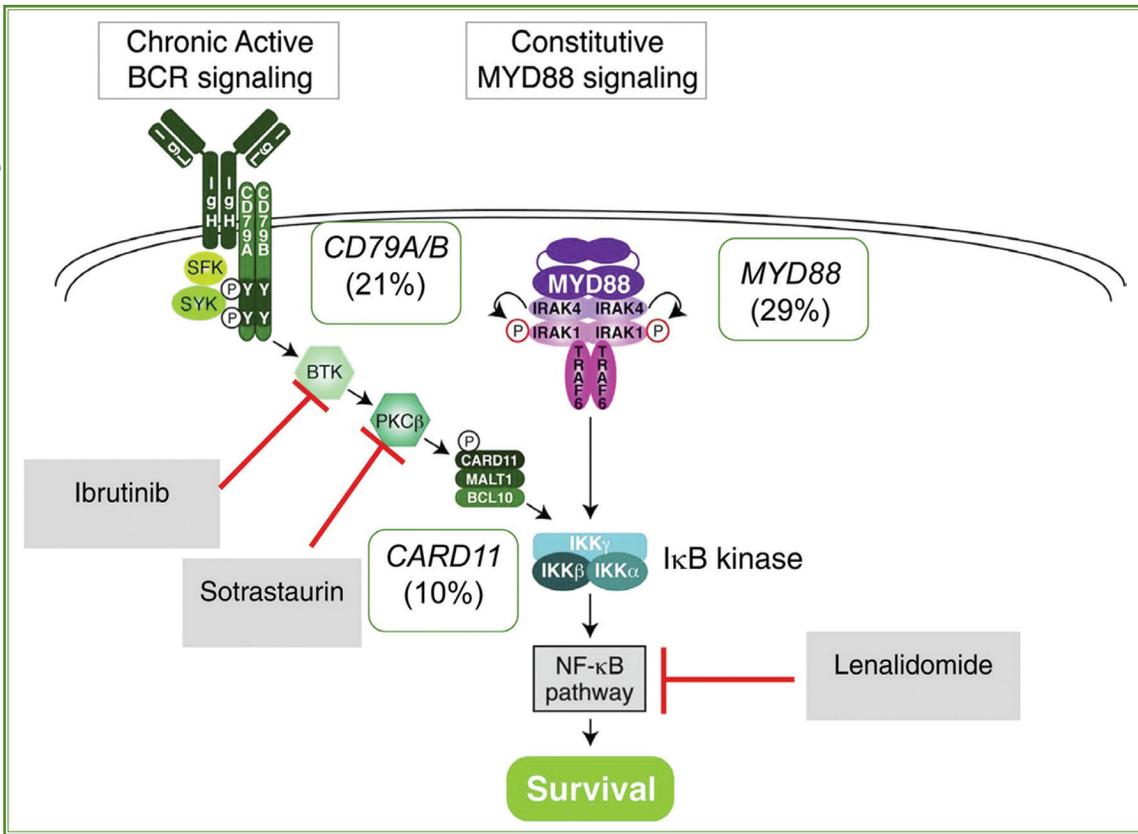
as compared to the germinal center B cell-like (GCB) subtype. We did observe a much higher response and survival rate in the ABC subtype in this proof-of-principle study, suggesting that preferentially targeting of a molecular subtype of DLBCL was possible. There are now ongoing randomized studies testing bortezomib in newly diagnosed patients with DLBCL.

Wyndham and Lou led a multi-institutional trial in systemic, relapsed diffuse large cell lymphoma that was just completed and demonstrated that the BTK-inhibitor, ibrutinib (imbruvica), is effective as a single agent in a significant proportion of ABC lymphomas. This is interesting when thinking about new ways to tackle PCNSL. These rare lymphomas constitute just 2–3 percent of all DLBCLs and importantly, outcomes for patients with PCNSL have been inferior to those with systemic DLBCL. There are many reasons for this, including the fact that drugs used in standard DLBCL platforms do not penetrate the blood brain barrier (BBB) very well. As a result, agents that do

penetrate the BBB well, such as methotrexate, have typically been used although they have low efficacy and are not routinely used in curable systemic lymphomas.

Recent work has demonstrated that most PCNSLs are of the ABC subtype and frequently harbor specific molecular aberrations that are 'targetable' with small molecule inhibitors. Based on its activity in systemic DLBCL, we hypothesized that ibrutinib could be effective in primary CNS lymphoma. We therefore designed a study that incorporated ibrutinib into a novel immunochemotherapeutic platform of drugs: TEDDI-R (Temozolomide, etoposide, doxil, dexamthasone, ibrutinib, and rituximab). The study is constructed so that there is a so called 'window' in which ibrutinib is tested as a single agent and various imaging and molecular analyses are performed within this period before the start of chemotherapy. Albeit early days in our study, the first two patients treated have demonstrated good responses and this is encouraging.

(Figure: L. Staudt, CCR)



The NF-κB pathway is activated through B-cell receptor signaling in activated B cell-like diffuse large B-cell lymphoma (ABC DLBCL). The mechanisms triggering activation may vary among tumors, and the position of molecular mutations in the tumors plays a role in treatment.

Low-Intensity Therapies in Aggressive Lymphomas

Our group also has a big interest in developing therapies for aggressive B-cell lymphomas that harbor a *MYC* rearrangement. Last year, we published a study demonstrating that in Burkitt lymphoma, DA-EPOCH-R was a highly effective treatment with very low toxicity. This represented a significant departure from standard Burkitt lymphoma strategies that are dose intense and are very toxic for patients, especially those who

are older or immunosuppressed. To validate our single-center findings, we are currently doing a multicenter risk-adapted study of the regimen in Burkitt lymphoma. As the regimen was so effective in Burkitt lymphoma, we retrospectively analyzed the outcome of our DLBCL cases that harbored a *MYC* rearrangement (about 10 percent of all DLBCL) following DA-EPOCH-R. In contrast to the experience with R-CHOP (where *MYC* portends a poor outcome), patients whose tumors had a *MYC* rearrangement had a

similar outcome to those without it. Hence, we have an arm in our multicenter study that will specifically evaluate the outcome of *MYC*-rearranged DLBCL cases with DA-EPOCH-R. Considering that R-CHOP is not effective for a high proportion of these patients, our multicenter results will be interesting. (See “On the Other Side of Cancer.”)

To learn more about Dr. Wilson’s research, please visit his CCR website at <https://ccr.cancer.gov/wyndham-wilson>.

To learn more about Dr. Dunleavy’s research, please visit his CCR website at <https://ccr.cancer.gov/kieron-m-dunleavy>.

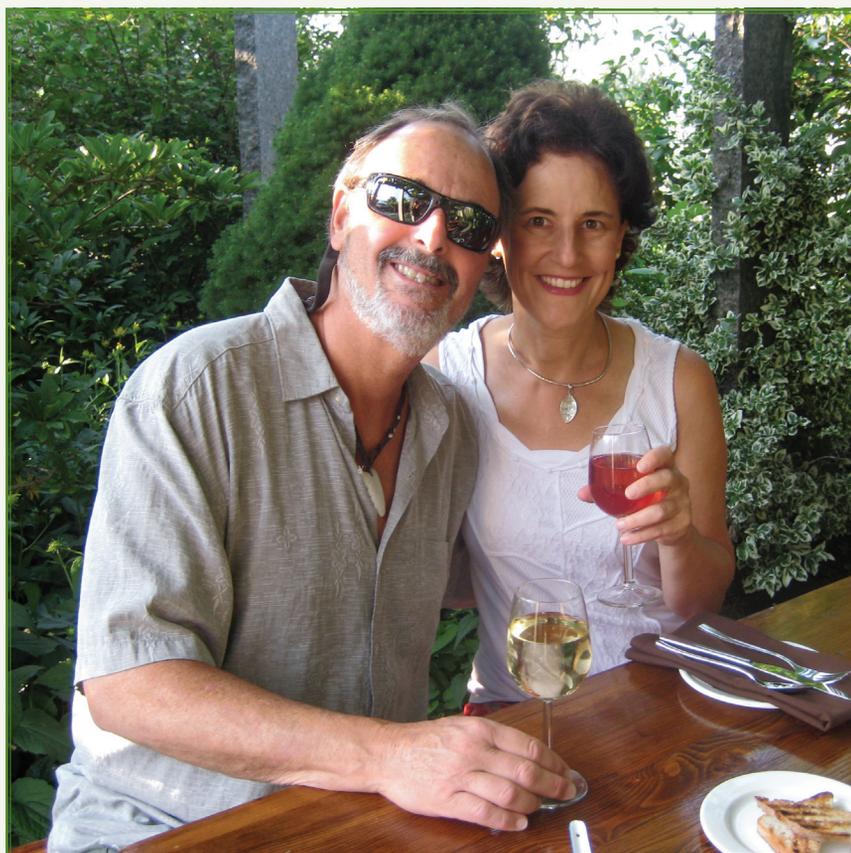
Albeit early days in our study, the first two patients treated have demonstrated good responses and this is encouraging.

On the Other Side of Cancer

At age 64, an active, healthy, 6'2", 210 lb Richard Spivack went to his doctor for a regular checkup. A blood test led to a sonogram, then an MRI, and finally a liver biopsy, which led to the diagnosis of large B-cell lymphoma with a rearrangement of the MYC gene. Then, it was time to find an oncologist. Through a network of friends, Spivack found a clinical trial with a new treatment for exactly this subtype run by Wyndham Wilson, M.D., Ph.D., and Kieron Dunleavy, M.D.

Spivack began an 18-week course of treatments at the NIH Clinical Center in July 2013. The DA-EPOCH-R treatment runs in six cycles of three weeks. "Part of the treatment consisted of me having a fanny pack strapped on to me full of chemo for five straight days, infused through a catheter placed in my bicep. Every 24 hours, I'd have it refilled," explained Spivack. "Going regularly to the NIH Clinical Center turned out to be a real blessing. I had many days where I was dehydrating and I needed blood infusions and I would just show up there. My whole existence was between my apartment and the hospital."

Spivack met with the medical team after every three-week cycle for review of blood tests and, PET/CT scan results when needed. "In between, I received all kinds of incredible medical care," said



(Photo: Courtesy of Richard Spivack)

Richard Spivack with Sandy Eisen

Spivack. "During my first cycle, I had a very adverse reaction to the chemo and ended up in the hospital for almost three weeks. Top medical specialists—cardiologists, infectious disease doctors—were evaluating me. Night and day, no matter what time it was, I had the best people."

The treatments ended in October 2013 and Spivack's cancer is in remission. He will return to NCI

every four months for the next two years to have scans and blood work, and sit down with the team to evaluate his case. For a subsequent three years, he will be followed at six-month intervals.

"Hearing that I had the Big C—that was the scariest part for me," said Spivack. "Once it was diagnosed and I had a treatment plan, I calmed down. Not once during the whole 18 weeks did I ever doubt what they were doing for me."

"At my very first meeting with Dr. Dunleavy, I asked him flat out what my chances were. You're not going to get to a promise, but he gave me every indication that this was something I could survive and get to the other side of."

"Not once during the whole 18 weeks did I ever doubt what they were doing for me."

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

<https://ccr.cancer.gov/office-of-the-director>

CCR News

<https://ccr.cancer.gov/ccr-news>

Office of Training and Education

<https://ccr.cancer.gov/training-office-of-training-and-education>

Patient Information on Cancer and Clinical Trials

Open NCI Clinical Trials

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

How to Refer a Patient

<https://ccr.cancer.gov/physicians>

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

<https://ccr.cancer.gov/clinical-trials-search-start>

Additional Links

National Cancer Institute (NCI)

<http://www.cancer.gov>

Working at NCI

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

National Institutes of Health (NIH)

<http://www.nih.gov>



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