NATIONAL CANCER INSTITUTE

Volume 10, No. 1 | 2016

CCR connections

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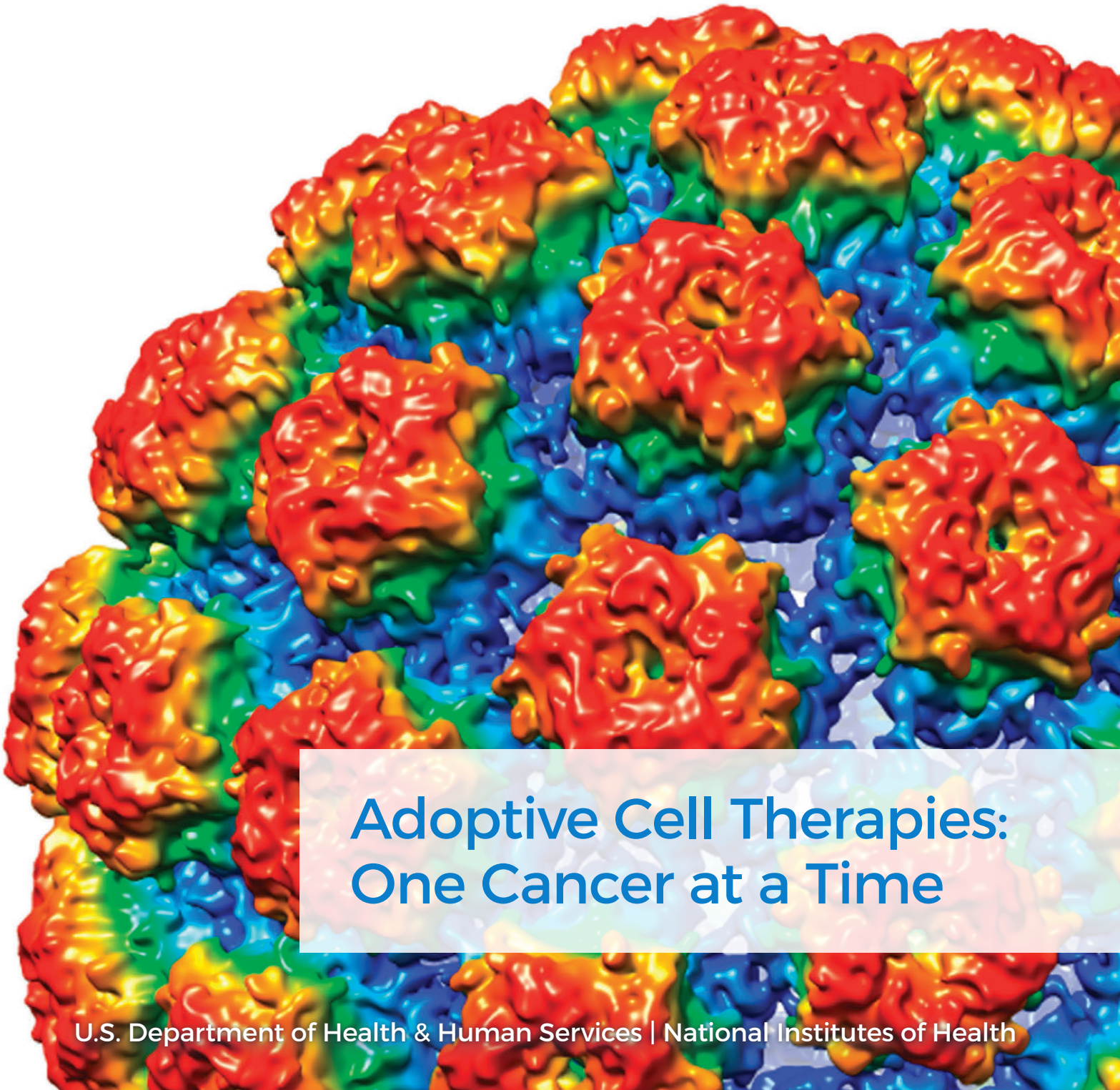

Adoptive Cell Therapies: One Cancer at a Time

U.S. Department of Health & Human Services | National Institutes of Health

We invite your comments and suggestions about *CCR connections*.

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Center for Cancer Research

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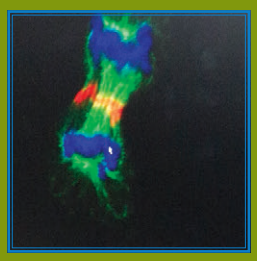

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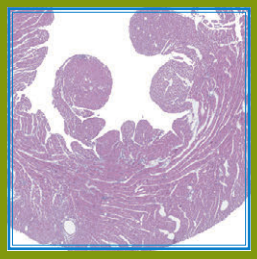

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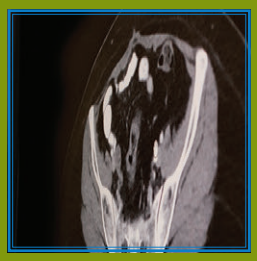

Adoptive Cell Therapies: One Cancer at a Time

NATIONAL CANCER INSTITUTE
Center for Cancer Research

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The mission of CCR is:

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

https://ccr.cancer.gov/connections

Cancer Research—The CCR Way!

More than 15 years ago, I joined the NCI Center for Cancer Research (CCR) as an eager and idealistic junior investigator. What attracted me to CCR were the stellar reputation of the NIH Intramural Program, the availability of state-of-the-art technology, and the certainty of having world-class colleagues by my side as I embarked on building my research program. While these are features common to most cutting-edge research institutions, I also recognized that CCR was a truly special place to do cancer research. My view has not changed a bit.

The scientific endeavor is driven by the free-ranging curiosity and creativity of individual basic and clinical researchers. CCR is one of very few institutions in the country where investigators can still freely pursue discovery, unencumbered by the need to justify our ideas in research grants. The CCR approach is complementary to many of the activities in academic institutions and allows us to pursue the most important, challenging, and provocative ideas in cancer research.

We have, for example, a long history of groundbreaking technology and methods development which, by definition, is high-risk and requires multiyear institutional commitments. Recent CCR breakthroughs include cryo-electron microscopy, which visualizes unprecedented detail in the structure of individual proteins to allow the identification and design of precisely binding, small-molecule inhibitors; and, UroNav, a magnetic resonance imaging method that enables high-precision prostate biopsies.

The intellectual liberty given to CCR investigators comes with responsibilities. It is our obligation as individual investigators, and as an institution, to take full advantage of our freedom to pursue the big questions in cancer research by creatively using our resources to push the boundaries of biomedicine and cancer research—across the spectrum from basic discovery to clinical practice and, increasingly, by building bridges between disciplines.

As the articles in this issue of *CCR connections* show, the CCR approach has been highly successful over the years. As CCR alum Bernard Fox, M.D., describes in "A Broader View of Immunotherapies," CCR was instrumental in pioneering some of the earliest immunotherapy strategies based on efforts to elucidate the fundamental mechanisms of how the immune system functions. Curiosity-driven studies laid the groundwork for the widespread use of this revolutionary therapeutic intervention. Similarly, the Human Papilloma Virus (HPV) vaccine, developed within CCR and now used routinely across the nation to prevent cervical cancer, was inspired by the curiosity to understand the life cycle of the virus. Now, CCR Investigator Christian Hinrichs, M.D., describes his use of immunotherapy to treat advanced HPV-related cancers in "Expanding the Use of Adoptive Cell Therapies, One Cancer at a Time."

(Photo: R. Baer)

Tom Misteli, Ph.D., CCR Director

We are continuing our tradition of doing impactful cancer research the CCR way. In "Inhibiting the Epidermal Growth Factor Receptor," we learn of three widely different approaches to understanding the network interactions and cell biology of this important cancer target, with a view to improving upon therapeutic strategies. In "The Secret Lives of Neurotrophin Receptors," we follow Lino Tessarollo, Ph.D., and the unexpected insights from his quest to understand a receptor family first identified here in Frederick as a fusion oncoprotein.

In the years since I joined CCR, my appreciation for the uniqueness and the importance of how we do things in CCR has only grown. Going forward, we will make every effort to ensure that we continue to do cancer research like nobody else!

The Effect of Memory

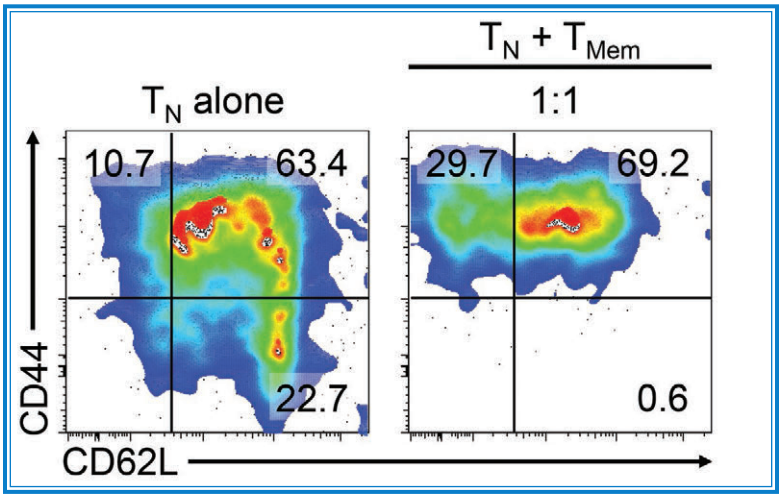
Adoptive cell transfer for cancer therapy may be hindered by memory T cells.

Though still experimental, adoptive cell transfer (ACT) to treat metastatic cancer has seen some dramatic successes (See "Going Home to Kansas," in this issue). In one form of ACT, a patient's own lymphocytes are extracted from their tumor and manipulated to mount a stronger attack against their cancer. The extracted T cells are stimulated with a tumor antigen; the cells that recognize that antigen survive and proliferate, whereupon they are re-injected into the patient.

Extracted cells are typically a mixed population of so-called naïve T cells (those that have not previously encountered antigen) and memory T cells. Once primed, naïve T cells progressively differentiate into memory cells of three varieties: stem cells, central cells, and effector memory cells.

A variety of evidence suggests that having more naïve T cells at the outset promotes a better outcome in ACT. In a recent issue of *Journal of Clinical Investigation*, Christopher Klebanoff, M.D., Assistant Clinical Investigator, and Nicholas Restifo, M.D., Senior Investigator, in CCR's Surgery Branch, led a study to ask what impact the inclusion of other T-cell populations may have during ACT.

First, using mouse models, the team found that mixing naïve T cells with memory T cells caused the naïve cells to differentiate at an accelerated pace into effectors, as reflected in key cellular markers, overall gene expression patterns, and physiological responses (e.g., secretion of IFN-γ). Moreover, when reconstituted into tumor-bearing mice, the mixed cell population was less able to reduce that burden. The effect of memory T cells was dependent on the ratio of memory to naïve cells, on antigen priming, and on direct contact between the memory and naïve T cells. The researchers identified FasL, a cell-surface signaling molecule that is normally associated with apoptosis, as the molecular mediator of this precocious differentiation of naïve T cells.



(Figure: C. Klebanoff, CCR)

Naïve T cells extracted from the blood form a distributed population of cells as measured by two key surface markers (CD44 and CD62L). When mixed 1:1 with memory T cells, they differentiate more rapidly. T_N = naïve T cell, T_{Mem} = memory T cell.

Finally, the team wanted to establish the relevance of this precocious differentiation to ACT in patients. They found the ratio of memory to naïve T cells in humans is greater than one, and may be as high as 18 in cancer patients, likely due to chemotherapies that are administered prior to ACT. By separating, labeling, and recombining these populations, they were able to repeat *in vitro* the precocious differentiation of naïve T cells observed in mouse cells.

"The direct interaction of T-cell populations to influence their collective behavior in response to priming is reminiscent of the ways in which single-celled organisms such as bacteria exhibit quorum sensing responses to optimize their behavior as a population," said Klebanoff. What may be effective for the normal immune response, however, is likely a problem for ACT. These findings have led directly to the initiation of a clinical trial to selectively enrich the population of naïve T cells before ACT.

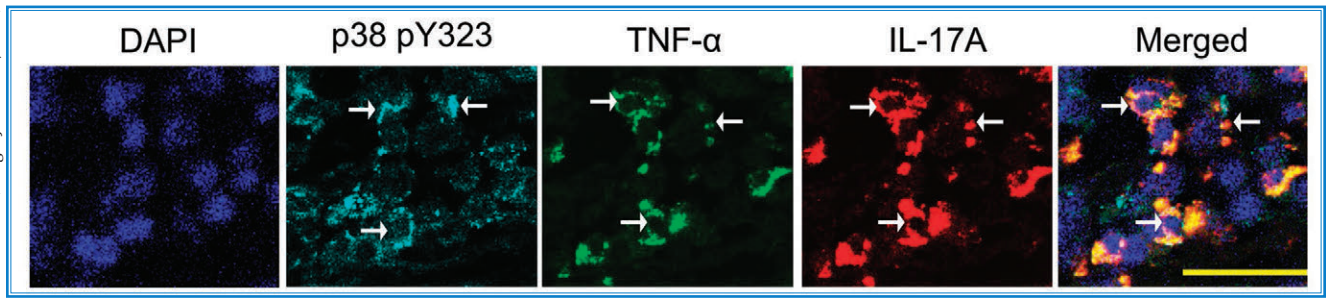
To learn more about Dr. Klebanoff's research, please visit his CCR website at https://ccr.cancer.gov/experimental-transplantation-and-immunology-branch/christopher-a-klebanoff

To learn more about Dr. Restifo's research, please visit his CCR website at https://ccr.cancer.gov/surgery-branch/nicholas-p-restifo.

Limiting Inflammation

CCR collaboration suggests new strategy for reducing tumor-promoting cytokines in pancreatic cancer.

(Image: J. Ashwell, CCR)



Human pancreatic ductal adenocarcinoma tissue in which three proteins are fluorescently labeled: p38 pY323 (cyan), TNF-α (green), and IL-17A (red). DAPI was used to stain the nucleus.

Pancreatic tumors, specifically pancreatic ductal adenocarcinomas (PDAC), are among the deadliest of cancers, in part, because of their unique environment. Surrounded by a dense tissue stroma, they develop in a milieu of chemical factors that promotes tumor growth, resistance to chemotherapy, and avoidance of immune targeting. Ironically, the many T cells infiltrating PDACs may promote tumor growth by secreting inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-17A (IL-17A).

Cytokine secretion is regulated by the p38 MAPK pathway, a molecular signaling cascade activated by a variety of cellular stresses via p38 phosphorylation at two classical residues, a threonine at amino acid position 180 and a tyrosine at position 182. Attempts have been made to therapeutically target p38, but toxicities resulting from inhibition of this ubiquitous pathway have thus far proven challenging. However, T-cell receptors also activate p38 through tyrosine phosphorylation at position 323 (pY323). To test whether this specificity might yield a strategy for dampening inflammatory cytokines, Postdoctoral Fellows Muhammad Alam, Ph.D., and Matthias Gaida, M.D., and Jonathan Ashwell, M.D., Chief of CCR's Laboratory of Immune Cell Biology, led a collaboration including Perwez Hussain, Ph.D., Investigator in CCR's Laboratory of Human Carcinogenesis, Serguei Kozlov, Ph.D., Principal Scientist in CCR's Center for Advanced Preclinical Research, as well as researchers in Heidelberg, Germany. Their results were recently published in *Nature Medicine.*

The research team subdivided PDAC patient samples into two groups depending on whether less or more than 10 percent of their T cells stained positively for pY323. The group with greater pY323 levels showed similar T-cell infiltration patterns, but a much greater percentage of TNF-α–, IL-17A–, and IL-21–producing CD4+ T cells. Moreover, this group had a poorer prognosis, with a median survival of 9.8 months as compared to 20.3 months.

In two different mouse models of pancreatic cancer, the researchers then replaced the critical Y323 residue with a phenylalanine residue to prevent phosphorylation. In both cases, they found that this double knock-in substantially reduced disease aggressiveness.

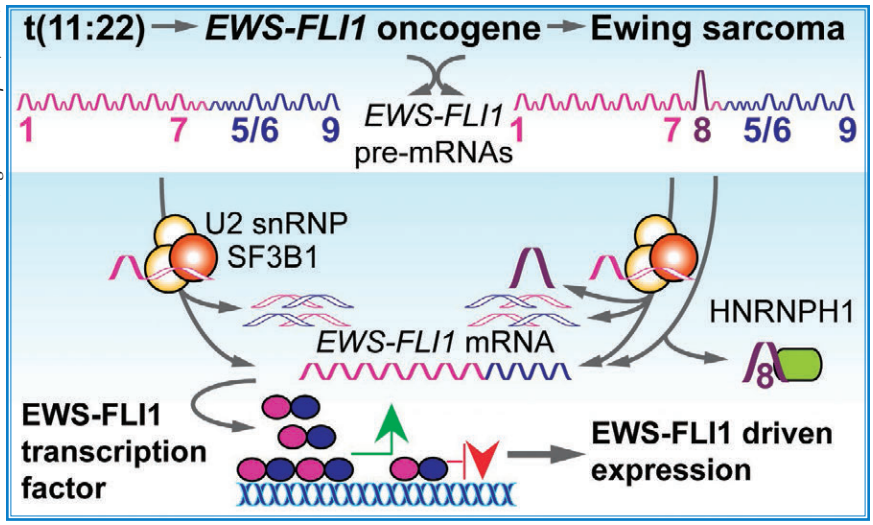
Finally, the team reasoned that a specific inhibitor might be devised to impede p38 activation selectively at Y323, i.e., in T-cell signaling. They designed a peptide based on a known endogenous inhibitor, GADD45-α, that would be taken up by cells and selectively interfere with Y323 signaling. They found that administration of this peptide inhibited tumor growth, in a manner consistent with the inhibition of inflammatory cytokine production by T cells in both mouse models of pancreatic cancer.

"We found that the presence of a high percentage of p38 pY323+ lymphocytes is a very strong negative prognostic factor in human PDAC, and that interference with this pathway in mouse PDAC was beneficial in both preventive and treatment models," said Ashwell. "A potential advantage of targeting the T-cell p38 alternative pathway in the tumor microenvironment, rather than a single cytokine or factor, is that it interferes with multiple downstream proinflammatory events that are involved in tumor progression."

To learn more about Dr. Ashwell's research, please visit his CCR website at https://ccr.cancer.gov/Laboratory-of-Immune-Cell-Biology/jonathan-d-ashwell.

Defusing a Fusion Gene

Genome-wide RNAi screening discovers splicing is a vulnerability in Ewing sarcoma.



(Figure: N. Caplen, CCR)

Splicing factors SF3B1 and HNRNPH1 are critical for the correct expression of the Ewing sarcoma protein EWS-FLI1.

Pediatric oncologists know what causes the majority of Ewing sarcomas (ES), cancers of the bone and soft tissue: a chromosomal translocation that joins together the opening sequences of the *EWSR1* gene on chromosome 22 with the closing sequences of the *FLI1* gene on chromosome 11. The resulting gene expresses a fusion protein, EWS-FLI1, responsible for tumor initiation and maintenance. Despite this knowledge, targeted therapies for ES have lagged, because drugs against the EWS-FLI1 protein have proven difficult to develop.

To discover new targets for the treatment of ES, researchers from CCR's Pediatric Oncology Branch and Genetics Branch worked with a trans-NIH team at the National Center for Advancing Translational Sciences (NCATS) to conduct a genome-wide RNAi screen designed to identify genes needed for EWS-FLI1 activity. As recently described in *Cell Reports*, the researchers homed in on genes that, when silenced by RNAi, selectively reduced EWS-FLI1 activity. Among the activities ascribed to these genes, the most common were RNA splicing and RNA processing, including SF3B1, a component of the spliceosome and HNRNPH1, an alternative splicing factor. Follow-up studies, conducted by Postdoctoral Fellow Suntae Kim, Ph.D., Investigator Natasha Caplen, Ph.D., and colleagues in CCR's Genetics Branch, in collaboration with a former CCR Pediatric Oncology Fellow and Assistant Clinical Investigator, Patrick Grohar, M.D., Ph.D., focused on testing the hypothesis that the *EWS-FLI1* transcript is vulnerable to the loss of proteins required for specific steps in splicing.

Previous studies have characterized the exact position of the chromosomal breakpoints that occur in ES and the structure of the *EWS-FLI1* transcripts expressed in ES cells. When the breakpoint is within *EWSR1* intron 8, exon 8 of *EWSR1* must be removed by splicing to generate a *EWS-FLI1* mRNA that will encode the fusion protein. Caplen and colleagues established that HNRNPH1 is required for this splicing event. Depleting HNRNPH1 in ES cells with a breakpoint in *EWSR1* intron 8 blocked the expression and the activity of the EWS-FLI1 protein and reduced cell survival.

The researchers also determined that silencing *SF3B1* results in mis-splicing of *EWS-FLI1*, irrespective of the position of the breakpoints in either fusion gene partner. This mis-splicing disrupts expression of full-length EWS-FLI1 protein, resulting in a change in EWS-FLI1 activity; however, variant EWS-FLI1 proteins were also generated. These variant EWS-FLI1 proteins still need to be studied in more detail, but targeting SF3B1 in ES cells may be an interesting therapeutic approach as this study reported a substantial decrease in the growth of ES cells when a compound that inhibits spliceosome activity was used.

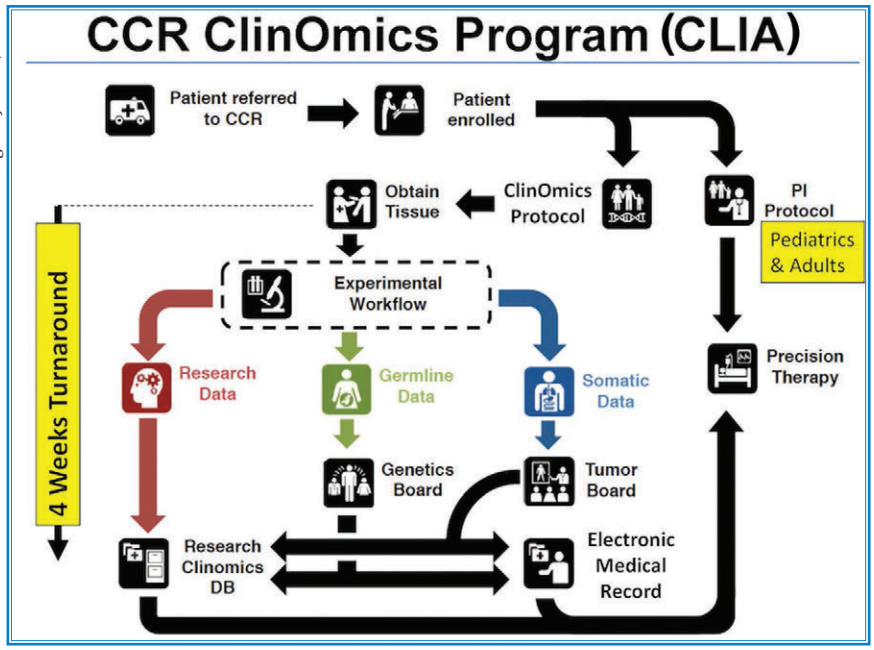
"Our goal was to identify therapeutic vulnerabilities in ES that might be more amenable to drug development. We found that the *EWS-FLI1* transcript is vulnerable to the loss of specific RNA-processing proteins, particularly those required to ensure the splicing of exons at and downstream of the fusion breakpoint," said Caplen. "This study opens up a potential strategy for the treatment of ES through disruption of the processing of the *EWS-FLI1* transcript itself."

To learn more about Dr. Caplen's research, please visit her CCR website at https://ccr.cancer.gov/genetics-branch/natasha-j-caplen.

Precision Pediatric Oncology

A new study paves the way for precision therapy trials at NCI.

(Figure: J. Khan, CCR)



Schema for CCR's planned genomics-guided CLIA sequencing program (ClinOmics)

Current excitement around tailoring treatments to abnormalities in tumor tissues is predominantly focused on adults. Pediatric tumors, so the literature argues, do not have the same range of somatic mutations as seen in adults, and so the value of an individualized, comprehensive genomic analysis may not be as obvious.

Javed Khan, M.D., Deputy Chief of CCR's Genetics Branch, and his colleagues decided to test the insights to be gained from a genomics approach to pediatric cancers. For 59 patients referred to the NIH Clinical Center between 2010 and 2014, with a range of 20 solid tumor types, they analyzed the sequences of all protein-coding genes in both tumor and nontumor cells through whole exome sequencing (WES). They also studied the mRNA profile through whole transcriptome sequencing (WTS), and copy number alterations in the tumor genome through single nucleotide polymorphisms (SNP) arrays. Their results were recently published in *Clinical Cancer Research.*

The majority of patients (73 percent) had recurrent/resistant cancers, which probably accounted for the higher number of observed tumor mutations than has previously been reported in pediatric tumors. About two-thirds of the mutations were identified through a combination of WES and WTS, but SNP arrays also accounted for a sizeable fraction. Approximately 50 percent had clinically actionable mutations in the tumor—that is, genetic alterations in the person's tumor that changed their diagnosis or that could be targeted with FDA-approved drugs or agents being tested in existing clinical trials—and 12 percent had a significant germline mutation that may be important in the management of the patient and their family.

The team described two cases in which their analyses could have informed the course of therapy. In the first, a patient diagnosed with epithelioid inflammatory myofibroblastic sarcoma, driven by a RANBP2-ALK fusion gene, was treated with the ALK inhibitor, crizotinib. When the patient relapsed eight months later, WTS and WES showed that the relapsed tumors acquired a secondary mutation in the ALK coding region previously linked to crizotinib resistance.

In the second case, a patient's initial diagnosis of melanocytic neuroectodermal tumor was later changed to melanoma, based on histology undertaken after the disease progressed despite chemotherapy. A mutation known as a common driver of uveal melanoma was revealed with WES and WTS of the metastatic tumor.

Based on the potential of this approach, CCR established the ClinOmics program to enable precision therapy trials in children and adults with cancer enrolled on NCI trials. However, other challenges remain for precision pediatric oncology. Only 24 patients in this study had a mutation with a corresponding drug, either approved or in clinical trials. "There are still many mutations that can be documented with a high degree of confidence, but whose significance is unknown and undruggable," said Khan. "Resistance can develop very quickly, even to targetable mutations. Therefore, future clinical trials should utilize immune-based or combination therapies—even for patients whose tumors harbor a genetic alteration for which a targeted therapy already exists."

To learn more about Dr. Khan's research, please visit his CCR website at https://ccr.cancer.gov/Genetics-Branch/javed-khan.

Good Neighbors

CCR and Walter Reed National Military Medical Center commit to sharing strengths.

Directly across Rockville Pike from the NIH Bethesda campus, stands the world's largest military integrated medical center, The Walter Reed National Military Medical Center (WRNMMC). WRNMMC provides first class care to over 800,000 beneficiaries each year. Walter Reed Bethesda encompasses what was once called the Bethesda Naval Campus. For many years a clinical branch of the NCI intramural program was physically located there. This tangible intersection led to many seminal advances in cancer research and treatment, including creation of the world's largest cell line bank in lung cancers and the first randomized trial demonstrating that standard dose therapy was as good as double dose in small cell lung cancer.

In 2011, the leadership of CCR and Walter Reed Bethesda came together to discuss the formation of the Murtha Cancer Center at Walter Reed Bethesda. They realized the time was right to work together on new initiatives that would capitalize on the strengths of each institution to improve cancer research and patient care.

"We began having more and more discussions to find ways to better integrate Walter Reed [Bethesda] and ourselves," said William Dahut, M.D., CCR's Acting Scientific Director for Clinical Research and Clinical Director, who is part of an oversight group including Deborah Citrin, M.D., Senior Investigator in CCR's Radiation Oncology Branch, Craig D. Shriver, M.D., COL, Director, Murtha Cancer Center, Rear Admiral David Lane, M.D., Director, WRNMMC, and Acting NCI Director, Doug Lowy, M.D.

The first initiative to come from these discussions was Activation Grants, funded from a joint pool of discretionary funds available to the Directors of each institution. Joint teams of investigators from each institution can apply each year for funding to conduct research. Two projects were awarded in 2014; three more were awarded in 2015.

"NCI has world-class scientists; we have world-class clinicians interested in translational research. The fund has enabled them to work together more collaboratively," said Shriver. "Each year Dr. Lowy and I decide how much we can put in the funding pool. We started three years ago and the quality of the proposals outstrips our ability to fund them."

"Walter Reed [Bethesda] has a cadre of very skilled physicians who are interested in clinical care and often in clinical research, but who have not historically had the research infrastructure that we have in CCR or the basic science component that we have here," said Dahut. "When they have clinical research ideas, we have a wider, deeper network of basic scientists with whom to collaborate."

Any interested investigator can send an email to their respective office asking about capabilities and skill sets at the other institution. "An NCI Investigator might have a great idea and say 'I wonder if there's anyone at the Murtha Cancer Center who would be interested in a collaboration,'" said Shriver. "It has happened more often than I can count and it is very rewarding. It's a forcing function, as we say in the military, for collaboration and better research."

The second initiative was shared biobanking. The Murtha Cancer Center has a biobank of tumor samples, whose origins date back to 1993. A protocol is in place whereby any patient who has cancer surgery is asked to contribute to research the excess tissue not needed for diagnostic pathology. The tissue is collected according to strict procedures and associated with a wealth of clinical data. Now, CCR will have a "mirror" of that repository.

"Because the NIH Clinical Center is limited to research protocols, NCI does not have access to 'run-of-the-mill' cancers. Yet for research, you need collections of samples ranging from early stage cancer to advanced

> "NCI has world-class scientists; Walter Reed Bethesda has world-class clinicians interested in translational research."

(Photo: Sharon Renee Taylor, WRNMMC PAO

The first transfer of biospecimens collected by the WRNMMC biobank to the biobank managed by CCR's Laboratory of Pathology. Left to right: COL Craig Shriver, LTC Joel Moncur, MAJ Patrick Malafronte, David Kleiner, M.D., and Fredric Barr, M.D.

cancer," said Shriver "We have worked with NCI scientists who needed such samples over the years, on a one-to-one basis, but each time, we had to put together a protocol that needed approval from both sites. Since, we have enough of each sample on our side, it made sense to split them. A simple idea, but it is huge conceptually as an agreement that we want to help each other to drive cancer cures forward, rather than being parochial about it."

On February 12, 2016, the first samples were transferred between Walter Reed Bethesda and NCI. Going forward, every two weeks, samples that have been collected (typically 20–50 tissues) will automatically be shared.

The military has 9.2 million beneficiaries, tracked through a single electronic health record system worldwide. When a patient enters the system, and even after they transition to the Veterans Administration, their data can be tracked over clinical changes, demographic changes, and outcomes. "When you align that data with tissues and put them in the hands of world-class scientists at NCI, the results could be transformative," said Shriver.

Finally, and most importantly from a patient perspective, Walter Reed [Medical Center] and the NIH Clinical Center have begun to collaborate on patient care. "Military patients could, of course, always come to the NIH Clinical Center if they matched our current protocols," said Dahut. "But our nonmilitary patients did not have access to care at Walter Reed [Bethesda]. Now, they can receive care across the street, even if they are not Department of Defense beneficiaries."

Since the agreement was signed in April 2015, 24 patients have been transferred between NIH and Walter Reed Bethesda. A quarter of them were under the age of 18. Moreover, a co-credentialing agreement has meant that physicians from NCI can go to Walter Reed Bethesda and continue to be involved in their patient care. "This has not only cemented our relationship, but it is good for patients," said Shriver. "There was one young man who had his leg amputated on a protocol at NCI as a result of a sarcoma, after multiple other treatments failed. Given our unique patient population, we are the amputee rehabilitation center of the world with a state-of-the-art prosthetic facility. He could not have asked for more expert care."

"Walter Reed [Bethesda] has a large network of patients, who have not always had the easiest access to NCI clinical trials," said Dahut. "By giving them clearer access, we can conduct and complete our trials more easily and with a broader population. Meanwhile, our patients have access to their skilled physicians. It is the best of both worlds."

Another critical component of this mutual relationship is taking shape as well. CCR is working towards allowing Clinical Fellows to train at Walter Reed Bethesda, to expose them to more routine cancers, rather than limiting their experience to patients who qualify for clinical research studies at NCI.

Recent CCR Awards

2016 Ramon Guiteras Award
American Urological Association

For identification of genes associated with different types of kidney cancer and developing new strategies for their management

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

2016 Failla Award
Radiation Research Society

For contributions to radiation research

Norman Coleman, M.D.
Adjunct Investigator, Radiation Oncology Branch

Distinguished Research Award in Retrovirology
Ohio State Center for Retrovirus Research

For significant research contributions to retrovirus biology

Genoveffa Franchini, M.D.
Senior Investigator, Vaccine Branch

Arthur Purdy Stout Annual Award
Arthur Purdy Stout Society of Surgical Pathologists

For outstanding achievements and tremendous contributions to pathology

Elaine Jaffe, M.D.
Senior Investigator, Laboratory of Pathology

2016 Special Achievement Award
University of Miami/Miami Winter Symposium

For his work in cancer research

Giorgio Trinchieri, M.D.
Director, Cancer and Inflammation Program

2015 Society for Endocrinology Transatlantic Medal
British Society for Endocrinology

For major contributions to the discipline of endocrinology

Gordon Hager, Ph.D.
Chief, Laboratory of Receptor Biology and Gene Expression

Silver Medal Award-Biology/Medicine
International EPR Society

For significant contributions to EPR (ESR) spectroscopy

Murali Krishna Cherukuri, Ph.D.
Senior Investigator, Radiation Biology Branch

2015 Betty Hay Award
Tumor Epithelial-Mesenchymal Transition International Association

For her work in the field of epithelial-mesenchymal transition

Claudia Palena, Ph.D.
Investigator, Laboratory of Tumor Immunology and Biology

Young Fluorescence Investigator Award
HORIBA Scientific

For novel and exciting applications of fluorescence in biology and biophysics

Kandice Tanner, Ph.D.
Investigator, Laboratory of Cell Biology

2016 Excellence in Federal Technology Transfer National Award
Federal Laboratory Consortium for Technology Transfer

For the technology *"Development of First Immunotherapy to Treat Chordoma, Rare Bone Cancer"*

James Gulley, M.D., Ph.D.
Chief, Genitourinary Malignancies Branch

Christopher Heery, M.D.
Associate Research Physician, Laboratory of Tumor Immunology and Biology

Claudia Palena, Ph.D.
Investigator, Laboratory of Tumor Immunology and Biology

Jeffrey Schlom, Ph.D.
Chief, Laboratory of Tumor Immunology and Biology

Elected to the American Academy of Arts and Sciences

Michael Lichten, Ph.D.
Deputy Chief, Laboratory of Biochemistry and Molecular Biology

Elected to the Association of American Physicians

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

Recently Tenured CCR Scientists

Mitchell Ho, Ph.D.
Laboratory of Molecular Biology

James Hodge, Ph.D., M.B.A.
Laboratory of Tumor Immunology and Biology

Yinling Hu, Ph.D.
Cancer and Inflammation Program

Li Yang, Ph.D.
Laboratory of Cancer Biology and Genetics

Staff News at CCR

Announcements

(Photo: R. Baer)

Tom Misteli, Ph.D.

Tom Misteli has been named CCR Director. He obtained his Ph.D. from the University of London, U.K., and was a Postdoctoral Fellow at the Cold Spring Harbor Laboratory in New York. He has served as CCR Senior Deputy for Research and was recently named an NIH Distinguished Investigator. Misteli is an internationally recognized leader in the field of genome cell biology and is best known for development of imaging approaches to study genomes and gene expression.

(Photo: B. Branson)

William Dahut, M.D.

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

(Photo: R. Baer)

Shyam K. Sharan, Ph.D.

Shyam K. Sharan has been named Director of CCR's Center for Advanced Preclinical Research. He received his Ph.D. from Case Western Reserve University. He completed postdoctoral training at Baylor College of Medicine as a Howard Hughes Medical Institute Associate. He then joined NCI's Mammalian Genetics Laboratory, which is now the Mouse Cancer Genetics Program. Sharan's laboratory has generated humanized and mouse models for functional dissection of *BRCA1/2* genes. His lab pioneered the use of mouse embryonic stem cells to evaluate the functional significance of human variants of *BRCA1* and *BRCA2* genes.

New Tenure-Track Scientists

(Photo: B. Branson)

Grégoire Altan-Bonnet, Ph.D.

Grégoire Altan-Bonnet joins CCR's Cancer and Inflammation Program as an Earl Stadtman Tenure-Track Investigator. His research focuses on systems immunology, i.e., the development of experimentally validated quantitative models of the immune response against tumors towards better cancer immunotherapies. Specifically, the Altan-Bonnet lab aims at integrating signal transduction, gene regulation, cytokine communications, cell differentiation, and proliferation/death across multiple spatio-temporal scales.

(Photo: B. Branson)

Natasha Caplen, Ph.D.

Natasha Caplen is now a Tenure-Track Investigator in CCR's Genetics Branch. She joined CCR in 2004 as a Senior Scientist, where she pioneered approaches for exploiting RNAi to investigate cancer biology and treatment and helped establish a trans-NIH facility for genome-wide RNAi screening. Her current research focuses on using functional genetic methods to interrogate specific aspects of the genetic, transcriptional, and signaling alterations observed in cancers driven by fusion oncogenes.

(Photo: Irismagic Photo Studios)

Mioara Larion, Ph.D.

Mioara Larion joins CCR's Neuro-Oncology Branch. Her research focuses on understanding the metabolic changes in brain tumors such as glioblastoma multiforme. She is also interested in the mechanism(s) by which mutations in the IDH1/2 enzyme lead to the formation of D-2HG and tumorigenesis, and in how to deplete D-2HG via the action of D-2HGDH enzyme.

(Photo: Wenjia Fan)

Chunzhang Yang, Ph.D.

Chunzhang Yang joins CCR's Neuro-Oncology Branch. His research focuses on signaling pathways, functional genetics, and metabolomics in cancers of the central nervous system. Yang leads collaborative translational research studies focused on understanding the molecular basis of brain tumor oncogenesis and malignancy, with an effort to identify novel therapeutic strategies.

In Conversation: Research Fellow Ngoc-Han Ha, Ph.D.

CCR: What sparked your interest in research?

Ngoc-Han: As an undergraduate at George Washington University (GWU) studying pharmacogenomics, I started volunteering in a lab. One of my projects was looking at polymorphisms in an enzyme involved in drug metabolism. It was a small project, but that is when I found out that I loved bench work.

CCR: How did you move from pharmacogenomics to metastatic cancer?

Ngoc-Han: I have always been fascinated by cancer and when I did my Ph.D. at GWU, my interest in oncology drew me to work on a protein called lactoferrin, which turns triple-positive breast cancer cells (expressing estrogen, progesterone, and HER2 receptors) into a more aggressive triple-negative form. On completing my degree, I saw a job advertised by Kent Hunter, Ph.D. (Deputy Chief, CCR's Laboratory of Cancer Biology and Genetics [LCBG]). His interest in germline polymorphisms and breast cancer metastasis seemed to be a perfect match, given my undergraduate and graduate degrees and my interest in personalized therapy.

CCR: What is the focus of your current research?

Ngoc-Han: The basic question of our lab is whether we can identify breast cancer patients who are at risk for metastasis by looking at germline mutations. We use a genetically modified mouse model and identify genes with differential expression that play a role in metastatic susceptibility.

CCR: Have you discovered new genes related to metastasis?

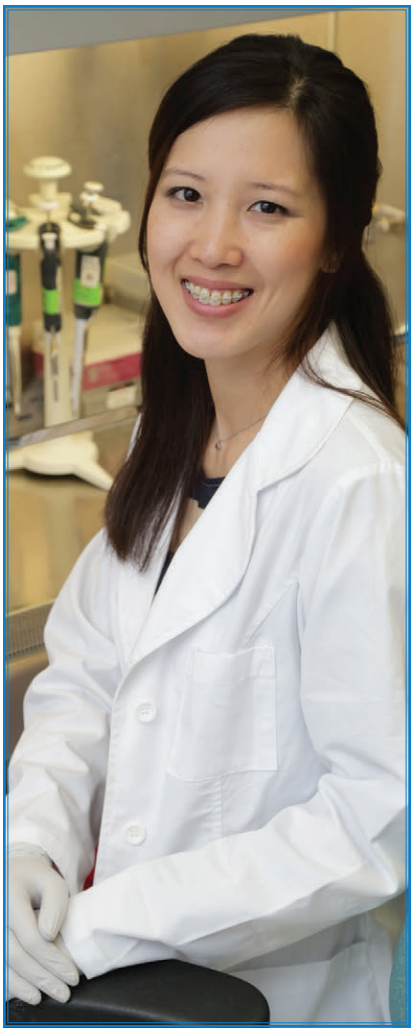
Ngoc-Han: The gene I found—ARNTL2—is actually a circadian rhythm gene. Studies have shown that women who work the night shift (i.e., alter their circadian rhythm) have an increased risk for breast cancer and/or metastasis. Specifically, our work shows that mutations in a putative promoter region change the transcriptional expression level of Arntl2, which in turn affects metastatic outcome. This demonstrates that not only protein-coding polymorphisms, but also those in regulatory regions, can affect metastasis.

CCR: To build on your research, how do you find the people and resources you need?

Ngoc-Han: Our Lab, LCBG, is very supportive when I have a technical question, need to borrow reagents, or need to discuss project ideas. Additionally, the NIH LISTSERVs are always helpful for resources such as reagents or protocols. The Foundation for Advanced Education in the Sciences (FAES) at NIH is also a good resource to advance your knowledge on various subjects. For example, to understand next-generation sequencing, I took an RNA-seq class last semester. Overall, NIH is a great place to learn and everyone seems eager to help and to start collaborations.

CCR: What is next in your career?

Ngoc-Han: When I first started my postdoc, I thought I'd become a Principal Investigator because I love academic science and bench work. Having been at the NIH for about four years, I could see myself as a Staff Scientist or equivalent, where I can spend more time at the bench, while still getting to mentor students.



(Photo: B. Branson)

Ngoc-Han Ha, Ph.D.

CCR: Have you done much mentoring?

Ngoc-Han: We have summer students every year and we also have a postbaccalaureate student whom I've mentored. I love mentoring students because I want them to understand and to be excited about science.

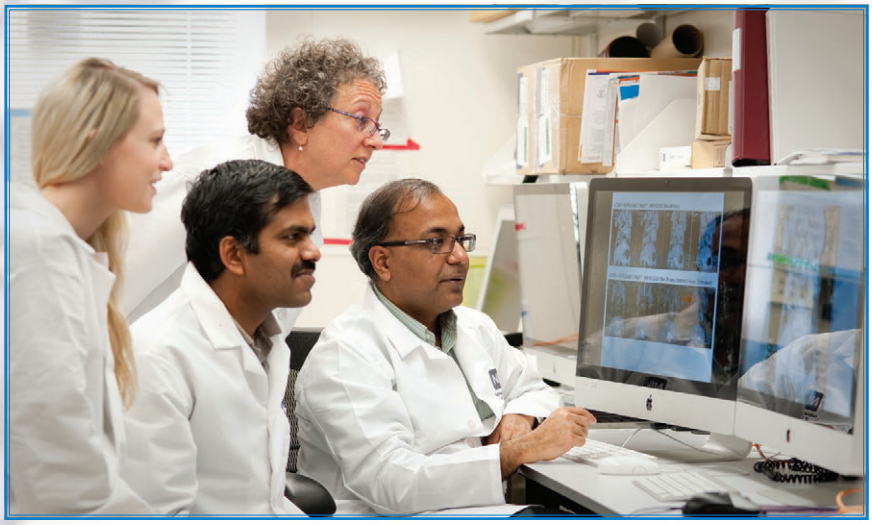
Inhibiting the Epidermal Growth Factor Receptor

The Epidermal Growth Factor Receptor (EGFR) is a widely distributed cell surface receptor that responds to several extracellular signaling molecules through an intracellular tyrosine kinase, which phosphorylates target enzymes to trigger a downstream molecular cascade. Since the discovery that EGFR mutations and amplifications are critical in a number of cancers, efforts have been under way to develop and use targeted EGFR inhibitors. These efforts have met with some spectacular successes, but many patients have not responded as expected, have subsequently developed drug-resistant tumors, or have suffered serious side effects from the therapies to date. CCR Investigators are studying EGFR from multiple vantage points with the goal of developing even better strategies to defeat EGFR-related cancers.

EGFR and Lung Cancer

"When I began my postdoc in Harold Varmus' lab at Memorial Sloan Kettering Cancer Center in 2004, *EGFR* kinase domain mutations had been discovered in lung adenocarcinoma patients," said Udayan Guha, M.D., Ph.D., Investigator in CCR's Thoracic and Gastrointestinal Oncology Branch. "Companies were developing drugs against EGFR and expecting that all patients would respond."

Unfortunately, only approximately 10 percent of lung cancer patients responded to tyrosine kinase inhibitors (TKIs) in the United States, and while those responses were striking early on, they soon led to relapse and drug resistance. Efforts ensued to sequence *EGFR* in tumors, and multiple mutations in the kinase domain were discovered. Guha wanted to know why tumors were so dependent on EGFR signaling and what was happening downstream of the wild-type receptor and of the different mutant receptors, and in response to TKIs, which target EGFR. He began looking at patterns of phosphorylation of proteins.



(Photo: R. Baer)

Nina Monkash, M.S., Abnilash Venugopalan, Ph.D., Constance Cultraro, Ph.D., and Udayan Guha, M.D., Ph.D., discuss lung scans.

"I started my own lab at CCR in 2011, and I continued to work on EGFR-dependent phosphorylation in human lung carcinoma cell lines. My lab has worked with first, second, and now third generation TKIs," said Guha. "We are trying to discover the differences between sensitive and resistant cells, and also how the dynamics of phosphorylation change with TKI treatment. Our overall goal is to identify actionable targets to overcome drug resistance."

Guha and his colleagues use mass spectrometry to identify phosphorylated proteins and to quantify the degree of phosphorylation as an initial unbiased proteomics screen for studying EGFR signaling. Using this approach, his team recently identified the protein MIG-6 as a suppressor of EGFR. They found it was constitutively phosphorylated on two particular tyrosine residues in cells engineered to express cancer-causing mutations of *EGFRs*; with the

> "... perhaps we can find actionable common drivers and then either in combination or through switching single targeted therapies, we can find successful treatments."

degree of phosphorylation correlated with drug sensitivity. From these initial observations, they went on to generate a series of genetically modified mice to show that mice lacking two copies of *Mig6* had accelerated lung tumor formation driven by mutant *Egfr* (See "A Brake for Cancer," *CCR connections* Vol. 9, No. 2).

Over the years, Guha's laboratory has used many mouse models in which mutant *Egfrs* are conditionally and selectively expressed in the lungs, so the mice develop lung tumors similar to patients. His laboratory has also generated models to conditionally express the mutant *Egfrs* in the context of heterozygous or null *Mig6*, the target of mutant Egfrs. More recently, they have explored using genetically modified fruit flies as screening tools. By expressing mutant *Egfrs* in the eye imaginal disc, they can distinguish functional changes as changes in the eye phenotype. "The idea is to use this model as a way to explore other targets we've discovered from our proteomics screen," said Guha. "We can make these transgenic flies in two to three months, and make genetic crosses with different targets. Moreover, we've started treating embryos or larvae with TKIs and in a lot of cases, the mutant phenotypes are reversed, giving us a potential drug screening tool."

In addition to cell and animal models, Guha has clinical protocols under way to study *EGFR* mutations in individual tumors and the heterogeneity of the tumors, which is likely key to cancer's ability to evade treatment. In a rapid autopsy protocol, tissues from hospice patients are collected within three hours of death. The team collects tissues from all sites of metastases and then does whole exome/transcriptome sequencing and proteomics to understand the tumor's heterogeneity and how it may have affected response to treatment. "Unfortunately, tumors are continuously evolving, but perhaps we can find actionable common drivers and then either in combination or through switching single targeted therapies, we can find successful treatments," said Guha.

In another clinical protocol, Guha and his colleagues are looking at tissues that develop resistance to the newest generation of TKIs targeted to a specific mutation of a threonine to a methionine in residue 790 of the ATP-binding pocket of the EGFR. The inhibitor, osimertinib, was developed because this mutation, T790M, accounts for 60 percent of the resistance that develops to the earlier, first generation of TKIs like gefitinib and erlotinib. Unfortunately, resistance develops to osimertinib, too, but is usually localized to a limited number of sites. The protocol calls for ablative surgery or radiation at those sites followed by continuation on the drug.

"In the meantime, we will do proteomic and genomic analyses, create cellular models, and try novel therapeutic combinations so that, if resistance reappears, we will have another shot at the tumor," said Guha. "The goal is to treat patients at different time points, but also to continuously do streamlined studies so there are some options for the patient at each step of resistance. You'd like to cure their cancer, but maybe it becomes chronic disease."

EGFR and Brain Tumors

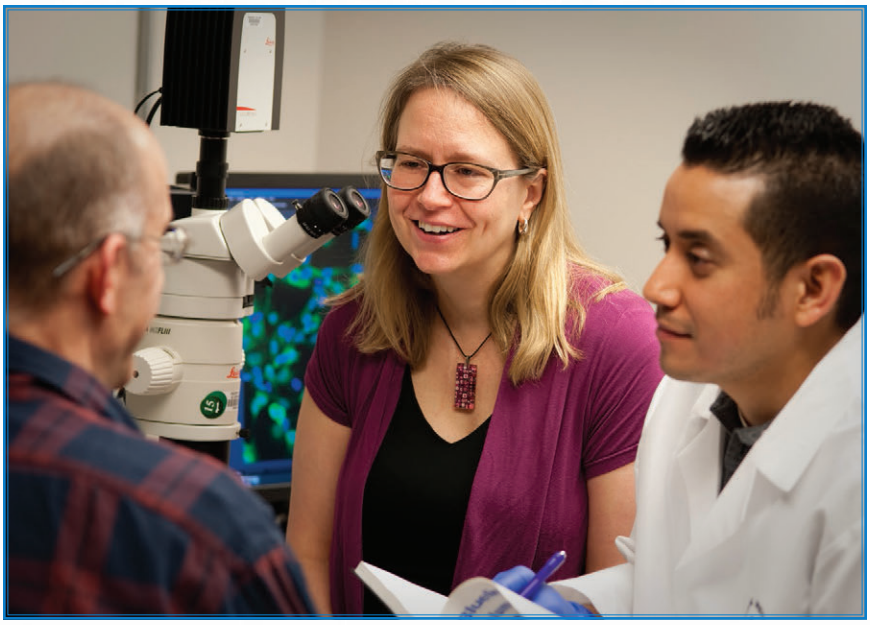
"*EGFR* is amplified and/or mutated in about half of all glioblastomas. It's the most common alteration. In 2004, I started my postdoc with Ron DePinho in Boston at a time when multiple clinical trials were under way to test TKIs in glioblastoma," said Jayne Stommel, Ph.D., Investigator in CCR's Radiation Oncology Branch. "Everyone thought this would be a home run because the *EGFR* mutation is such an important alteration in glioblastoma. The TKI trials all failed and the neuro-oncologists were devastated. My postdoc project was to try and figure out why they weren't working."

The brain has many unique biological features, but even at the level of EGFR activity, clear differences between glioblastoma and other tumors exist. Unlike tumors that do respond to TKIs, such as lung cancers and chronic myelogenous leukemia, glioblastoma does not appear to have as strongly activating mutations in the *EGFRs*. Moreover, most mutated EGFRs in the brain seem to cooperate with the wild-type receptor, requiring coexistence in the same cells.

"We still have no idea how EGFR is really working in these tumors," said Stommel.

However, in culture, EGFR inhibition will kill glioblastoma cells, so Stommel believes that something about the environment *in vivo* is

(Photo: R. Baer)



Jayne Stommel, Ph.D., and Carlos Tristan, Ph.D., discuss their work.

forestalling the effectiveness of TKIs. Using a novel cell-based model, her laboratory is trying to discover sensitizers to TKIs.

"We are using a system in the lab that consists of comparing biological differences between sparsely and densely plated cells. Low-density cancer cells respond to TKIs just fine, but when you plate them at high density, the cells are resistant. We see this in all cells lines—colon and lung cancer—too. It's a very interesting system for dissecting the biological requirements for TKIs to work."

Stommel's cells are derived from patient tumors; they are primary brain tumor cultures. "We are specifically looking at multiple lines," said Stommel. "We want to find something in common for all the lines. We are not looking for a specific genomic background; we are hoping to find something useful for as many patients as possible."

> "...EGFR is one of the most important and successful targets for cancer treatment in several major organ sites."

Stommel's work is still very much in progress. She has partnered with the National Center for Advancing Translational Sciences (NCATS) to do a whole genome screen with small interfering RNAs for genes that, when knocked out, would sensitize densely plated glioblastoma cells to TKIs. Her team is currently working on the hits identified in that screen. Their work on the special properties of densely packed cells has also taken them in the direction of molecules not obviously related to cancer, namely those associated with lipid and cholesterol metabolism.

"The biology of dense cells is very interesting; not many people are studying it in the context of cell culture. Making an impact on tumor growth and sensitivity to drugs does not necessarily involve genes associated with specific oncogenic mutations," said Stommel. "There are multiple ways of approaching the problem, from precision medicine to targeting biological processes required for cancerous cells to stay alive."

EGFR and Skin

"Anybody who is interested in cancer research, cancer treatment, and patient welfare has to be interested in EGFR because it is one of the most important and successful targets for cancer treatment in several major organ sites," said Stuart Yuspa, M.D., Co-Chief of CCR's Laboratory of Cancer Biology and Genetics. His laboratory has been studying EGFR as part of their focus on skin development and carcinogenesis for over 20 years.

Yuspa and his colleagues started working on EGFR in the 1990s, with a lot of their work focused on the effects of EGFR downstream from RAS signaling. They found that in cells lacking functional EGFRs, tumor formation induced by the *Ras* oncogene was inhibited. Eventually, they produced a knockout of *Egfr* in mice and showed that *Ras*-driven tumors either do not form at all or, if they do, are very small.

"Typically, when you look at a signaling diagram, RAS is downstream of EGFR, so our findings are somewhat counterintuitive," said Yuspa.

RAS, however, induces the expression of the ligands that activate EGFR, including TGFα, which was shown to induce skin tumors by Glenn Merlino, Ph.D., who shares with Yuspa the title of Co-Chief of CCR's Laboratory of Cancer Biology and Genetics. "We think EGFR ligand production is required for transformation by RAS because, normally, *RAS* mutations on their own without amplification don't drive signaling strongly enough to cause tumor formation. The signal strength has to be enhanced by activation of EGFR," said Yuspa.

Meanwhile, Yuspa and his colleagues have also been studying the role of EGFR in skin homeostasis and immune function. Many years ago, Yuspa decided that in order to understand deviation from normal (i.e., early events in skin cancer or epithelial carcinogenesis, in general), he first had to understand what was normal. Thus, he focused much of his attention on skin growth and differentiation and, more recently, the skin's role as an immune organ.

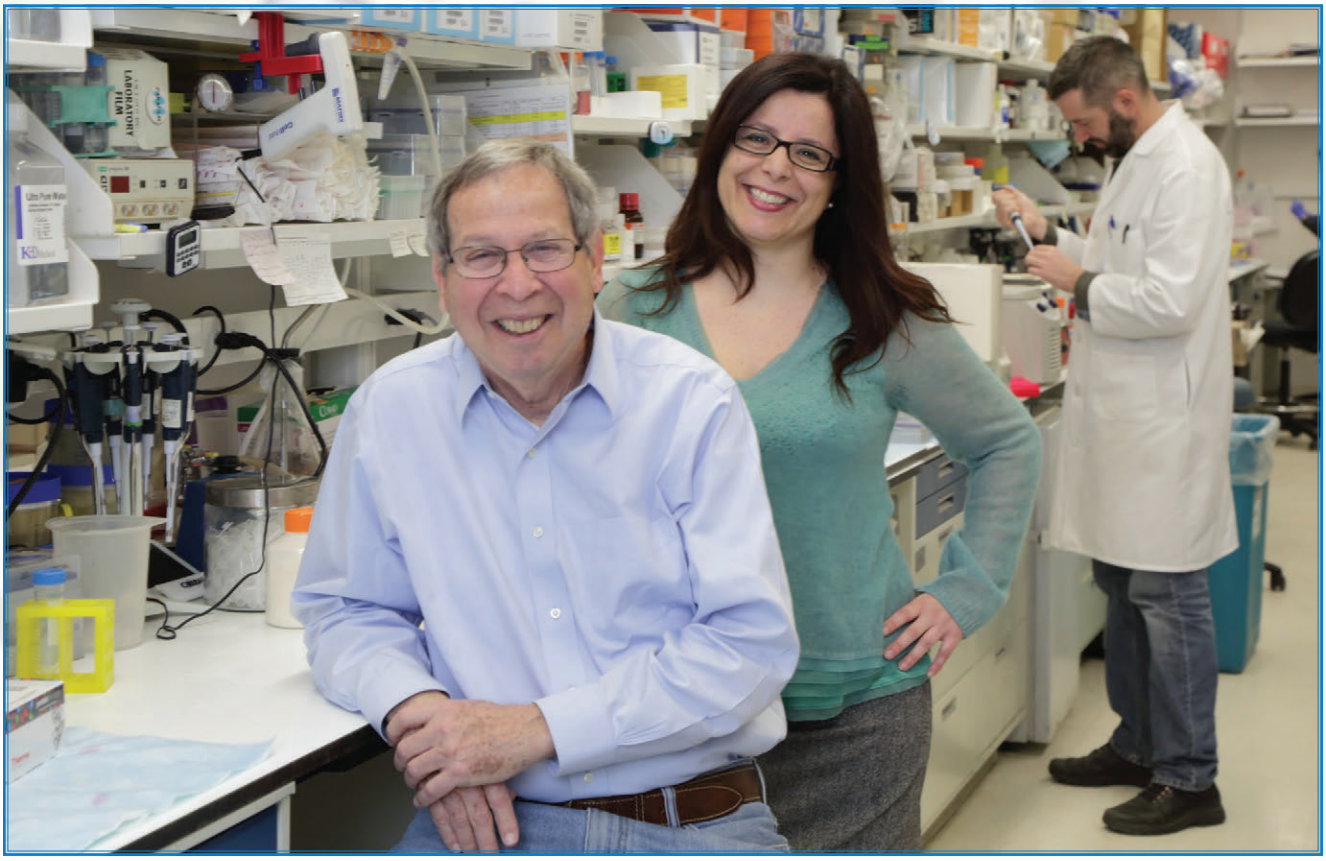
"Skin is the major immune organ of the body, by virtue of its size," said Yuspa. "Homeostasis of immune function in the skin is very important, and plays a role in skin cancer. In particular, we've known for many years that inflammation in the skin plays a role in tumor formation. People think of EGFR and downstream signaling as a proliferation stimulus in general, but in the skin it has a more important function in immune homeostasis."

A few years ago, Francesca Mascia, Ph.D., joined the laboratory from Italy, as a Postdoctoral Fellow. During her doctoral work, she had studied immune homeostasis in keratinocytes, and had a wealth of information on cytokines and chemokines that are influenced by the status of the EGFR. Mascia, Yuspa, and their colleagues began studying the effects of EGFR inhibitors—TKIs—on the skin inflammatory response.

"Almost all the targeted cancer drugs have a skin problem as one of their major adverse effects," said Yuspa. "TKIs are a prime example. The skin response is so dramatic that it can stop patients from taking a drug or cause the oncologist to reduce the dose," said Yuspa.

To better understand the cause of the skin response, Mascia and Yuspa first obtained clinical samples from their colleagues Elise Kohn, M.D., formerly an Investigator in CCR's Medical Oncology Branch and now in NCI's Cancer Therapy Evaluation Program, and Seth Steinberg, Ph.D., in CCR's Biostatistics and Data Management Section. They found increases in leukocyte counts and chemokines in samples treated with the first generation TKI gefitinib that paralleled the clinical occurrence of skin rashes and pruritus.

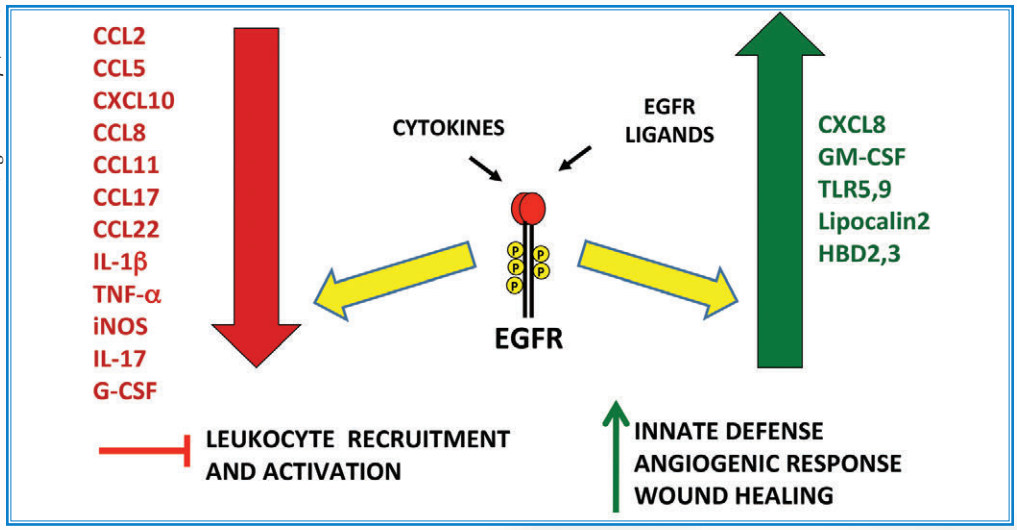
"The clinical material was 10 years old," said Yuspa. "At the beginning of the first studies using EGFR inhibitors, many clinicians were looking for what it did to their tumor cohort. Elise had a very active ovarian cancer clinic, and I think it was an attempt to see whether or not these drugs could have an effect on ovarian cancer. We were very fortunate to have these samples and that her team had had



(Photo: B. Branson)

Stuart Yuspa, M.D., and Francesca Mascia, Ph.D.

(Figure: S. Yuspa, CCR)



The direct activation of EGFR in the epidermis in response to ligand release or cytokines elicits a dual response to protect tissue integrity and repair damage. A subset of innate immune effectors (green) is positively regulated while other pro-inflammatory factors are suppressed (red). Inactivation of EGFR signaling undermines this protection and can result in immune cell infiltration, barrier and differentiation defects, infection, impaired wound healing, and severe cutaneous inflammation.

a dermatologist characterize the skin response."

In parallel, Mascia and Yuspa created a mouse model in which *Egfr* was selectively ablated in the epidermis. The mice developed skin lesions similar to those seen clinically, and before the lesions developed, the team found an upregulation of circulating chemokines and changes in blood counts that also echoed results from patient samples. Crossing the mice with mutant mice deficient in each of several immune-related factors (TNF-α, MyD88, NOS2, CCR2, T cells, or B cells) failed to affect the skin response, but local depletion of macrophages was partially effective.

"Whenever skin is perturbed in any way, it releases large amounts of antimicrobial peptides, cytokines, and chemokines that circulate to produce systemic effects," said Yuspa. "And that's really what we are seeing in patients who are on TKIs. We are seeing systemic release of a large number of cytokines and chemokines from the EGFR-depleted skin that results in infiltration of the primary cellular fighters of infection/inflammation coming back to the skin."

Yuspa and his team are investigating avenues that could help prevent skin side effects that are associated not just with TKIs, but with other targeted therapies including MEK and VEGF inhibitors. In addition, they are pursuing evidence suggesting that part of the therapeutic effect of TKIs may be mediated via the immune system and not simply by blocking the proliferative effects of oncogenic EGFR.

"We have data pointing to an altered immune response in the tumor milieu, which may also play a role in antitumor effects of TKIs," said Yuspa. "Some data suggest that a worse skin response to TKIs is associated with a better tumor response. In our current studies, we have preliminary evidence that in a tumor lacking EGFR, the immune environment of the tumor is altered. It's possible that the immune response in skin is paralleled by a response in the tumor milieu that contributes to the antitumor activity. Basically, our next step is to try to characterize and understand whether the immune system is playing a role in the antitumor activity of these drugs."

Now in their third generation, TKIs to inhibit EGFRs are a powerful tool for fighting many kinds of cancers. Through better understanding of their biological actions, CCR Investigators will continue the effort to further improve on their therapeutic efficacy.

To learn more about Dr. Guha's research, please visit his CCR website at https://ccr.cancer.gov/thoracic-and-gastrointestinal-oncology-branch/udayan-guha.

To learn more about Dr. Stommel's research, please visit her CCR website at https://ccr.cancer.gov/radiation-oncology-branch/jayne-m-stommel.

To learn more about Dr. Yuspa's research, please visit his CCR website at https://ccr.cancer.gov/laboratory-of-cancer-biology-and-genetics/stuart-h-yuspa.

FEATURE

Notable Expressions: Transcriptional Regulation from Biochemistry to Immunology

Dinah Singer, Ph.D., came to NCI in 1975 as a Postdoctoral Fellow in the Laboratory of Biochemistry, but soon created a career for herself in the Experimental Immunology Branch. Her interest in how genes are regulated to control biological function led her to focus on major histocompatibility complex class I genes (MHC Class I)—molecules critical to immune system function—as a model system for complex regulation of ubiquitously expressed genes across cell types and molecular contexts. Using this system to study the sequence elements and factors that control transcription, her laboratory continues to uncover fundamental principles of gene regulation. In addition to her active research career, Singer has served since 1999 as Director of NCI's Division of Cancer Biology, which manages a portfolio of over 2,200 grants to extramural investigators.

"Since I was a graduate student, I have been interested in understanding the mechanisms that regulate gene expression," said Singer. "It is not only an issue of understanding the biochemistry, but how gene regulation is really linked to the function of the gene product. That is how I got interested in immunology."

(Photo: B. Branson)

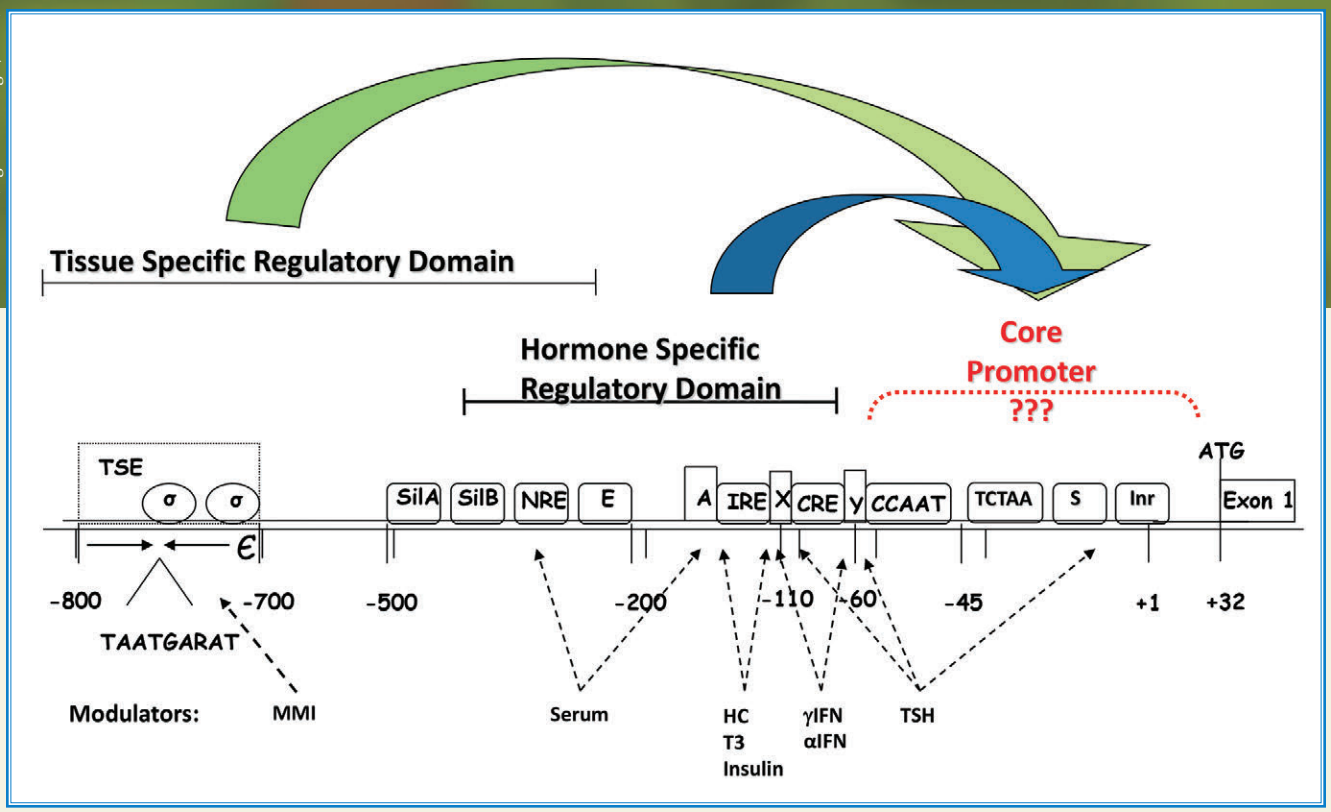
Sabina Hlavaty, B.S., and Dinah Singer, Ph.D., discuss a gel.

Regulation of MHC Class I Gene Transcription

MHC Class I proteins bind intracellular peptides and present them on the surface of cells for immune surveillance. This presentation is critical for the development of immune cells, which learn to identify "self" from "nonself," and for their ability to mount an appropriate defense upon presentation of foreign antigens. MHC Class I proteins are expressed ubiquitously, but at different levels in different cell types. They can also be induced by various immune triggers and hormones. Singer chose this model system to ask how the regulation of gene transcription reflects its biological function and context.

"Our major contribution has been to make that linkage between regulation and function," said Singer. "We've shown that MHC Class I gene transcription is regulated both in a tissue-specific fashion and in response to hormone and cytokine signaling. Moreover, transcription is very tightly regulated and any perturbation leads to an aberrant immune response. Low levels of MHC Class I gene expression are

(Figure: D. Singer, CCR)



Intracellular and extracellular pathways target distinct domains of the MHC Class I upstream regulatory region but are integrated at the core promoter.

associated with defective immune surveillance. Overexpression also leads to a defective immune response, but in the form of autoimmunity."

Singer's group has extensively studied the role of individual transcription factors, coactivators, and sequence elements regulating the MHC Class I genes, in the context of cellular phenotype and function. They have found, for example, that constitutive expression and activation by chemokines are under the control of different transcriptional cofactors.

Key to their work has been a combination of *in vitro* and cellular studies with the development of genetically engineered mouse models. Singer's laboratory was the first to make a transgenic MHC Class I mouse, in order to understand the minimum requirements of gene expression *in vivo*. "Over the last 20 years, we have developed an emphasis on verifying *in vivo* what we observe *in vitro* and in cell lines. There aren't many labs studying transcriptional regulation that use this approach as extensively as we have," said Singer.

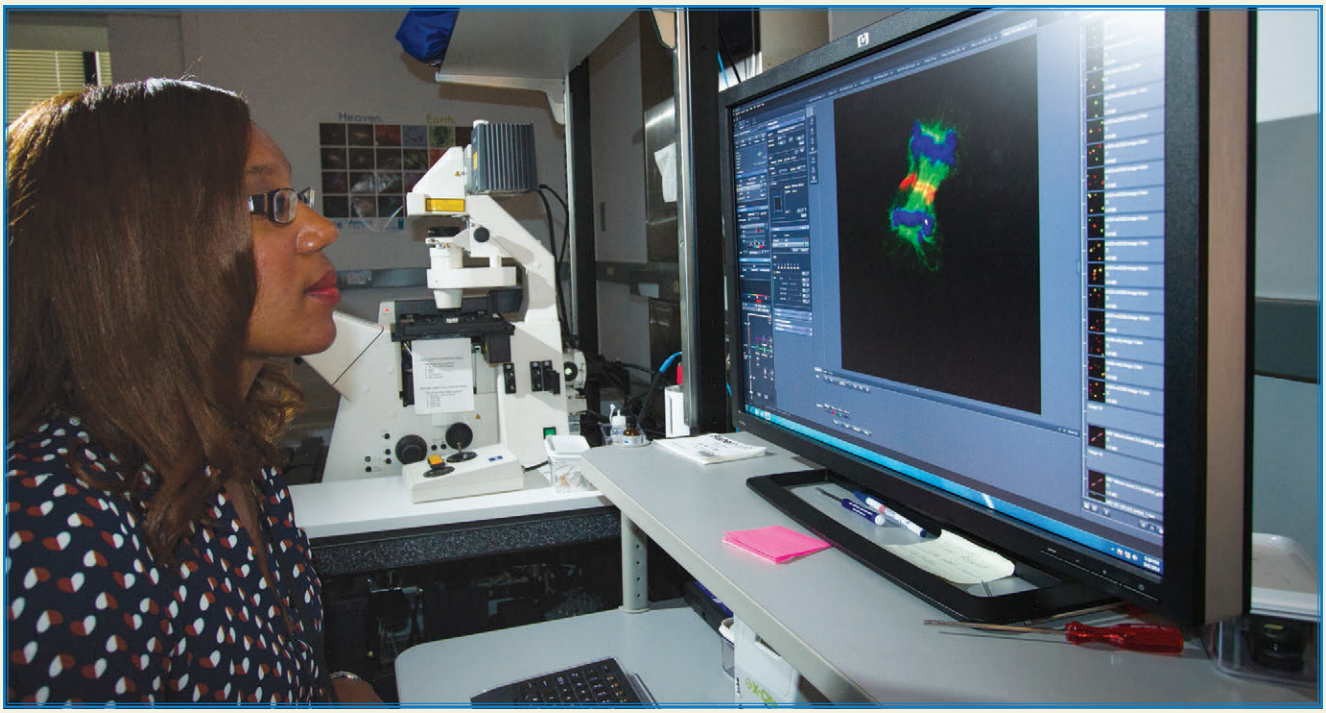
Singer's work has recently led her to reconsider the role of core promoters in transcription. Classical descriptions of transcriptional regulation point to a core promoter region, defined as the smallest piece of DNA necessary to direct transcriptional initiation by the enzyme RNA polymerase II and containing specific DNA sequence elements. However, recent genome-wide analyses have generated a much more complex picture.

To examine this complexity at the MHC Class I promoter, Postdoctoral Fellow Zohar Barbash, Ph.D., and Staff Scientist Jocelyn Weissman, Ph.D., led a study to assess the functional roles of each of the core promoter sequence elements in transgenic mice expressing the MHC Class I gene, *PD1*. They found that none of the four known core promoter sequence elements were required *in vivo* for transcription initiation. Instead, each element had a distinct role in regulating tissue-specific expression or modulating responses to cytokines.

"We have come to see even the core promoter as a more flexible platform for transcriptional regulation, which does not depend on a single element to support initiation," said Singer.

From Transcription to Chromatin Structure

"When I started out, there were basically two fields of molecular biology," said Singer. "One was looking at transcription, really with a focus on bacterial and viral transcription. The goal was to identify and characterize promoters, core promoter elements, upstream regulatory elements, and so on. In parallel, and to a large extent independently, the field of chromatin biology was describing nucleosomes, histones,



(Photo: D. Sone)

Chanelle Case-Borden, Ph.D., in the lab

and packaging of the different chromatin fibers. Over the last 5 to 10 years, those two fields have come together in a very real way. The conceptual synthesis has been a fundamental change in our thinking about nuclear biology, chromatin, and transcription. One of the big questions right now is how nuclear organization affects regulation of gene expression."

Singer's work on MHC class I gene regulation uncovered an unexpected player in the bromodomain protein, BRD4. BRD4 was first identified as a scaffold that binds to chromatin and recruits additional molecules involved in DNA regulation. BRD4 became an important drug target when it was found to be overexpressed in some cancers, where it enhances the expression of cell-cycle-related genes thought to contribute to the cancerous phenotype.

> "Singer's work on MHC class I gene regulation uncovered an unexpected player in the bromodomain protein, BRD4."

When Chanelle Case-Borden, Ph.D., came to Singer's laboratory as a Postdoctoral Fellow, she brought her previous experience in imaging and molecular biology to delve into the complex biology of BRD4. (See "In Conversation: Postdoctoral Fellow Chanelle Case-Borden, *CCR connections* Vol. 9, No. 1).

"We discovered that BRD4 is a kinase and phosphorylates RNA polymerase II, to aid in transcription and elongation," said Case-Borden. "We are trying to validate that biochemical process to see how it affects cell cycle and global gene expression. We are also developing mouse models that express a mutant BRD4 to further elucidate its biological function."

Led by Staff Scientist, Ballachanda Devaiah, Ph.D., Singer's team has also described a new role for BRD4 as a histone acetylase transferase (HAT). Transcriptional activation depends on acetylation of histone proteins, which loosens their hold on DNA, allowing the transcriptional machinery access to target genes. Acetylation at one particular amino acid reside, lysine 122 on histone H3 (H3K122), destabilizes the bond with DNA sufficiently to evict the histone. Singer and her colleagues have shown that BRD4 not only acetylates a unique pattern of lysine residues on multiple histones, it is also capable of acetylating H3K122, which ultimately leads to an unpacking of the chromatin and increased transcription.

In another series of experiments, a collaboration with David Levens, M.D., Ph.D., Senior Investigator in CCR's Laboratory of Pathology, and Keji Zhao, Ph.D., Senior Investigator in the National Heart, Lung, and Blood Institute's Laboratory of Epigenome Biology, has demonstrated that BRD4 cooperates with the

enzyme topoisomerase I to relieve the physical stresses of coiled DNA to aid in transcriptional elongation.

"BRD4 has become a very popular drug target," said Case-Borden. "Many studies have demonstrated that inhibiting BRD4 binding biochemically, decreases disease-related gene expression in the cells and ultimately reduces the disease phenotype. Considering that BRD4 is a multifunctional protein—a scaffolding protein, a kinase, a HAT—it is important to study how these functions are also affected in these conditions. Moreover, the long-term effects of BRD4 inhibition have yet to be fully elucidated. One of our goals is to determine how these functions fit together and consider the bigger picture, not just one aspect of BRD4 function."

Extramural Activities

Singer became involved in scientific policy and administration many years before she became Director of NCI's Division of Cancer Biology, first through activities with the NIH Biosafety and Cooperative Research and Development Agreement (CRADA) committees and later at the Howard Hughes Medical Institute as a senior science officer.

"As a scientist, I felt I had a responsibility and interest in giving back to the community. At first, I felt I needed to give back locally, and then, realizing that being here at NIH as a scientist is a very fortunate position, I wanted to extend my efforts beyond the intramural community," said Singer.

As Division Director, Singer is responsible for managing a multimillion-dollar portfolio of investigator-initiated cancer biology grants. Her goal is to ensure continuation and stability within that portfolio through interactions with grantees. In addition, she uses her perspective over the full landscape of cancer biology to try to anticipate where the field is going, to merge new areas, and to help establish funding opportunities and consortia.

"Fifteen years ago, we set up a consortium for mouse models of human cancer; that really spurred the field to use and develop those models," said Singer. "We developed a network for tumor microenvironment research, an area that was recognized as important but had not yet flowered completely. We've also had a successful program in systems biology and predictive computational models of cancer."

Earlier this year, Acting NCI Director Doug Lowy, M.D., asked Singer if she would agree to take on an additional role as Acting Deputy Director to help with Vice President Biden's Cancer Initiative. She will co-chair a Blue Ribbon Panel to develop scientific recommendations for the Cancer Initiative. She retains her position as Division Director, but her deputy has taken the reins as Acting Director.

"The Cancer Initiative could create a lot of exciting opportunities at a time when the field is really ripe for taking advantage of all the things we've learned," said Singer. "This will give us a chance as a community to articulate our priorities and invest in them."

Doing It All

Singer splits her days between her laboratory and the Division. She places a strong emphasis on mentorship, harkening back to her experiences as a mentor in high school. "I used to teach swimming to both kids and adults. I learned how to deal with people's fear and to recognize what they need to learn. I tutored in high school and in college and that carries over."

She credits the success she has had to the people she works with. "I have excellent people in my Division—a wonderful staff of program directors that function quite well in my absence. And in the laboratory, I have staff scientists who have been with me for many years, who know what my philosophy is and provide mentorship that complements mine," said Singer.

(Photo: D. Sone)

Dinah Singer, Ph.D.

Singer is in a unique position to see the merits of both the intramural and extramural programs. She values both, but feels fortunate to be in CCR. "We have opportunities to pursue areas of research not easily supported extramurally," explained Singer. "Our reviews are retrospective, so we have more flexibility to try things that are new and innovative. Our first transgenic mice and our studies of BRD4 would have been much harder on the outside. The intramural program plays a special role in supporting both science and the people who do it."

To learn more about Dr. Singer's research, please visit her CCR website at https://ccr.cancer.gov/Experimental-Immunology-Branch/dinah-s-singer.

FEATURE

The Secret Lives of Neurotrophin Receptors

Neurotrophins are a family of growth factors that are critical to the proper development and functioning of the nervous system. Neurotrophins activate a family of tyrosine receptor kinases (Trk), which typically initiate signaling cascades through phosphorylation. This axis is important for central nervous system (CNS) drug development efforts, ranging from pain management to neurodegeneration. However, neurotrophin-activated pathways are important for a variety of cancers and their metastatic properties. Indeed, TrkA, the prototype of the neurotrophin receptor family, was first identified at NCI as part of a fusion oncogene. Moreover, Trks are widely expressed in many different organs where their misactivation has been associated with tumor formation. Trks are also present as truncated receptor isoforms, lacking kinase activity, and these forms are particularly prominent in adult tissues. Little is known about the role of neurotrophins and Trk receptors outside the nervous system. Lino Tessarollo, Ph.D., Director of CCR's Mouse Cancer Genetics Program, uses his expertise in developing genetically modified mouse models to dissect the functions of these receptors, with the goal of developing insights that will guide the successful targeting of therapeutic interventions.

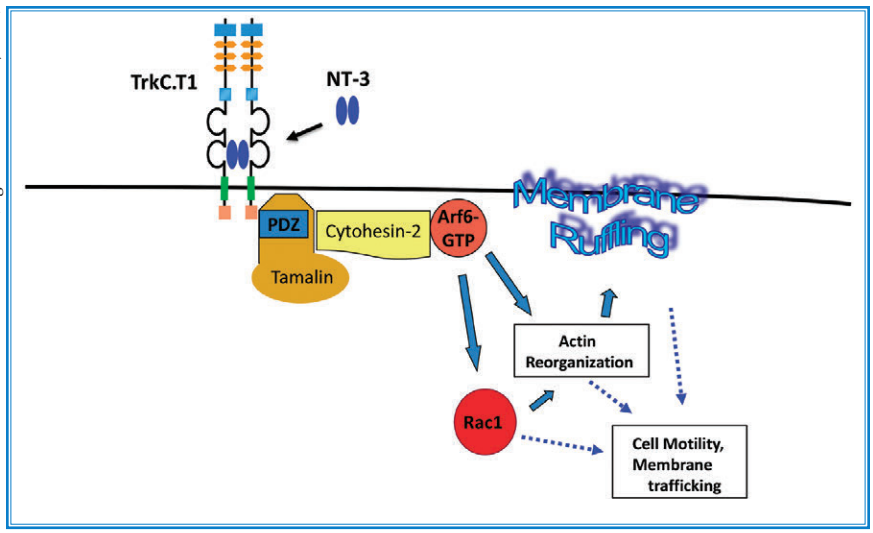
Imprinting on Neurotrophins

Tessarollo came to the Frederick Cancer Research Facility in Maryland (now known as NCI at Frederick) at a scientific moment that has defined his career. He joined the laboratory of Luis Parada, Ph.D., as a Postdoctoral Fellow in 1990, just a few years after Dionisio Martin-Zanca, Ph.D., Stephen Hughes, Ph.D., and Mariano Barbacid, Ph.D., also working in Frederick, cloned a human fusion oncogene from colon carcinoma cells and discovered one of the first transforming genes in a human malignancy, TrkA. When Tessarollo arrived, Martin-Zanca had just joined Parada's lab and was searching for the ligand that could bind and activate TrkA.

Meanwhile, a young Principal Investigator, just recruited to Frederick, David Kaplan, Ph.D., wanted to identify the proteins that were phosphorylated in PC12 cells in response to nerve growth factor (NGF), thinking that one of them was its receptor. Kaplan and Martin-Zanca soon realized they had something in common: one had a protein around 140–150 kilodaltons in size that was phosphorylated in response to NGF, the other had TrkA, a receptor of about 150 kilodaltons. Their pivotal discovery that NGF is the ligand for TrkA paved the way to an entire field of research into related neurotrophin receptors and their actions, including TrkB and its ligand, brain-derived neurotrophic factor (BDNF), as well as TrkC and its ligand, neurotrophin-3 (NT-3).

"Dionisio was an amazing molecular biologist, who taught me a lot during many long nights at the bench, and David was an amazing biochemist. Without these skills coming together, the finding would have probably taken longer," said Tessarollo. "I was just a spectator in the unfolding story. But it was a very exciting time and I was hooked. Eventually, as the others moved on, I kept the lights on for neurotrophins and Trk receptors at Frederick."

Although the field in general has focused on the role of Trks in the nervous system, Tessarollo knew from the first studies he performed on Trk gene expression that the receptors were found in many different organs outside the nervous system. Moreover, *TrkB* and *TrkC* genes are alternatively



(Figure: L. Tessarollo, CCR)

Truncated Trks signal independently of the Trk tyrosine kinase receptors.

spliced into multiple isoforms, but the two most common are the full-length tyrosine kinase form and a truncated form. The truncated forms are the predominant forms in adult tissues, but their function is almost entirely unknown.

"Basically, what I want to do with my research is to achieve a molecular dissection of Trk signaling," said Tessarollo. "We know that these genes control many different physiological aspects in mammals. If we can dissect how these pathways are activated, then maybe we can generate druggable targets specific for desired effects."

Beyond Development

Tessarollo uses genetically modified mice as his model of choice for understanding the physiological functions of neurotrophins and their receptors. "I like to look at the physiology first. I want to have a phenotype and then try to understand the molecular mechanism," said Tessarollo.

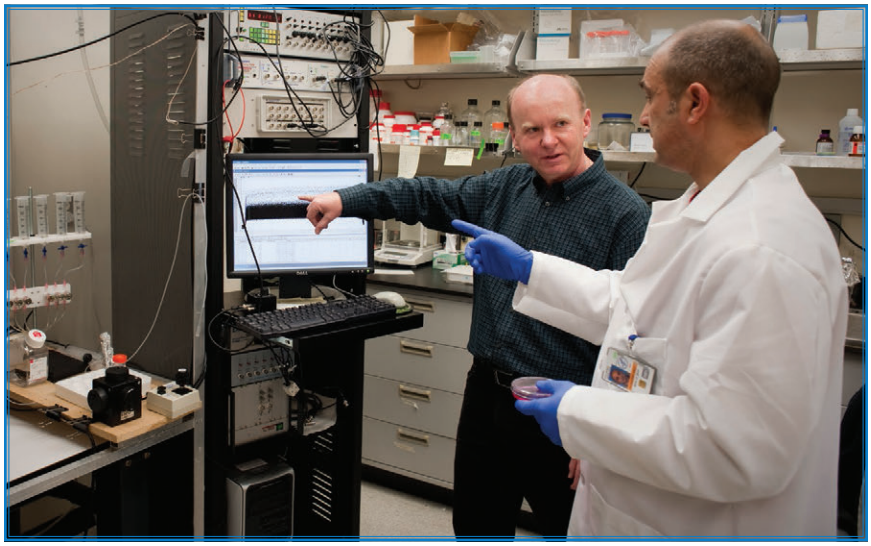
In 1999, Tessarollo and his colleagues published the first evidence that BDNF regulates food intake and obesity in mice. They showed that a single copy of the gene produced subtle physiological alterations that may be more meaningful to human physiology than a simple knock out, which is lethal. These alterations included changes in serotonin neurotransmission and in serotonin-related behavior, such as food intake and aggression. The work presaged later findings of heterozygosity in human *BDNF*. "It really showed that a neurotrophin can be involved, not just in development, but in other aspects of mammalian physiology," said Tessarollo.

As painstaking as mouse model approaches can be, Tessarollo finds this careful work is ultimately rewarding. In 2004, Tessarollo and his colleagues challenged 20 years of literature based on indirect evidence, reporting that NGF was critical for the proper development and functioning of the immune system. They developed a genetically modified mouse model in which they first deleted TrkA and then reintroduced it selectively to cells of the nervous system. This reverse conditional gene targeting strategy generated a mouse with only a very mild disturbance of specific immune cell populations.

"I had a very hard time publishing that paper; it was essentially negative data," said Tessarollo. "But recently at a Gordon Conference, I met a senior pharmaceutical industry scientist who told me that our data was used in their argument to the FDA that an anti-NGF drug would likely not have immune side effects. We worked rigorously to find major immune defects, but simply did not. That turned out to be useful. Sometimes you don't know where science will take you."

Truncated Receptors

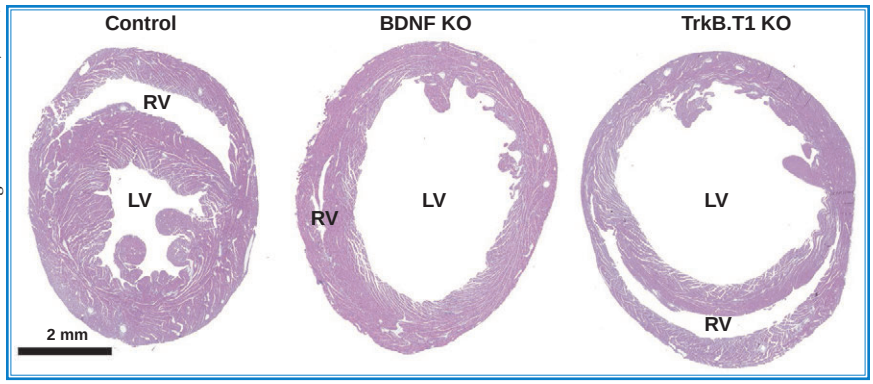
Most recently, Tessarollo and his colleague, Gianluca Fulgenzi, Ph.D., have uncovered an unanticipated



(Photo: R. Baer)

Lino Tessarollo, Ph.D., and Gianluca Fulgenzi, Ph.D., discuss results on cardiac function recordings.

(Figure: L. Tessarollo, CCR)



Deletion of BDNF or its receptor TrkB.T1 in cardiomyocytes causes cardiomyopathy. LV, left ventricle; RV, right ventricle

role for truncated TrkB receptors in cardiac function.

Truncated Trk receptors are difficult to study. The short intracellular tail is extremely conserved across mammals and chickens. Many researchers have tried and failed to find binding partners. So far, Tessarollo has identified a pathway activated by the truncated form of TrkC, but has been unable to create a mouse model to verify the importance of that pathway for technical reasons. Deletion of the exons encoding the truncated isoforms of TrkC resulted in an upregulation of the long, kinase isoform. His laboratory was successful, however, in deleting the truncated isoform of *TrkB*.

"One day we were using a heart as a control for an expression study and discovered that cardiac TrkB receptors are truncated," said Tessarollo. "Gianluca asked me if I had ever looked at the hearts in mice lacking truncated TrkB."

The team discovered that mice lacking truncated TrkB had altered cardiac muscle tissue. This launched them on an investigation of the role of its ligand, BDNF, working through truncated TrkB, on cardiac output. They found that BDNF regulates heart contractile force without involvement of the nervous system. Instead, the truncated TrkB receptor appears to modulate calcium signaling in cardiomyocytes.

"This paper will put the heart on the radar screen of researchers studying therapeutic interventions with neurotrophins," said Tessarollo. "There is a big push in biotech to target TrkB, which is very important for synaptic plasticity and brain function. People are looking for good agonists to ameliorate neurodegeneration. But if the drug is delivered systemically, cardiac toxicity has to be considered."

Sudhirkumar Yanpallewar, M.D., has worked with Tessarollo, first as a Postdoctoral Fellow, now as a Staff Scientist, for 11 years. His work also focuses on truncated Trk receptors. A few years ago, he published a paper in which he deleted both copies of the truncated TrkB receptor from a genetically engineered mouse model of amyotrophic lateral sclerosis (ALS). He showed that ablating

Building Mouse Models

"The reason I landed my first real job is because I learned homologous recombination," said Lino Tessarollo, Ph.D. "It was a very imperfect science at the time, but by pulling a lot of all-nighters, I got the technology working reliably to create genetically modified mouse models."

In 1994, in addition to his own research, Tessarollo set up a Gene Targeting Facility to assist investigators in creating mouse models. Tessarollo works collaboratively with investigators across CCR to develop mouse models of cancer and related systems. For example, he worked with David Levens, M.D., Ph.D., Senior Investigator in CCR's Laboratory of Pathology, on a mouse engineered to express a fluorescent Myc protein upon activation of the *myc* gene, in order to monitor *myc* expression in living cells.

"A great deal of cancer research is done in tissue culture, usually in transformed cells. But, if you want to know what is happening in normal cells, which was key to our work with *myc*, how do you get cells that you are 100 percent sure are normal? To get close to a true physiological state, you need to develop an animal model," said Levens.

Levens consulted with Tessarollo on how to design the recombination vectors to target the gene, and once this work was completed, he handed off the mouse generation and breeding.

"There was both a lot of science and a lot of art to it, and Lino guided us through that. There's no way we could have done this on our own," said Levens. "Lino's expertise is broad and he gets things done quickly. He's got an ability to inform and instruct in a very collegial manner."

> "It's Lino's work *in vivo* that has highlighted the significance of these [Trk] receptors."

the truncated form of the receptor delayed onset of motoneuron degeneration and muscle weakness, suggesting that the truncated form was normally acting to limit BDNF's neutrophic actions through the kinase form of the receptor.

"People always knew the truncated Trk receptors were there, but no one knew what they were doing," said Yanpallewar. "These receptors don't have kinase or other functional domains. It's Lino's work *in vivo* that has highlighted the significance of these receptors."

In the last few years, a new gene editing technology, CRISPR, has taken genetic engineering by storm, making it much easier to make targeted changes in genomic sequences. Using the new technology, Yanpallewar and Tessarollo are looking again at creating a selective knock out of the truncated form of *TrkC*.

A CRISPR Future

"I have always been fascinated by genetic engineering," said Tessarollo. "CRISPR has created a bonanza. It is really amazing."

Recently, using CRISPR technology, his team has made a series of mice by modifying different domains of *Trks* to increase the activity of these receptors. These gain-of-function mutations are typically more difficult to achieve than loss-of-function mutations; they often result in inadvertent inactivation. CRISPR technology speeds up the trial-and-error process. The models will help Tessarollo and his colleagues explore what happens in the nervous system if one is really able to increase neurotrophic function, as people have been trying to do with drug interventions for decades.

These mice cannot only help to determine the therapeutic possibilities of augmenting neurotrophin signaling, they can also address the longstanding question of whether boosting neurotrophin signaling can cause cancer. So far, Tessarollo believes it unlikely. In cancers, when Trk genes undergo mutations that make them constitutively active, they either die or acquire other mutations that allow them to become neoplastic.

"With the type of mice we are now generating, we can address the real utility of therapeutic intervention through neurotrophin signaling," said Tessarollo.

To learn more about Lino Tessarollo's research, please visit his CCR website at https://ccr.cancer.gov/mouse-cancer-genetics-program/lino-tessarollo.

A Mouse-Based Community

Tessarollo's laboratory became part the Mouse Cancer Genetics Program in 1999, under the direction of Neal Copeland, Ph.D., and Nancy Jenkins, Ph.D. He served as Deputy Director of the Program for eight years, and he became Director in 2013. "I was reluctant to take the job, but Bob Wiltrout, CCR's Director at that time, convinced me, and it was exciting," said Tessarollo. "You can make more of a difference for the new generation of scientists. You also have the opportunity to influence the overall direction of the science."

The program is diverse, united in the use of genetically engineered mouse models as a tool for understanding function. The eight additional investigators in the Program have research interests as diverse as cancer stem cells, angiogenesis, epigenetics, and transcriptional regulation.

The emphasis is on basic research, but Tessarollo is always on the lookout for and ready to support translational opportunities. "Shyam Sharan, for example, developed a beautiful system based on embryonic stem cells, in which he can screen hundreds of *BRCA* polymorphisms found in humans whose physiological significance is unknown. He developed that in this program and it has strong translational potential," said Tessarollo. (See "Breast Cancer Genes: When the Sequence Is Not Enough," *CCR connections* Vol. 3, No. 2).

"Often, we generate mice and their phenotypes are not predictable from the functions put forward in the literature for the gene of interest. Having many different areas of expertise around the table can really help people to dissect phenotypes and understand the function of specific genes. The mouse brings us together."

COMMENTARY

A Broader View of Immunotherapies

Fresh out of graduate school at Wayne State University in Michigan in 1985, Bernard Fox, Ph.D., landed a coveted fellowship with Steven Rosenberg, M.D., just as the first patients were being treated with cell-based immunotherapies at NCI. Now the Harder Family Chair for Cancer Research and Chief of the Laboratory of Molecular and Tumor Immunology in the Earle A. Chiles Research Institute at Providence Cancer Center, Fox combines basic and translational research to develop new forms of immunological interventions for cancer. Fox mentors students through the Oregon Health and Science University and has played a prominent leadership role in the Society for Immunotherapy of Cancer (SITC). He is also President and CEO of UbiVac, a company he cofounded to bring the tools forged from his research directly into the clinical armamentarium.

My laboratory is part of the Robert W. Franz Cancer Research Center, directed by Walter Urba, M.D., Ph.D. (who also spent 10 years at NCI), which has grown from three people, when we started 22 years ago, to 90 people focused on cancer immunotherapies. Currently, we are running 16 investigator-initiated trials, some of which are first-in-human studies. I've watched cancer immunology go from un-fundable to unstoppable, thanks in no small part to NCI.

My work is divided into a preclinical group studying basic mechanisms of T cell-mediated tumor elimination and developing therapeutic interventions in animal models, and a translational group that takes our discoveries into clinical trials and feeds back clinical information into the preclinical process. I manage a human applications lab that makes cell lines and products, which complies with practices required for administration to humans. The Center is also part of Bristol Myers Squib's International Immuno-Oncology Network (II-ON), which brings together 10 leading institutions to pursue clinical trials based on combination immunotherapies and multidimensional monitoring.

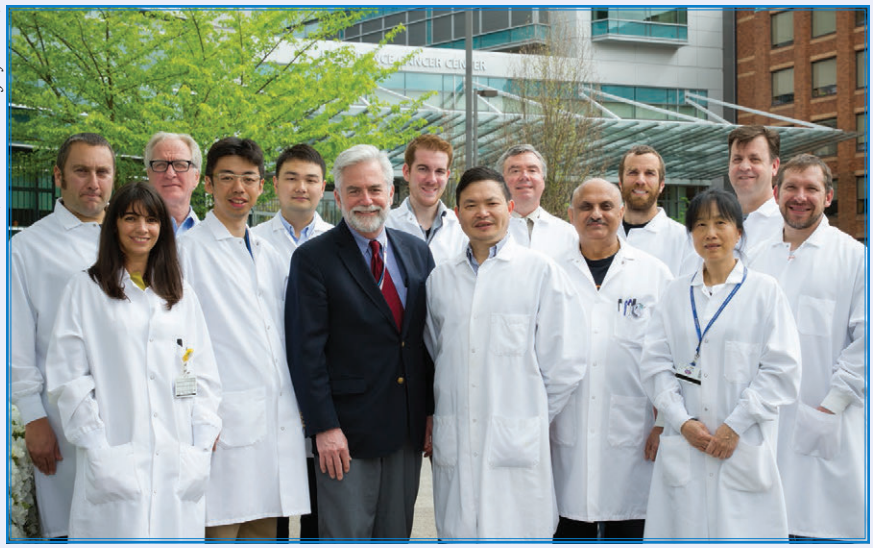
Bringing immunotherapies into the clinic is clearly not a solitary pursuit. It requires teamwork on multiple dimensions, across basic and clinical research, across nonprofit and commercial sectors, and across international boundaries. I view myself as kind of a bridge builder. When I was President of SITC, we brought together leaders of 15 different international societies to identify the major hurdles preventing the successful translation of immunotherapies. We identified issues ranging from limitations in animal models to limitations in training for scientists focused on translational research.

SITC has also been instrumental in supporting work across 13 countries to validate a prognostic biomarker in colon cancer that was first developed at INSERM. In 2006, Jerome Galon and colleagues published a remarkable correlation between the presence of specific T-cell infiltrates in an excised tumor and lack of recurrence. Such a biomarker, if validated, will not only have game-changing clinical implications, it also speaks to the increasingly clear link between the patients' immune response, therapeutically stimulated or not, and cancer morbidities.

Expanding Targets

We know that solid tumors are heterogeneous, continue to mutate, expand clonally, and spread to other parts of the body. As Bob Schreiber elegantly laid out in the Elimination-Equilibrium-Escape hypothesis, if your immune response is limited to a small number of targets, tumors will eventually escape from equilibrium. We probably aren't going to be able to find a single antigen to combat that diversity, but will need a broader immunotherapy strategy and multiple targets to which the host is not already tolerant.

In 2008, my former student and now colleague, Hong Ming Hu, Ph.D., developed a new vaccine strategy based on short-lived proteins (SLiPS) and defective ribosomal products (DRiPs). Normally, SLiPS and DRiPs are degraded by the proteasome and, we believe, are typically not cross-presented by antigen-presenting cells. When cells



(Photo: Courtesy of B. Fox)

Bernard Fox, Ph.D., and Hong Ming Ho, Ph.D. (center), and members of their laboratories at Robert W. Franz Cancer Research Center in the Earle A. Chiles Research Institute located within the Providence Cancer Center in Portland, Oregon

die and release proteins into the milieu, there are very few short-lived proteins around to generate peripheral tolerance. Hong Ming showed that if you blocked the proteasome (with bortezomib), SLiPS and DRiPs would be diverted into the autophagy pathway and end up in microvesicles which we know are studded with ligands for receptors found on antigen-presenting cells.

Moreover, we've shown that if you take these proteasome-blocked autophagic microvesicles from a tumor created in one mouse, you can use them to vaccinate another mouse against a somewhat related tumor. Back in 1957, Prehn and Main established that tumor-derived vaccines only protect against the specific tumor from which the vaccine was developed, and that has been dogma for 50 years. They took 10 identical mice (A, B, C, etc...) and gave them all methylcholanthrene to induce individual sarcomas. Vaccinating mice with tumor A only protected against tumor A, and the same was true of B, C, etc. In contrast, we were able to show that vaccinating mice with proteasome-blocked microvesicles from tumor A protects from a challenge with other sarcomas, in eight of nine tumor combinations studied, changing the Prehn and Main paradigm. Much of this work was done in collaboration with Hong Ming's lab, and we continue to work together on several joint projects.

Our preclinical results led to support from NCI for a phase 1, and then a phase 2 clinical trial as an adjuvant study in definitively treated Stage IIIA/B non-small cell lung cancer, where the recurrence rate is 70 percent. We have reported on our first nine patients. All have developed strong immunity to many targets, which we know from The Cancer Genome Atlas (TCGA) are overexpressed in lung cancer. We have used mass spectrometry to study the protein content of the microvesicles and looked at whole exome sequencing. Our primary goal is to understand whether mutated or overexpressed nonmutated proteins induce the strongest antitumor responses.

With a new way to induce broad immunity, anti-PD1 or other costimulatory molecules could be synergistic. Some years ago, our institute developed anti-OX40, an antibody that can stimulate CD4 and CD8 T cells, as an anticancer therapy. We have shown in mouse models that anti-OX40 boosts microvesicle-primed immunity in mice. We are very excited to move that data from the mouse into clinical trials.

The Patient Connection

There is a photo that I still see occasionally in papers, which shows the unbelievable shrinkage of metastatic melanoma nodules—it belongs to Linda Taylor (see "Immunotherapy's First Cure," *CCR connections* Vol. 8, No. 1). Taylor was treated with lymphokine-activated killer (LAK) cells and IL-2, shortly before I arrived at NCI as a Fellow in 1985, but she would come back periodically for monitoring. No one expected her remission to last 30 years. She is part of the linkage between laboratory and patients that was cemented for me during my years in Steven Rosenberg's laboratory. You knew that if it was promising, Steve would find a way to move your work into clinical trials. As a Ph.D. scientist, you can get busy and lose a little bit of that energy. So I try to make sure all my students have clinical experiences that relate to their translational projects.

Seven years ago, we had a patient with metastatic prostate cancer, they took him off ipilimumab when his enzyme levels spiked and he had a flare of hepatitis. However, he had a complete response and is still cancer free. Using protein arrays, we asked what proteins he was making antibodies against. One protein was the mitochondrial enzyme 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) and we've identified HIBCH as overexpressed in his tumor. Less than 10 publications have referred to HIBCH, and none have identified HIBCH as a potential tumor antigen. The individual patients also drive us to think outside the box. With more time and resources to study these super-responders, eventually we will make everyone an exceptional responder.

Adoptive Cell Therapies: One Cancer at a Time

After completing medical school and a general surgery residency at the University of Missouri, Kansas City, Christian Hinrichs, M.D., planned on doing cancer research at the start of his fellowship at Roswell Park Cancer Institute in 1996. However, a detour sent him into surgical oncology, and Hinrichs only returned to his research interests through a subsequent surgical oncology fellowship at NCI. Then, cancer had an unexpectedly personal impact on Hinrichs' career when an ocular melanoma compromised his eyesight and cut short his potential as a surgeon. Undeterred, Hinrichs shifted his focus to internal medicine as a resident at George Washington University and a medical oncology fellow in CCR. Now a Lasker Clinical Research Scholar in CCR's Experimental Transplantation and Immunology Branch, he is using his knowledge of cancer immunotherapies to help patients with metastatic cancers caused by the human papilloma virus (HPV).

During my surgical oncology fellowship, I worked in the laboratory of Nicholas Restifo, M.D., Senior Investigator in CCR's Surgery Branch. The branch was studying the use of adoptive cell transfer (ACT) for melanoma. In ACT, cancer-killing immune cells are harvested from the patient's tumor, grown outside the body, and then reintroduced. These lymphocytes (T cells) carry receptors which allow them to identify abnormal cells and other threats to the body that express specific protein markers, or antigens. We were expanding populations of the native tumor infiltrating lymphocytes (TIL) from patients and we were trying to engineer T cells with receptors designed to recognize specified antigens found on the melanoma cells. Unfortunately, antigens are rarely found exclusively on cancer cells and one of the key toxicities we were seeing in our melanoma protocols was the destruction of normal tissues that also contained melanocytes, e.g., the skin, eyes, and ears.

TIL for HPV

When I began my own research as a CCR Investigator, I had already thought a lot about the kinds of tumor antigens we could use for ACT that would be effective against cancers, while sparing more normal tissue. Although most cancers are caused by mutations of genes found

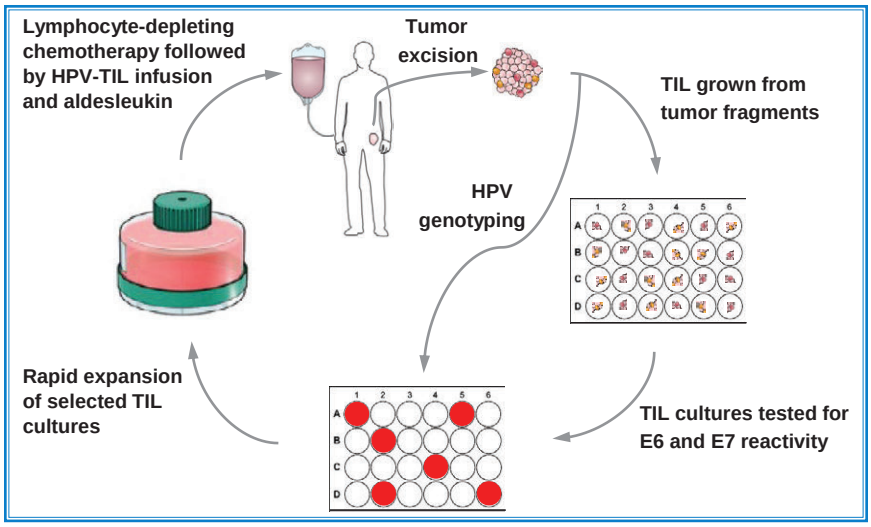
(Photo: R. Baer)

Christian Hinrichs, M.D.

normally in the body, some are caused by viruses, which introduce oncoproteins. Certain strains of HPV (HPV-16 and HPV-18), for example, cause cervical cancers as well as oropharyngeal, anal, vulvar, vaginal, and penile cancers. Although cervical screening and now anti-HPV vaccines may one day make these cancers obsolete, cervical cancer alone causes 4,000 deaths per year in the United States. Like many cancers, once cervical cancers reach advanced stages, they do not respond well to chemotherapy.

In 2012, we opened a protocol to treat women with cervical cancer, using essentially the same procedures that were initially pioneered for metastatic melanoma, but with an added layer of complexity. We tested our TIL cultures for reactivity to specific HPV antigens (E6 and E7) and selectively expanded the most HPV-reactive populations before reintroducing them into patients.

Of the nine women we treated on this protocol initially, three saw their tumors shrink. In one patient, the response was only partial and lasted for three months. But, the other two patients remain cancer free to this day (see "Going Home to Kansas," in this issue). We have since treated a total of 29 patients with HPV-related cancers with TIL. It has taught us some important concepts, namely that immunotherapy can mediate complete regression of cervical cancer. This is the most compelling evidence to date that cellular therapy can cause complete regression of an epithelial cancer. The trial numbers are small, so we cannot accurately assess the overall response rate. Fundamentally, TIL is also limited because we have to do surgery before we can even make a cell product for these patients. And once we do, the cell products are quite variable. For example, some cells are very reactive against HPV antigens, while others are completely unresponsive. We cannot control whether the patient has reactive cells, but our data indicates that the degree of reactivity of a patient's cells is related to the success of the procedure.



(Figure: C. Hinrichs, CCR)

Outline of first HPV-TIL protocol. A tumor is selected, excised, and split into tiny fragments which are grown in individual wells of cell culture plates. After initial expansion of two to three weeks, individual TIL cultures are tested for HPV-type specific E6 and E7 reactivity. Selected TIL cultures (red wells) are further expanded using a rapid expansion protocol. Expanded TIL are administered to patients, who have first been treated with lymphocyte-depleting chemotherapy to "clean the immunological slate" and allow the TIL to dominate.

Engineering Better T Cells

As part of our protocol, we not only wanted to assess the potential of TIL therapy for HPV-related cancers, we also wanted to study the populations of T cells in patients that were reactive against HPV. Our hope was that we could identify a good T-cell receptor that could be used to engineer lymphocytes extracted from patients' blood to respond to their tumors, thereby avoiding the issues associated with isolating TIL. In one of our patients who responded well to the TIL therapy, we identified a T-cell receptor against the E6 protein of HPV-16.

Armed with the genetic sequence for this receptor, we have now begun a protocol to treat patients whose tumors were caused by the HPV-16 strain, without having to surgically extract cells in the hope that they contain reactive TIL. Instead, we can use leukapheresis to extract lymphocytes from the blood, engineer those lymphocytes with the T-cell receptor sequence, and expand the cells. Leukapheresis can be performed at any medical center that already handles hematopoietic stem cell transplants. The leukapheresis product can be shipped to a commercial facility for genetic engineering then returned to the medical center to be given to the patient. However, at this stage, we

> This is the most compelling evidence to date that cellular therapy can cause complete regression of an epithelial cancer.

(Photo: R. Baer)

Arrica Wallace and Christian Hinrichs, M.D., discuss her scans.

can only treat patients that 1) have the HPV-16 strain (65 percent of cervical cancers, 70 percent of oropharyngeal cancers, and 90 percent of anal cancers) and 2) are immunologically matched to the original T-cell receptor donor (HLA-A2, about 40–50 percent of Caucasians).

Patients are always very excited to see their cells infused, but it is actually kind of anticlimactic for me. I think what is important is seeing the scans when they come back for their first follow-up appointment. Patients return six weeks after the cell infusion; and, if the treatment is going to work, we usually see some shrinkage. However, at the first visit, it can still be difficult to tell if the treatment is working. We really only understand how well the cells are working after the second, third, or even fourth monthly visit.

From Clinic to Laboratory

I see my patients in clinical trials and I cover the medical oncology service for two weeks each year; the rest of my time is in the laboratory. I have four people working directly with me and two additional cell processing technicians who work in my lab. The Experimental Transplantation and Immunology Branch is developing a critical mass of cell therapy researchers, e.g., Jim Kochenderfer, M.D., who works on very similar approaches but for hematological cancers, and Luca Gattinoni, M.D., who works at a more basic science level. The three of us have a similar interest in cellular therapies; and Luca and I have a joint lab meeting and joint journal clubs.

I mostly work with human cells, studying why treatments work in some patients and not in others, trying to discover new T-cell receptors for gene therapy-based approaches, and investigating ways to improve the function of the T cells that we give to patients. Projects in my lab include efforts to delineate the landscape of T-cell responses against tumor antigens in the patients with cervical cancer who we have treated successfully. We are also working to identify new T-cell receptors that can be used to treat patients with HPV-related cancers and other types of cancer. Finally, we are seeking to improve

> We are also looking at the tumor side of the equation to understand better how tumors might evade our treatments.

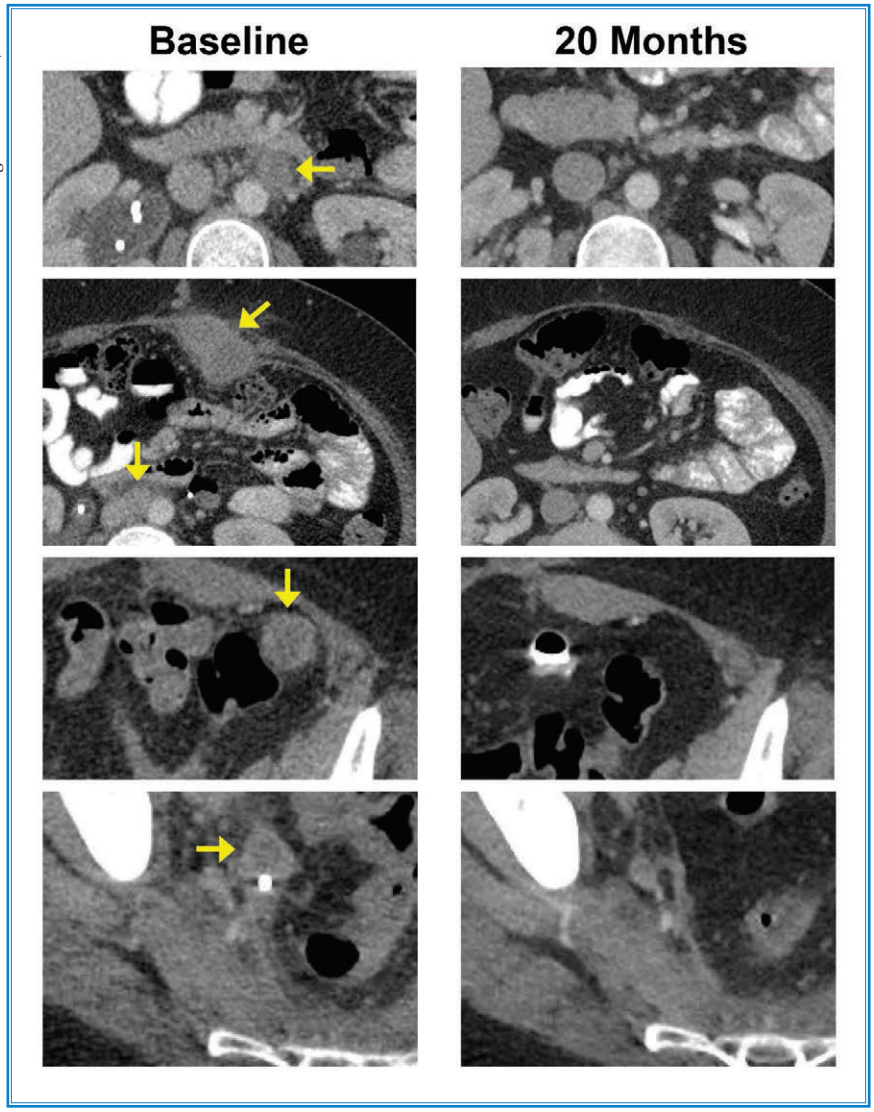
> I think we will make more progress by finding a really good target in a smaller subset of cancers.

the function of therapeutic T cells, either by increasing expression of stimulatory genes or by decreasing expression of inhibitory genes.

We are also looking at the tumor side of the equation to understand better how tumors might evade our treatments. Tumors can lose expression of molecules that are needed for recognition by the immune system. They can also produce molecules that inhibit T cells, like PD-1. Understanding these factors can help us to select patients who are most likely to respond to treatment and to design rational combination therapies.

As it stands right now, it is proving difficult to get a single therapy to work in many different kinds of cancers, especially in the realm of solid tumors or epithelial cancers. It might be that we do not find a single magic bullet, a highly effective cellular therapy that can be broadly applied to different types of cancer; rather, we may move forward in increments, where we find a target for a particular type of cancer that makes sense, works well, and has low toxicity. And then, we repeat the process for the next type of cancer or even a particular subset of a type of cancer. Instead of looking for one target expressed by all cancers and targeting that, I think we will make more progress by finding a really good target in a smaller subset of cancers.

For the kind of research I do, working with complex cell therapies, there is no other center in the world that can do it as well as the NIH Clinical Center. It is the mission of the Clinical Center to do these kinds of cutting-edge clinical trials that would be very difficult to conduct elsewhere, with high impact and important laboratory science. We need to be able to move between patient protocols and research into the cellular mechanisms associated with patient outcomes. I cannot imagine doing that more efficiently anywhere else.



(Image: C. Hinrichs, CCR)

Computed tomography (CT) images of a 36-year-old patient with adenocarcinoma (HPV-18+) before and after treatment with adoptive cell transfer immunotherapy.

To learn more about Dr. Hinrichs' research, please visit his CCR website at https://ccr.cancer.gov/Experimental-Transplantation-and-Immunology-Branch/christian-s-hinrichs.

Going Home to Kansas

In June 2011, at the age of 34 and despite regular screenings, Aricca Wallace was diagnosed with stage 3 cervical cancer. Raising two small children with her husband, and otherwise healthy, she was ready to fight. "I was young and was able to handle the aggressive chemotherapy and radiation. I thought everything would be fine," said Wallace.

Instead, by January of 2012, her doctors found that the cancer had spread to her chest. She was referred to a clinical trial at M.D. Anderson Cancer Center, but the form of treatment was milder than she had already experienced. Chemotherapy could "control, but not cure," and she was told she might not survive a year.

"I knew there had to be something else," said Wallace. "So I started the chemotherapy and hoped another option might appear." From February through April of that year, she went in three days per week, every three weeks. She was getting weak and her doctor, Verda Hunter Hicks, M.D., suggested a break to let her body recover.

At about the same time, Christian Hinrichs, M.D., got approval for a protocol to treat advanced cervical cancer with cellular immunotherapy. He started reaching out to colleagues, letting them know about the study.

"Minutes after she got off the phone with Dr. Hinrichs, Dr. Hicks called and said, 'You're going to the NIH in Bethesda,'" said Wallace. "I'd never heard of the NIH before, but I said okay."

Wallace met Hinrichs and his team in May 2012. Their study was brand new; one patient had signed on but she had not yet received tumor-infiltrating lymphocyte (TIL) therapy. "I remember the doctor telling me that the only thing he knew for sure is I would lose my hair. I pulled my wig off and said, 'Been there, done that. If it doesn't work, maybe it will buy us more time,'" said Wallace.

(Courtesy of A. Wallace)

Arrica Wallace and her family at the beach

Wallace had surgery the following month to remove a large lymph node in her chest, near the aorta. After recovering from the surgery, she returned to her home in Manhattan, Kansas, and enjoyed the next month with her family. "I remember it like it was yesterday," said Wallace. "Every time I passed a certain ballfield in Kansas City, I would remember my son pitching in a championship game. I had to go back to the NIH that afternoon, so I had the coach call a time out and all the boys came to give me a big hug."

That August, Wallace had the single infusion of TIL grown from her tumor and returned one month later for her first scan. The scan showed shrinkage of most of the tumors by over 50 percent. Some were completely invisible. "That was amazing," said Wallace. "Finally, what I believed in my heart was actually showing up on scans."

By December 2012, no tumors were visible and her scans have been clear ever since. She started having some symptoms resulting from the 31 doses of radiation and 18 doses of chemotherapy she went through since her diagnosis. "When you put that much poison in your body, you're bound to have other things happen," said Wallace. "But the NIH has been phenomenal about answering my calls, working with my doctors here, and making sure that everything can be as normal as possible."

"I am a huge NIH advocate," she concluded. "It's a place you go when there is no hope, and they give you hope."

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

Websites with More Information about CCR

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How to Refer a Patient
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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

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