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CCR connections

CENTER FOR CANCER RESEARCH

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Preventing Cervical Cancer:
The HPV Vaccine

U.S. DEPARTMENT
OF HEALTH AND
HUMAN SERVICES

National Institute
of Health

Center for Cancer Research

National Cancer Institute | National Institutes of Health | Building 31 – Room 3A11 | 31 Center Drive | Bethesda, MD 20892

Table of Contents

03 Editorial: Our Passion and Our Mandate

NEWS

- 04** Rapamycin: An FDA-approved agent that may prevent lung cancer?
President Bush Visits CCR
- 05** Cutting in: Getting between HIV and Chromosomes
- 06** Silent No More
Newly Tenured CCR Scientists
- 07** 2007 Fellows & Young Investigators Retreat
- 08** Probing the Workings of an Ancient Antiviral Weapon
- 09** Uncovering Cancer's Molecular Switches
- 10** Re-educating Stem Cells
- 11** New Recruits at CCR

FEATURES

- 12** A Victory Against Cancer: 25 Years in the Making
- 16** Viral Deception: The Promise of Adenovirus as an HIV Vaccine
- 20** A War on Kidney Cancer

COMMENTARY

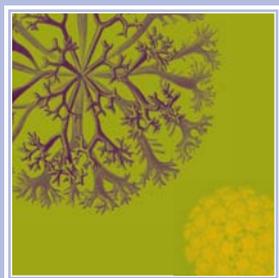
26 A CCR in Belfast

IN THE CLINIC

28 CCR Team Tackles Neurofibromatosis

12

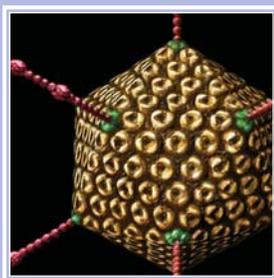
FEATURE



A Victory against Cancer

16

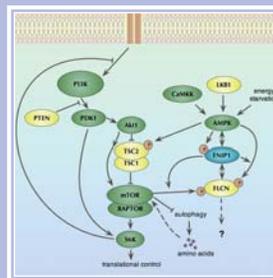
FEATURE



Viral Deception

20

FEATURE



A War on Kidney Cancer

28

IN THE CLINIC



Tackling Neurofibromatosis



The mission of the 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

Contributors:

L.M. Bennett, Ph.D.

D. Kerrigan, M.S.

M. Randazzo, M.A., M.A.

S. Fox, B.A., B.S.W.

Baer Photography

Palladian Partners

SPG&M, SAIC-Frederick, Inc.

Vanchieri Communications

Designed and Produced by:

Feinstein Kean Healthcare

Our Passion and Our Mandate

For the first time in human history, we have the fundamental biological knowledge we need to fight devastating diseases such as cancer and HIV/AIDS within our reach. This knowledge is important, but it is not enough: it also needs to be tested and translated from basic scientific insight to treatment and even prevention. This Herculean feat can only come about in a scientific environment that encompasses a diversity of talent, open sharing of results and insights, and a united passion to understand, treat, and prevent disease.



The Center for Cancer Research (CCR) provides such a unique environment. As the basic and clinical research arm of NCI's intramural program, CCR is a distinctive organization of scientists, physicians, trainees, and support staff dedicated to informing and empowering the entire biomedical community by making fundamental discoveries in cancer and HIV/AIDS and rapidly translating those findings into novel therapies for patients suffering from those diseases. In addition, as part of the NIH, CCR has a mandate to confront the special challenges pre-sented by relatively rare, but devastating cancers, as well as cancers that may be predominant in medically underserved populations.

CCR has prodigious strengths in both cutting-edge basic science and clinical research. The deep integration of our basic and clinical efforts drives and contributes to our success. Our unique position within NCI enables us to be innovative and agile in our explorations, allow-

ing us to commit resources for longer-term and relatively high-risk studies that—despite their potentially high impact—would be difficult for other research institutions to undertake. Furthermore, our researchers collaborate not only with their CCR colleagues, but also with many external researchers in both the private and public arenas. We draw on their expertise, and they on ours, to make the fastest progress possible, and to ensure that innovative findings and technologies are rapidly dispersed throughout the cancer research community.

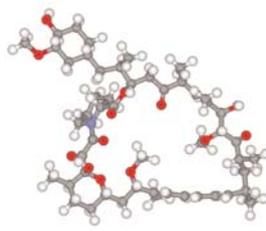
Everything we do at CCR begins and ends with our patients. We recognize them as full partners in our work, and we seek to offer them an unsurpassed level of care. As active participants in our research efforts, in the flow of information from bench to bedside and back, they often learn as much about their disease as we do! This close collaboration is deeply embedded in our culture, is empowering for both patient and

physician, and adds to the unique richness of the CCR environment.

It is one thing to describe the uniqueness of CCR, and another to show it. To that end, you hold in your hands the first issue of *CCR Connections*. Here you will find information about our basic and clinical research, our patients and scientists, and our alumni. Within the pages of each issue are only a few of the hundreds of CCR “stories” we could tell that support our vision and mission. Although each story is distinct, each one also shares with all the others the underlying passion for our work and our mandate, derived from the founding mission of NCI 70 years ago, to understand, diagnose, treat, and prevent cancer and HIV/AIDS.

We hope you find *CCR Connections* interesting, informative, and even challenging, and we welcome your thoughts and comments.

Robert H. Wilttrout, Ph.D.
Director, Center for Cancer Research



(Graphic: Wikipedia)

Rapamycin:

An FDA-approved agent that may prevent lung cancer?

Even though smokers who quit reduce their chances of developing lung cancer, their risk will always remain higher than that of the general population. To reduce that risk, clinicians and researchers are on the hunt for effective interventions capable of countering tobacco's lingering carcinogenic effects.

One attractive chemoprevention target is mTOR, a protein linked to cell proliferation and the synthesis of new proteins. The cellular pathways connected to mTOR are stimulated by tobacco components such as nicotine and one of its byproducts, a potent tobacco-specific carcinogen called NNK. However, mTOR's relative importance in tumor development and progression has been unclear, clouding its potential as a prevention target for lung cancer.

After examining the interplay of mTOR, NNK, and rapamycin (a well-known mTOR-blocking drug that is FDA-approved as an immunosuppressant), a collaborative effort led by CCR's Phillip A. Dennis, M.D., Ph.D., Senior Investigator, Medical Oncology Branch, has established mTOR's critical role in the formation of tobacco carcinogen-induced lung tumors. In addition, the researchers also showed that rapamycin might be an effective agent for preventing lung cancer. The results were published in the April 1, 2007, issue of *Clinical Cancer Research*, in a paper by lead author Courtney Granville, a doctoral student in Dennis' laboratory.

Mice given rapamycin shortly after exposure to NNK and kept on the drug continuously for several weeks had 90 percent fewer tumors than untreated mice, and the tumors that did develop were 74 percent smaller, highlighting mTOR's importance in NNK-induced lung tumors. In a different experiment, tumors that developed after NNK administration withered in size, but not in number, when treated with rapamycin, verifying their dependence on mTOR to maintain growth, even after they are formed. The team also found an every-other-day dosage schedule—as opposed to five-days-on/two-days-off—to be the most effective at quenching mTOR activity. Importantly, the levels of rapamycin using this schedule were comparable to what is achieved when humans take rapamycin as an immunosuppressant.

But can rapamycin's effectiveness in mice translate into a new intervention for people at risk for lung cancer? That question remains open. Dennis and his team point out that while rapamycin was profoundly effective, well-tolerated, and reached levels in the mouse that are achievable in people, the drug's immunosuppressive effects in humans could limit its use as a lung cancer intervention. For these reasons, the researchers are currently studying the effects of this schedule of rapamycin on immune function in this mouse model system. If profound immunosuppression is not observed, the Dennis team will translate these findings into a clinical trial focused on rapamycin's effectiveness at eliminating premalignant lesions in smokers with the highest lung cancer risk, such as those carrying cancer-predisposing polymorphisms or having the greatest exposure to tobacco carcinogens.

President Bush Visits CCR

On January 17, 2007, U.S. President George W. Bush paid a visit to CCR's patients and staff as part of a day-long trip to the NIH to participate in an NCI roundtable on cancer prevention. The visit, which was the President's fifth to the NIH since taking office in 2001, included a tour of the laboratories of CCR's Urologic Oncology Branch.

Moderated by U. S. Department of Health and Human Services Secretary Michael O. Leavitt, the roundtable—which included NIH Director Elias Zerhouni, M.D., National Human Genome Research Institute Director Francis Collins, M.D., Ph.D., NCI Director John Niederhuber, M.D., and cancer survivors and advocates Grace Buler, M.D., and Becky Fisher—coincided with the American Cancer Society's announcement that cancer deaths in the U.S. had fallen for the second year in a row (and only the second time ever). The President, addressing the gathering, said "I love coming to the NIH; it is an amazing place. It is an amazing place because it is full of decent, caring, smart people, all aiming to save lives. And I truly believe the NIH is one of America's greatest assets."



(Photo: White House)

Urologic Oncology Branch Chief W. Marston Linehan, M.D., talks with President Bush during the President's visit to the NIH in January.

Silent No More

(Graphic: Feinstein Kean Healthcare)

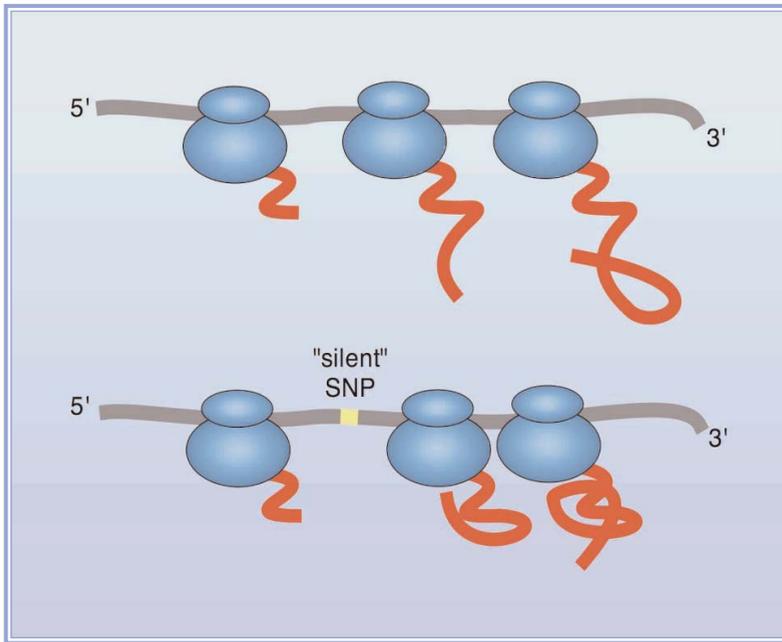


Illustration showing potential effects of silent SNP on protein translation. Ribosomes (blue) “read” the mRNA (gray) and add amino acids to the growing protein chain (red). The presence of a silent SNP (yellow) does not change the amino acid sequence, but alters the kinetics of the protein translation, changing the folding structure—and potentially the function—of the resulting protein.

For decades, it had been widely understood that certain genetic mutations were silent. Within genes, three-base blocks called “codons” direct the addition of a specific amino acid to a protein under construction. The third base position in the codon can sometimes change without affecting the protein’s amino acid sequence in a so-called “synonymous” or “silent” mutation. In other words, the gene can change, but the protein and its function remain the same. In a paper published in the January 26, 2007, issue of *Science*, a CCR research team led by Michael Gottesman, M.D., Chief of the Laboratory of Cell Biology, revealed that synonymous mutations may not be merely “silent,” but can actually cause some proteins to behave differently by changing how they fold.

The gene they studied, multidrug resistance 1 (*MDR1*), codes for a protein called P-glycoprotein (P-gp) that can pump molecules—including anticancer drugs—out of a cell. This protein pump can contribute to the drug resistance that arises in a tumor over time. Such resistance

can sometimes be reversed with P-gp inhibitors (e.g., cyclosporine A or verapamil). Gottesman’s group found that a synonymous mutation, C3435T, when combined with one or two other mutations, can interfere with P-gp inhibitors and frees P-gp to expel anticancer drugs from the cell. Experiments showed that the change does not result from chemical alterations or expression changes in P-gp, but rather from changes in the protein’s conformation. This phenomenon can occur if a synonymous mutation

like C3435T can substitute a rarer codon for a more common one. This substitution can affect the rate at which the P-gp is synthesized during translation of the mRNA into protein, which in turn affects the dynamics of how it folds.

The results of this study suggest that researchers should take a harder look at the large number of mutations that were once assumed to be “silent.” Such mutations may have renewed relevance to understanding the origins and treatment of cancer.

Newly Tenured CCR Scientists

Rémy Bosselut, M.D., Ph.D.

Laboratory of Immune Cell Biology (Nov/Dec 2006)

Ira O. Daar, Ph.D.

Laboratory of Cell and Developmental Signaling (March 2007)

Carole A. Parent, Ph.D.

Laboratory of Cellular and Molecular Biology (Nov/Dec 2006)

Ying E. Zhang, Ph.D.

Laboratory of Cellular and Molecular Biology (March 2007)

(Graphic: CCR)



2007 Fellows & Young Investigators Retreat

The 7th annual CCR Fellows and Young Investigators (FYI) retreat was held to much fanfare in Ocean City, Md., from February 27th to March 1st, 2007. Organized by the CCR-FYI Steering Committee and sponsored by CCR's offices of the Director and Training & Education, the event brought more than 300 young CCR scientists together for 48 hours of scientific discussion, career exploration, and networking.

This annual meeting provides the 1,000-plus young scientists at CCR an opportunity to meet, learn from each other, and develop a sense of unity. Organized by CCR young investigators for CCR young investigators, the meeting is part career fair, part scientific conference, and part networking event. Eleven months of planning by four members of the steering committee (Veronica Hall, Ph.D.; Veronique Pascal, Pharm.D., Ph.D.; Girish Patel, M.D.; and Gillian Whittaker, Ph.D.) went into this event, though all 31 committee members participated in its execution. There was so much to do, and many mailboxes remained overloaded throughout, prompting one committee member to comment, "When you volunteer, you just don't realize how much work is involved!"

The Career Fair—a first-time offering for this retreat—was a major achievement and success. Sixteen organizations from academia, government, and industry came searching for scientific talent, and a resume critiquing service was available to help prepare fellows to take their next important career steps. Many thanks are due to Veronique Pascal for her hard work in organ-

izing the fair, and we congratulate all who left the fair with job offers in hand.

Educationally, there were four fantastic keynote lecturers: Max Wicha, M.D. ("Cancer Stem Cells: a New Paradigm in Cancer Research"), Polly Matzinger, Ph.D. ("Conversations between Tissues and T cells"), Lalage Wakefield, D.Phil. ("TGF- β , in Cancer: From Bench to Bedside"), and Steven Holland, M.D. ("From Immune Deficiency to Immune Therapy"). Most important was the celebration of research conducted by CCR young scientists. There were 35 oral presentations and over 100 poster presentations during the retreat; the eight presenters judged the best received travel awards. We also wish to congratulate and thank Anurandha Budhu, Ph.D., of the Laboratory of Human Carcinogenesis, who delivered this year's postdoctoral plenary lecture, "Lessons Learned from Molecular Profiling of Hepatocellular Carcinoma." And CCR Director Robert Wiltrot, Ph.D., was on hand to answer questions and congratulate the committee on a job well done!

What now for the CCR-FYI Steering Committee? In addition to organizing next

year's retreat, they will be busy helping young investigators achieve their career aspirations with variety of programs, including orientations, seminar series, networking brunches, a newsletter, and a Web site. If you are interested in helping, join the CCR-FYI Steering Committee by emailing nciccrfyi@mail.nih.gov.

Girish Patel, M.B.B.S., M.R.C.P., M.D.

Vice Chair & 2007 Retreat Chair

Fellows and Young Investigators Association

Center for Cancer Research

National Cancer Institute

Probing the Workings of an Ancient Antiviral Weapon

The HTLV-1 virus (round objects with a dense core) may thwart one of the body's ancient antiviral defenses by a novel mechanism.

The best known weapons of the human immune system are its T cells and antibodies. However, its arsenal also includes much more ancient weapons, held over from our evolutionary ancestors. These primordial agents of immunity lack the specificity of their more advanced relatives, but provide a powerful first line of defense against whole classes of pathogens.

The immune system is the evolutionary result of an elaborate molecular sparring contest: as the host develops new ways of countering a pathogen, the pathogen evolves new evasions and tactics. This interplay is well illustrated by the unfriendly relationship of retroviruses like HIV or HTLV-1 to the APOBEC3 family of antiviral restriction proteins—components of what has come to be called the “intrinsic” immune response, a form of antiviral defense possibly older than the innate or adaptive responses—and, in particular, APOBEC3G (hA3G). This host protein halts retroviral replication by packing itself into nascent virions and forcing hypermutation of reversed-transcribed viral cDNA.

Not to be outdone, HIV and HTLV-1 have both devised strategies for circumventing hA3G. HIV does so by producing Vif, a viral protein that tags hA3G for degradation. HTLV's choice of strategy, however, has only recently become clear.

In the February 20, 2007, issue of the *Proceedings of the National Academy of Sciences*, David Derse, Ph.D., Head of CCR's Retrovirus Gene Expression Section, and his colleagues

revealed that rather than actively eliminating its opponent, HTLV-1 (which can cause adult T-cell leukemia) prevents hA3G from being packaged into new virus particles. The virus accomplishes this interference with a pair of highly conserved motifs located near the C-terminus of the NC domain of Gag, the viral structural protein that builds HTLV's shell. The Derse team found that the two motifs—a small cluster of acidic amino acids followed by a leucine-rich motif—together are required for HTLV to keep hA3G (and other APOBEC3 proteins) out of the virus.

The work sheds light on an additional mystery: how hA3G gets packaged into HTLV (and other retroviruses) in the first place. Current wisdom holds that both viral RNA and NC are required for hA3G to gain entry, but it is unclear whether the host protein attaches to viral RNA alone or to NC-RNA complexes. Derse's work suggests the latter, that hA3G binds to NC-RNA complexes. This revelation opens the possibility of finding a general—and potentially therapeutically exploitable—mechanism by which hA3G enters and interferes with all retroviruses.

Uncovering Cancer's Molecular Switches

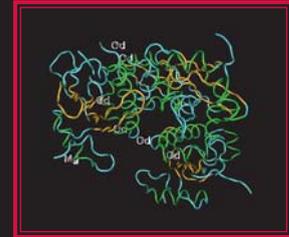
In many forms of cancer, the molecular machinery that is supposed to protect a cell from heading down the pathway to malignancy is subverted, actually contributing to a process it is supposed to prevent. Of the many different changes that can have this effect, most are poorly understood. However, investigators at CCR have illuminated the ways in which one such molecular guardian can unwittingly force healthy cells into malignancy, thereby pointing to a potential new pathway for cancer drug development.

As cells turn cancerous, the mechanisms that control their growth and division go awry. Thus-altered cells produce too many proteins that signal growth, a build-up that cells could reverse by breaking down the surplus. But cells also manufacture so-called chaperones which, under healthy conditions, bind to and protect “client” proteins from breakdown. This protection is supposed to stabilize a healthy cell during stressful conditions. Unfortunately, the clients of a chaperone called heat-shock protein 90 (Hsp90) include signaling proteins. Thus, Hsp90 inadvertently promotes undue cell growth and cancer by protecting the excess proteins.

For this reason, researchers have long sought not only an understanding of how the cell controls Hsp90 activation, but also ways of stopping it early in tumor formation. For example, a group of researchers led by Leonard Neckers, Ph.D., Senior Investigator in CCR's Urologic Oncology Branch, identified geldanamycin, a small molecule inhibitor of Hsp90, as early as 1994; in 1998 they brought to the clinic the first-in-class inhibitor called 17-AAG (now being evaluated in more than 20 phase II human cancer trials).

The Neckers team is now focusing its efforts on Hsp90 acetylation—the reversible addition of acetyl (-COCH₃) groups to specific amino acids. In a *Molecular Cell* paper authored by Post-doctoral Fellow Bradley Scroggins, Ph.D., (released January 12, 2007) the investigators revealed that acetylation of a specific lysine (K294) within the Hsp90 protein is crucial for many of its binding and stabilizing properties. And in a yeast-based model system, they showed that acetylation of K294 enhances Hsp90's ability to promote the same kind of aggressive growth that marks renegade cancer cells in humans—making the process an attractive target for drug development studies.

Although acetylation of Hsp90 is not known to be linked to cancer, these results are highly suggestive that it plays a role in the cancer process. “Acetylation is another layer of control of Hsp90 in response to environmental signals,” Neckers said. “We want to further understand the role of this regulation.”



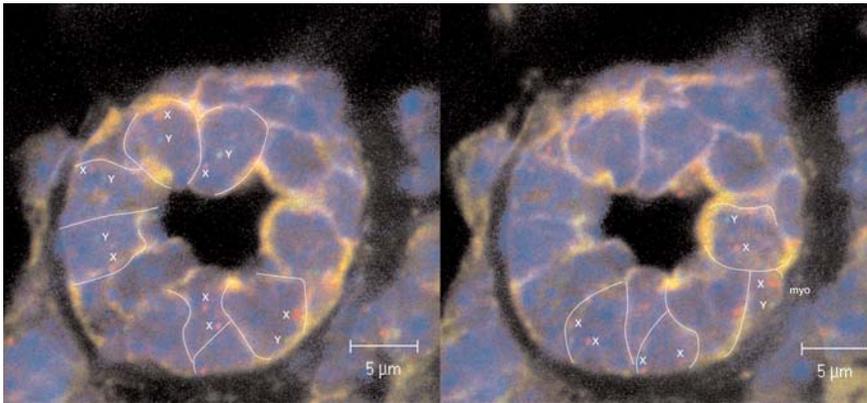
Instead of protecting cells from damage, Hsp90 (middle domain pictured) may inadvertently help them become cancerous; understanding its control mechanisms could help put cells back on track.

(Graphic: National Center for Biotechnology Information)

Re-educating Stem Cells

Coaxing partially committed adult stem cells to transdifferentiate—to develop into progeny cells and tissues other than those they are initially fated to become—is the holy grail of adult stem cell research. Reprogramming a skin stem cell to produce neurons and their supporting cells, for instance, would be a boon for stroke and Parkinson's disease patients.

(Graphic: C. Boulanger and G. Smith, CCR)



The presence of X (red) and Y (green) chromosomes in these chimeric mammary glands shows that spermatogenic cells transplanted into a mammary microenvironment can adopt characteristics and produce progeny cells specific to their new host tissue.

But to make this transformation with efficiency and specificity, researchers need a deeper understanding of the fate of adult stem cells and their progeny when placed into a different environment. Do the stem cells retain the ability to self renew? Do they gain tissue-specific multipotency, developing into all of the constituent cell types of their new host tissue? Can their progeny react in the same way as native host tissue cells to the normal hormonal or biochemical changes in their new surroundings?

In the March 6, 2007, issue of the *Proceedings of the National Academy of Sciences*, senior research assistant Corinne Boulanger, of CCR's Mammary Biology and Tumorigenesis Laboratory, and Gilbert Smith, Ph.D., Head of CCR's Mammary Stem Cell Biology Section, illustrate the extent to which a cell's microenvironment can influence its fate. The Smith lab showed that

mouse spermatogenic stem cells can be introduced into the mammary microenvironment and take the place of parity-induced mammary epithelial cells (PI-MECs): the native progenitors of all cellular subtypes found in mammary glands.

When transplanted into PI-MEC-depleted mammary fat pads in juvenile mice, the spermatogenic cells produced progeny that differentiated into several mammary-specific cell types, helping generate chimeric, fully functional mammary glands. The team also found that the transplanted male cells retained the ability to self renew, and continued producing mammary-specific progeny after experiencing the significant tissue remodeling seen in the mammary gland caused by pregnancy and lactation—just as PI-MECs do.

The significance of this work lies in its definitive proof of the power of the

microenvironment: that the signals specific to the milieu in which a semi-differentiated cell resides can override its previous programming. This outcome has obvious implications for cancer research as well as normal cell biology. However, the Smith team, including Postdoctoral Fellows Brian Booth, Ph.D., and David Mack, Ph.D., have not yet identified the particular cellular cues (other than pregnancy and lactation) that mediated the interactions they observed; that, they noted, is a subject for further study.

New Recruits at CCR

Giuseppe Giaccone, M.D., Ph.D.

(Photo: R. Baer)



Dr. Giaccone was recently appointed Chief of the Medical Oncology Branch. He is the former Head of the Department of Medical Oncology at the Free University Medical Center in Amsterdam. His work at CCR will focus on early drug development, molecular targets that manipulate

cellular processes, such as apoptosis, and how they relate to lung cancer. "I am very excited about the opportunity to work in the NCI environment," Giaccone said, "and about the great possibilities for having an impact on cancer research and treatment at this institution."

Dennis M. Klinman, M.D.

(Photo: SPG&M, SAIC-Frederick, Inc.)

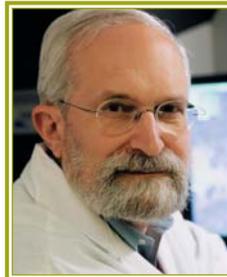


Dr. Klinman is a Senior Investigator in the Laboratory of Experimental Immunology and Chief of the Immunoregulation Group. Previously, he led the Section of Retroviral Immunology at the U.S. Food and Drug Administration's Center for Biologics Evaluation and Research.

Klinman's laboratory focuses on the mechanisms by which immunomodulatory DNA sequences alter host susceptibility to cancer, inflammation, and infectious diseases. His work on immune-stimulating CpG and immune-suppressing TTAGGG oligodeoxynucleotides is uncovering ways for clinicians to boost vaccine responses or curb inflammatory or autoimmune reactions by altering the host's inflammatory and immune milieu.

Paul Meltzer, M.D., Ph.D.

(Photo: R. Baer)



Dr. Meltzer came to CCR from the Cancer Genetics Branch of the National Human Genome Research Institute (NHGRI) in May 2006. He now serves as Chief of CCR's Genetics Branch, Head of the Clinical Molecular Profiling Core (CMPC), and attending physician in the Pediatric Oncology

Branch. At CCR, he is using genomic technologies, such as microarrays, to profile the cancer genome in both clinical and laboratory-based investigations and basic research. Via the CMPC, he is also helping to bring the latest genomic technologies to clinical investigators both within his branch and throughout NCI. "I hope to have a stronger interaction with the NCI Intramural Research Program and to help bring important genomic technologies to clinical and basic laboratory investigators within the NCI," Meltzer said.

Giorgio Trinchieri, M.D.

(Photo: SPG&M, SAIC-Frederick, Inc.)



Dr. Trinchieri was most recently the Director of the Schering Plough Laboratory for Immunological Research in Dardilly, France, and an NIH Fogarty Scholar at the Laboratory for Parasitic Diseases, National Institute of Allergy and Infectious Diseases. Since August 2006, he has

been the Director of the new Cancer and Inflammation Program (CIP) and Chief of the Laboratory of Experimental Immunology. Trinchieri discovered interleukin-12 (IL-12) in 1989 while at the Wistar Institute, and he has since been characterizing the molecular mechanisms underlying this cytokine's production, function, and activity. At CCR, he will continue his work on the interplay between inflammation/innate immunity and adaptive immunity, and on the roles played by IL-12 and other pro-inflammatory cytokines in the regulation of carcinogenesis, innate resistance, and anti-tumor immunity. "I am very optimistic that the CIP will bring to CCR new lines of investigation," said Trinchieri, "that will allow investigators to shed light on mechanisms of carcinogenesis and tumor progression and to identify new targets for cancer therapy or prevention."

Chondrus crispus, source
of carrageenan.

(Graphic: E. Haecel, via Wikipedia)

A Victory against Cancer

25 YEARS IN THE MAKING

In the early 1980s, CCR scientists heard the news that researchers had established the link between human papillomavirus (HPV) and cervical cancer, including those in NCI's Division of Cancer Epidemiology and Genetics (DCEG). This announcement set off a new quest: to craft a vaccine against a form of cancer that, at the time, claimed the lives of more than 5,000 American women each year.



(Graphic: C. Buck, B. Trus, CCR)

Nearly 25 years later, success: in 2006, the U.S. Food and Drug Administration (FDA) approved the first HPV-blocking vaccine to protect against cervical cancer. Approvals in Canada and Europe soon followed.

Apart from its significance as a landmark event in women's health, the FDA's approval also marked a victory for CCR scientists Douglas Lowy, M.D., and John Schiller, Ph.D., who laid the biological foundation for the HPV vaccine. Rather than rest on their laurels, these two lead scientists from CCR's Laboratory of Cellular Oncology and their colleagues are already looking toward alternate ways of fighting or preventing cervical cancer, including the next generation of HPV vaccines and topical microbicides that might address some of the significant challenges of delivering a vaccine in the developing countries where it is most needed.

A Vaccine with Impact

HPV vaccines promise to make a big difference in women's health. The American Cancer Society estimates that 11,150 American women will be diagnosed with invasive cervical cancer this year; nearly 3,700 will succumb to it.

A unique feature of HPV is the ability of one of its component proteins, L1, to assemble itself into empty shells called virus-like particles (VLPs). Because they contain no viral genomic material, these particles cannot cause infection on their own. What they can do, though, is mimic the presence of a viable virus and trick the immune system into mounting an anti-HPV immune response. The pair found that immunization of animals and even human volunteers with these L1-based particles could stimulate production of large numbers of antibodies; serum taken from vaccinees



(Photo: R. Baer)

Two decades of work by Douglas Lowy, M.D. (left), John Schiller, Ph.D. (right), and their collaborators and students could help protect millions of women around the world from HPV's cancerous consequences.

protected cultured cells from HPV infection in the laboratory.

NCI licensed the VLP technology to two pharmaceutical companies, Merck and GlaxoSmithKline (GSK), both of which subsequently developed HPV vaccines for clinical use. Both vaccines protect against HPV types 16 and 18, which cause up to 70 percent of all cervical cancer cases worldwide. Merck's vaccine, marketed under the name Gardasil®, also protects against HPV types 6 and 11, which cause 90 percent of genital warts. Both vaccines were remarkably successful in Phase III clinical trials, showing themselves to be 100 percent effective at preventing the premalignant cellular changes caused by the relevant virus types. Thus far, protection has remained solid after four years of follow-up. However, it appears that the vaccine cannot clear HPV infections that have already become established.

The FDA approved Gardasil in June 2006 for women and girls ages nine to 26

years. The vaccine, which is given in a series of three injections, was evaluated and approved in six months under the FDA's priority review process, which is used for products with potential to provide significant health benefits. GSK is expected to apply for approval of its vaccine, Cervarix™, in 2007.

Taking it Further

The considerable public health impact of this work has introduced NCI researchers to issues that most basic scientists do not face. How will the vaccine be delivered? Will the people who need it be able to get access to it? Those issues have spurred them on ever since.

HPV vaccines promise to make a big difference in women's health.

“The current vaccine has implementation limitations that will make it difficult for poor women to get it—and they’re the women who need it most because they have no Pap screening,” Schiller said. “It’s expensive to make and deliver this vaccine. We’re trying to make better approaches that are very simple to deliver.”

A new approach can be highly complex when developed in the laboratory, but has to be simple to produce and distribute. Lowy and Schiller are developing new therapeutic technologies capable of meeting those goals while still providing effective protection against HPV (see “A Topical Option”).

While the current Merck and GSK vaccines are based on L1, the virus’s L2 protein appears able to confer broad protection against more types of HPV. However, it is less effective than L1 VLPs at triggering virus-neutralizing antibody responses. Lowy and Schiller are working with Richard Roden, Ph.D., at the Johns Hopkins University—a former fellow in the Lowy lab—to improve the protein’s ability to spur on these responses.

Fighting two diseases with one vaccine is an efficient way of keeping costs down. Schiller is working with Denise Nardelli-Haefliger, Ph.D., at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland, to see if L1 can be incorporated into the typhoid vaccine, which is still used in developing countries. It may also be possible to formulate the dual-action vaccine as a nasal spray, easing the logistics of transport and administration. Such a preparation elicited strong immune responses in preclinical tests in mice.

“We can easily grow liters and liters of this. It doesn’t require high-tech production methods,” Schiller said. “Plus, a small oral dose for every villager would be much easier than giving three intramuscular injections.” They are moving forward with companies in India to conduct clinical trials of both vaccines.

Helping This Generation

While preventing future HPV infections remains a central goal, Lowy does not want to abandon the millions of women who are already infected. The Centers for Disease Control and Prevention reported in February, 2007, that about one in four females ages 14 to 59—the equivalent of 25 million American girls and women—are infected with HPV.

“We need to recognize that the vaccine is not going to do anything for the millions of women who are already infected with HPV and who remain at increased risk for cervical cancer,” Lowy warned. “The vaccine is for the next generations of women. But let’s not lose sight of the current generation and the need to help them reduce their incidence of cancer.”

He is hoping to see inexpensive DNA-based HPV tests made available to women globally, along with appropriate follow-up

“The vaccine is for the next generations of women. But let’s not lose sight of the current generation and the need to help them reduce their incidence of cancer.”

A Topical Option

In their continued search for alternate and easier ways to block human papillomavirus (HPV) infection, John Schiller, Ph.D., and his colleagues designed a high-throughput screening assay to test a wide range of compounds in hopes of finding ones that can prevent HPV infection. So far, a widely-used thickening agent extract from seaweed, called carrageenan—which can be applied as a topical gel or cream—has shown the most promise in laboratory tests.

“The screen let us test lots of drugs and other compounds very easily to see if they can act as a topical microbicide,” Schiller said. “Carrageenan popped out as something that worked amazing well, and it’s already [used] in some topical products.”

The fact that carrageenan is inexpensive and “generally recognized as safe” by the U.S. Food and Drug Administration (FDA) for food

and topical, including vaginal, applications increases its appeal to the scientists who are eager to find products that are safe and effective for humans. However, further studies are needed to determine whether products containing carrageenan can inhibit HPV infection and prevent sexual transmission of cancer-associated HPV in humans.

In preclinical animal studies, Schiller’s group tested carrageenan-based over-the-counter sexual lubricants in a mouse cervico-vaginal infection model developed in his laboratory. Even at a one million-fold dilution, these lubricants could prevent genital HPV infection in these mice. As for human studies, the Schiller lab has teamed with Terri Cornelison, M.D., Ph.D., and colleagues in the Division of Cancer Prevention to clinically test carrageenan’s ability to prevent sexually transmitted HPV infections.

Although a topical microbicide would be a significant addition to the anti-HPV arsenal, it does have some drawbacks. For example, it would have to be used prior to every occasion of sexual contact, as opposed to a vaccine, which requires just a few injections. “I don’t see it as a replacement for the vaccine,” Schiller said, “but it may be a complement to the vaccine to give coverage against more types of HPV or where the vaccine is not available.”

Carrageenan may have other uses as well. HPV can also be passed from mother to child during birth and, in rare cases, lead to serious diseases, including juvenile onset recurrent respiratory papillomatosis. The relative safety of carrageenan-containing infant formulas suggests that a cervical gel containing carrageenan may be a viable option for preventing HPV transmission during childbirth.

Recognition

The 2006 approval of the first human papillomavirus (HPV) vaccine ushered in a host of awards for the NCI scientists who made possible development of the HPV vaccine.

For their work on HPV, Douglas Lowy, M.D., and John Schiller, Ph.D., of the Laboratory of Cellular Oncology received a 2006 DHHS *Secretary's Award for Distinguished Service*. The award was shared with other NCI investigators who made significant contributions to HPV research including: Allan Hildesheim, Ph.D., and Mark Schiffman, M.D., M.P.H., in the Division of Cancer Epidemiology and Genetics, and Diane Solomon, M.D., in the Division of Cancer Prevention.

Lowy and Schiller's efforts also earned them the *3rd Annual David Workman Award* from the Samuel Waxman Cancer Research Foundation, the *2007 Dorothy P. Landon-AACR Prize for Translational Cancer Research*, the *2007 Award for Excellence in Technology Transfer from the Federal Laboratory Consortium for Technology Transfer*, and the *2007 American Medical Association Nathan Davis Award for Outstanding Government Service*.

"We are simply symbols of the many people who have made critical contributions to understanding the relationship between papillomavirus infection and cervical cancer," Lowy said. Both Lowy and Schiller are quick to point out that the recognition has been nice, but that they remain humbled by the insightful research done by so many of their colleagues.

treatment. (To put the need in perspective, more than half a million women worldwide are currently stricken with invasive cervical cancer each year, with a quarter million deaths.) Thanks to work done by DCEG's Mark Schiffman, M.D., M.P.H., and Diane Solomon, M.D., HPV-DNA tests are known to be cost effective when used in women with inconclusive Pap test results. DNA-based HPV testing is currently approved for screening of women over age 30, with some insurers now providing coverage. Digene Corporation, which has partnered with the Bill & Melinda Gates Foundation, is field-testing a new HPV-DNA test for use in developing countries. If proven effective, it will be available at a cost much lower than current DNA-based tests, according to Lowy.

Beyond Cancer

To capitalize on the fact that VLPs are "really good at inducing antibody responses," Schiller is exploring their ability to fool the immune system into making antibodies that target "self" proteins—which the body normally tolerates—that play roles in chronic diseases, such as Alzheimer's disease or HIV infection. For example, in the context of Alzheimer's, Schiller can imagine sopping up the beta-amyloid protein that makes up Alzheimer's-causing plaques with antibodies before it can coalesce in the brain.

In HIV, he is aiming at the CCR5 receptor. People lacking this receptor do not

develop AIDS after infection with HIV, indicating that this receptor is necessary for the virus to take hold. If the body could be tricked into making autoantibodies that compete for the receptor, perhaps HIV infection could be prevented or controlled.

VLPs may even work for contraceptive vaccines. With researchers in India, Schiller is testing a vaccine that targets a protein required for a fertilized egg to implant in the uterine wall, preventing pregnancy without affecting women's hormone levels or menstruation.

Right Place at the Right Time

Schiller, who began as a Postdoctoral Fellow in Lowy's lab, credits CCR's research environment for a lot of the progress they have made. "I stayed because CCR is a very good place to do research," explained Schiller. "We were studying the basic biology of a virus, but we had no experience in vaccines or immunology. However, no one said we couldn't try to develop this vaccine." He is convinced that if he and Lowy had been in the extramural program at the beginning of their search, they would have had to write a grant, which likely would have been rejected because they did not have the relevant track record in immunology or virology. Within CCR, however, they had the freedom to operate with confidence that they could justify their work in the long-term.

Leveraging CCR Progress through His Students

John Schiller, Ph.D., encourages his trainees to follow their own paths. "We try to set the postdocs up with broad, open-ended projects, where we can build an enabling technology in an area that's not fully explored," he said. He keeps his lab small enough to enable his postdocs to direct their projects and take them with them when they continue their careers at other institutions. He generally has three postdocs, two technicians, and a staff scientist. A great deal of responsibility falls to the postdocs.

He can list several success stories. One fellow, Bryce Chackerian, Ph.D., took work on virus-like particles (VLPs) and autoantibodies with him to the University of New Mexico. Richard Roden, Ph.D., who worked on the L2 vaccine in Schiller's lab, took that technology with him to the Johns Hopkins University.

"The nice thing is we're not competing for grants with my former postdocs," Schiller explained. "If we were in the extramural community, we'd be applying for the same research funds." But being in the intramural program, Schiller can let each fellow get a good head start and expand on a technology that they launched in Schiller/Lowy lab.

Viral Deception: The Promise of Adenovirus as an HIV Vaccine

Despite advances in drug treatments, HIV prevalence continues to grow at an alarming rate around the globe. The World Health Organization estimated that 4.3 million more people worldwide were infected with HIV in 2006, bringing the number of those living with HIV/AIDS to a staggering 39.5 million people. This figure does not count the three million who lost their lives to the disease that same year. The human and societal cost of HIV is incalculable.

These numbers underline the need to both treat HIV and prevent its further spread. To that end, researchers in the Vaccine Branch of the CCR are pooling knowledge of cancer and retrovirus vaccines to understand not only what immune system responses are required to fight off infection with the virus, but also how to develop a vaccine that will elicit those responses.

Researchers in the Immune Biology of Retroviral Infection Section of CCR's Vaccine Branch, led by Marjorie Robert-Guroff, Ph.D., have been pursuing these goals for the last two decades, with a singular focus on developing an effective HIV vaccine that can be easily delivered and made widely available. For the fundamental building block of their vaccine strategy, they have turned to the adenovirus, a common human virus that can be genetically altered to trick the immune system into thinking it is seeing HIV and thus into building robust HIV immune responses. There is a unique approach among many other HIV vaccine efforts, and their recent work in non-human primate models of HIV suggests that it has a high potential for success. Given the positive results to date,

they have set the aggressive goal of beginning human testing of this adenovirus-based HIV vaccine within the next two years. "It has become clear as we have studied the properties of this vaccine approach that we have to move it along," Robert-Guroff said. "Other approaches were not working, and the need is tremendous."

Why No Vaccine Yet?

The lack of an HIV vaccine more than 25 years after the first cases of AIDS were described is not due to a lack of research effort. Indeed, developing an HIV vaccine has been one of the largest—and thorniest—scientific problems of the last 20 years.

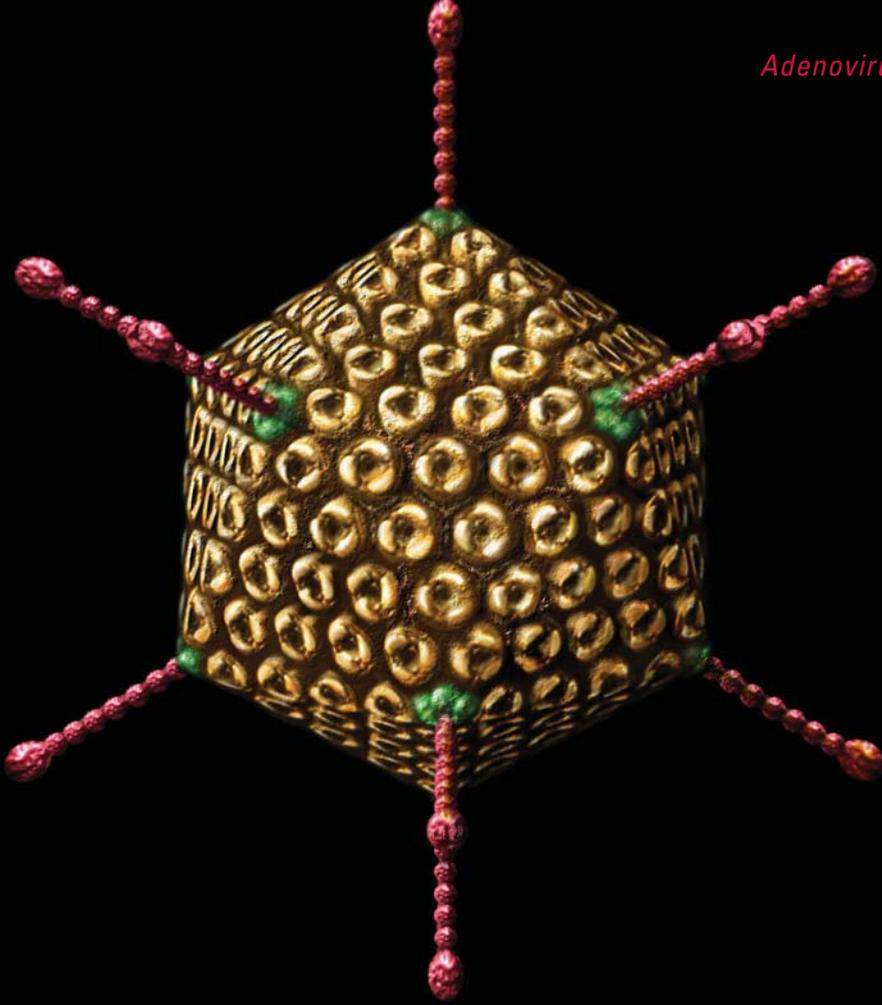
Perhaps the biggest obstacle is the nature of HIV itself. In addition to attacking the very cells the body relies on for defense against infection, HIV replicates rapidly and inaccurately, allowing new strains to arise quickly. This characteristic allows HIV to evade the immune response by quickly changing its protein makeup, a viral advantage that also explains the appearance of drug-resistant strains of HIV following a period of treatment. In other words, the virus always manages to stay a few steps ahead of

the immune system or drug treatment attempts to control it.

Thus, past HIV vaccine efforts had two strikes against them: they were poorly immunogenic and—because they targeted only one or a few HIV proteins—too narrowly focused.

Just as problematic for vaccine development efforts is an incomplete understanding of what immune responses actually control virus infection and replication, or the so-called "correlates of immunity." Scientists know that antibodies are important, as are cell-killing or "cytotoxic" responses. They also know that these immune responses need to be present not only in blood, but also at the mucosal surfaces where HIV most often gains its entry. People naturally infected with HIV have great antibodies and fairly robust cytotoxic immune responses against the virus, yet these defenses still fail to control its devastating effects. It may be that the initial burst of HIV replication following initial infection gives the virus enough of a lead that the immune system is forever in the futile position of playing "catch-up."

The evidence for this viral "head start" is strong, and the goal of many vaccine efforts is to block this advantage. A live but weakened (or "attenuated") vaccine (i.e., one that replicates slowly enough for the immune system to establish a response and survives long enough to make that response



lasting) has been the most successful vaccine tested in animals to date. This approach to vaccine design has worked in humans before, the best example being the Sabin oral poliovirus vaccine. The immune response triggered by such a vaccine may not prevent infection, but may control the initial infection enough to allow the immune system to win the race.

Unfortunately, an attenuated HIV vaccine approach is far too dangerous to attempt in people, as even an attenuated virus can potentially regain its normal virulence. Thus, much research effort, including that of the Robert-Guroff lab, has gone into investigating the notion of altering other infectious viruses to elicit the right anti-HIV immune responses without the risks of attenuated HIV. Adenovirus as an HIV “pretender” (see “Adenovirus Reconstructed”) is a particularly attractive basis for an HIV vaccine. It can readily be engineered to express HIV-specific proteins that elicit an immune response from the host against native HIV, without the use of

actual live HIV. Also, adenovirus does not insert itself into the host’s DNA, as HIV does, but is content to pursue its lifecycle without disrupting the host’s normal genes or genome functions.

Perhaps one of the most attractive features of an adenovirus-based vaccine is the virus’ ability to infect many different cell types without requiring that the cells be dividing. These types include epithelial cells, such as those that line the upper respiratory tract and gut and which can engender the mucosal immune responses necessary to fight back against sexually transmitted infections such as HIV. This characteristic opens the way to oral or inhaled delivery of the primary vaccine—a critical element to

consider when developing a vaccine that would need to be distributed in remote parts of the world where HIV is endemic.

Finally, adenovirus has been substantially studied for therapeutic purposes in other settings, including its uses as a vaccine against adenovirus infection for millions of military personnel and as a gene therapy vector. For these reasons, among others, Robert-Guroff and her colleagues are focusing on an adenovirus-based HIV vaccine.

Although others have also used an adenovirus approach, they have concentrated on “replication-incompetent” adenoviral vectors (adenovirus that cannot replicate and is thus eliminated fairly quickly from the host after its initial infection). The problem with this approach is that it requires a high dose of the replication-incompetent adenovirus in order to elicit an immune response. Because this dose can cause a significant inflammatory response, there is an upper limit to the amount of replication-incompetent virus a person can be given.

Thus, the researchers in the Robert-Guroff lab have focused on replication-competent adenovirus, or adenovirus that is able to replicate in the host. Replication-competent adenoviruses provide two significant advantages. First, vaccine effectiveness is improved because the virus is “real,” drawing on all of the elements of immunity necessary to fight infection. This method gives the immune system greater exposure to the HIV elements the virus is carrying and results in more robust immune responses. The scientists in Robert-Guroff’s group demonstrated this enhanced effectiveness convincingly in studies that compared replicating and nonreplicating adenovirus-HIV vaccines in nonhuman primates (the results were published in the August, 2005, issue of the

No other vaccine approach—except for live attenuated HIV or SIV virus—has resulted in this level of protection to date.

Journal of Virology), showing that replicating adenovirus-based vaccines resulted in significantly more potent antibody and cytotoxic immune responses.

The second, considerable advantage of replicating adenovirus is that one can administer a much lower dose of the vaccine to get sufficiently robust immune responses. This fact will have a significant positive impact on both manufacturing and cost issues.

In addition to administering the adenovirus-based HIV vaccine, the researchers have found that a traditional “boost” element a few weeks later of only the HIV proteins encoded by the genes engineered into the original adenovirus vaccine is necessary for strong antibody responses.

This “prime-boost” strategy is a common one in many vaccine protocols, although it is not ideal, given that the need for a follow-up booster shot decreases the simplicity of providing effective vaccination.

From Non-Human Primates to People

The real proof-of-principle for the replicating adenovirus vaccine approach to controlling HIV infection is described in papers published in the March, 2004, issue of the *Journal of Virology* and in the September, 2006, issue of *Virology*, both from Robert-Guroff’s group. In the first paper, the team outlines a series of studies in which rhesus macaque monkeys were primed either orally or by inhalation

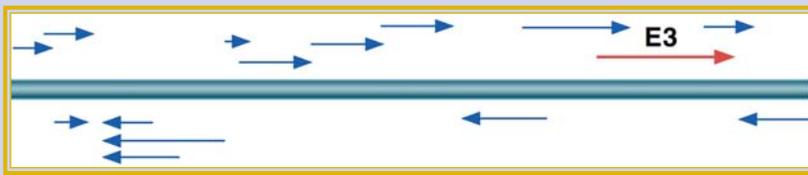
with an adenovirus vaccine containing the simian immunodeficiency virus (SIV, the monkey equivalent of HIV) genes encoding SIV core and coat proteins, followed by a booster shot of the actual proteins themselves. The vaccinated monkeys were subsequently challenged with a high dose of a particularly virulent form of SIV. The results were striking: all of the monkeys showed some degree of protection, with about 40 percent of them showing exceptionally strong protection, including a complete lack of viremia (virus in the blood stream) or levels of SIV viremia at the lower edges of detection limits.

The 2006 *Virology* paper describes further the research where 11 of the same vaccinated monkeys were challenged again intrarectally with a high dose of the same pathogenic SIV. All of the vaccinated monkeys again showed some degree of protection, and eight of the 11 were still strongly protected, demonstrating that not only was this vaccine approach effective soon after vaccination, but it also evoked a durable immune response. No other vaccine approach—except for live attenuated HIV or SIV virus—has resulted in this level of protection to date.

There are significant challenges still to be faced with moving this approach into human trials. Robert-Guroff and her colleagues are currently addressing a host of “traditional” drug development challenges—such as choosing the right adenovirus and HIV type (they are using “clade C,” the most common strain of HIV worldwide, particularly prevalent in Asia and southern Africa); determining which HIV proteins will elicit the best response; making the adenovirus in sufficient quantity and purity according to strict regulatory guidelines; and preparing it properly for oral, inhaled, or injected administration; toxicology testing, etc. They believe it will take a year to make the vaccine properly and another year to thoroughly test for toxicity.

“My goal is to get this moving along with strong enough data to interest a company in developing it further,” said Robert-Guroff, envisioning the need for extramural partnerships in the world of vaccine development. “First it needs to be tested in humans.”

Adenovirus Reconstructed



(Graphic: Feinstein Kean Healthcare)

Adenoviruses are found in a wide range of animals and derive their name from human adenoid tissues, from which they were first isolated and described in 1953. The different strains can cause significant diseases, yet only about a third of the 50 or so known human strains, or serotypes, are known to be disease-related. The majority of adenovirus infections do not require clinical attention.

The combination of its ability to infect non-dividing cells and the opportunity to substitute non-adenovirus genes in its DNA genome for carrying into host cells has made adenovirus an attractive vector for gene therapy research. The size of the gene that can be inserted is limited, due to the rigid structure of the adenovirus vector and the requirement for most of its own genes to remain intact. Inserts of up to ten kilobases of DNA have been successfully transferred, though so-called “gutless” adenovirus can accommodate about 35 kilobases. (However, they require a “helper” virus to infect host cells.)

Gene therapy uses have focused on the delivery of large viral doses in order to optimize expression of the therapeutic transgene. However, adenovirus can cause

Representation of the adenovirus genome. Blue arrows represent transcribed adenoviral genes. The red arrow represents the adenoviral E3 genes, which can be removed and replaced with HIV-related genes without interfering with the ability of the adenovirus to replicate in its host cells.

significant inflammatory responses at higher doses, limiting its utility and safety in most gene therapy approaches. This makes adenovirus ideally suited for vaccine use, where obtaining an immune response is the goal. In particular, replication-competent adenovirus (adenovirus that has had a transgene added that does not disrupt its ability to replicate after infection) can provoke sufficient immune responses even at relatively low doses. In addition, the adenovirus predilection for epithelial cells, such as those that line the gut and respiratory passages, allows it to be administered orally or intranasally, routes that promote the mucosal immune responses needed to prevent HIV infection.

Vaccine Hunters

The laboratories of CCR's Immune Biology of Retroviral Infection Section are tucked away in one of the smaller buildings on the edge of the NIH campus in Bethesda, Md. In those laboratories are 11 researchers all working together to move the adenovirus-based HIV vaccine from the laboratory into the clinic. Several of the scientists are profiled briefly here.



(Photo: R. Baer)

Marjorie Robert-Guroff, Ph.D.

The pill she is holding is a prototype version of the adenovirus-based HIV vaccine.



(Photo: R. Baer)

Jean Patterson, Ph.D. and Ruth Florese, Ph.D.



(Photo: R. Baer)

Thorsten Demberg, Ph.D.

Marjorie Robert-Guroff, Ph.D., Chief

Marjorie Robert-Guroff joined Robert Gallo's CCR lab in the early '70s, prior to the epidemic appearance of HIV. At that time, the Gallo lab was looking for retroviruses associated with cancer. She joined the lab as an enzymologist and was charged with looking for the telltale biochemical "footprint" of reverse transcriptase, an enzyme specific to retroviruses. Using the techniques of enzymology was difficult at best, as other DNA polymerases had similar properties. Thus, she and her colleagues set out to make specific antibodies against the different enzymes in order to more easily identify and characterize them in tumor tissue. "An enzymologist basically became an immunologist by necessity," said Robert-Guroff, a transition which led naturally to her current interest in retrovirus biology and immunology, virus-cell interactions, and AIDS vaccine work. In 1989, she began a collaboration with Wyeth on adenovirus vaccine protocols which continues to today.

Robert-Guroff received her Ph.D. degree from Georgetown University, and she was a Postdoctoral Fellow of the Leukemia Society of America at the NCI and of the Friedrich Miescher-Institut, Basel, Switzerland.

Jean Patterson, Ph.D., Staff Scientist

Jean Patterson joined Robert-Guroff in 1996 as a Postdoctoral Fellow, and she stayed on to become a full research associate in 2000 and then a staff scientist in 2004. "This work has exciting potential to make a difference," she said, crediting the steadfast determination of Robert-Guroff to pursue this approach even as others were focusing elsewhere. Her doctoral work in microbiology and immunology, at Ohio State University, prepared her for the science, but she now focuses on translating the science into clinical research and practice.

"It has been incredibly interesting to see how the vaccine pipeline works," Patterson said. "I have been involved in the process since its beginning, and I want to see where it will go." She is working on making the recombinant adenoviral vector that will be used in the clinical trial, as well as the background work to identify appropriate cell lines to produce the virus. "Vaccine work integrates so many diverse fields," she said.

In addition to her laboratory work, Patterson plays a major role in the teaching and training of new scientists in the laboratory.

Ruth H. Florese, Ph.D., Visiting Fellow

Ruth Florese joined the Robert-Guroff lab almost three years ago, coming from the University of the Philippines, Manila, where she was teaching microbiology to advanced students. "I'm here trying to learn as much as I can," she said. "I believe that doing cutting-edge bench work will make me a better teacher, because I can talk from experience," she said. "The NIH provides an unequalled environment to do this kind of work."

Although Florese is involved in the vaccine work of the lab, her primary focus is on identifying the immune responses that are important for controlling HIV infection. In particular, she is studying whether an antibody-mediated form of immunity called "antibody-dependent cellular cytotoxicity," which does not prevent HIV from entering the cells, may nevertheless play a critical role in limiting HIV's spread in the body or slowing the progression to AIDS.

Florese received her doctoral degree from Kobe University in Japan in 2002.

Thorsten Demberg, Ph.D., Visiting Fellow

Thorsten Demberg joined the laboratory two and a half years ago after completing his doctoral work at the Georg-August-Universität of Göttingen in Lower Saxony, Germany.

Demberg's work in the lab touches on several projects, including a recent study published in the *Journal of Virology* demonstrating the efficacy of a combination of two different adenovirus-HIV constructs (one with HIV-env and one with HIV-tat), followed by boosts with the related proteins, in rhesus macaques. However, his main expertise is in technical wizardry, developing and perfecting the assays needed to understand immune responses to vaccine regimens as well as the virus itself.

Demberg is also excited about a state-of-the-art fluorescence-activated cell-sorting (FACS) facility soon to be installed at the lab. "One of the major differences at CCR, compared to other places, is the resources they provide to help you do the science quickly and completely," he said. "This is a great work environment."

The Division of AIDS of the National Institute of Allergy and Infectious Diseases is contributing funds and collaborating with CCR to produce and test the clinical-grade adenovirus-based HIV vaccine in an NIH clinical trial.

A War on Kidney Cancer

A decade after President Richard Nixon's 1971 declaration of war on cancer, urological surgeon W. Marston Linehan, M.D., at CCR, declared his own battle against kidney cancer. The disease afflicts nearly 40,000 adults in the U.S. annually, a third of whom will die from the illness or its complications. Then, as now, the incidence of kidney and similar renal pelvic cancers was rising by 2 percent each year.

Linehan saw the devastation firsthand while caring for patients at Duke University Medical Center. Thus, by the time he arrived at NCI in 1982, he was already compelled to take action.

"I looked at these people with kidney cancer, dying even after we had tried 300 different kinds of chemotherapy agents," said Linehan, who is now Chief of CCR's Urologic Oncology Branch (UOB). "We had to come up with a better approach."

And so began the 25-year story of a group of CCR investigators who dedicated their careers to doing just that.

All Kidney Cancers Are Not Equal

All kidney tumors are not created equal. Clinicians only began to understand this concept when genetic analysis was applied to kidney cancer. This new knowledge gave scientists a map with which to track the genetic roots for human disease. Whereas oncologists used to classify cancers by the tissue within which they first emerged, the emphasis on genes revealed the relative naiveté of this organ-based scheme.

Tumors arise from once-normal cells that begin dividing uncontrollably because of alterations in their genes. Many genes play a role in normal cell division. For example, certain genes in a skin

follicle cell prompt cell division in order for a person to grow hair. But that same follicle cell has a restraining system to stop cell division when inappropriate. (This is why eyelashes stop growing at a certain length, for example.)

Every cell has many genes at its disposal, each capable of prompting division or restraining it. Mutations in any single gene or set of them can cause cells to go renegade. And because different genes might become mutated in different, even adjacent,

All kidney tumors are not created equal.

cells, different tumors within the same tissue, and even different cells within the same tumor, can be genetically different from one another. Therefore, treatments that target one person's kidney cancer may not affect that of another.

Unraveling Kidney Cancer Gene-by-Gene

Early on, Linehan's group did not know the extent of this genetic diversity. Thus, they treated all kidney cancer as similar. But, after partnering with Berton Zbar, M.D., Chief of

the Laboratory of Immunobiology, in 1983, Linehan realized that the genetic knowledge could now be transformed into a groundbreaking new weapon against cancer.

"We had hoped that if we understood the genes that when mutated cause these cancers," Linehan said, "we might understand how they provide the foundation for the development of targeted therapies."

And the CCR researchers developed a new logic: if one wants to find the genes that cause kidney cancers, one must look for cancers that seem to be genetically inherited. Although these malignancies may occur rarely, one can extrapolate the information gleaned from the rare cases to all patients with more common forms of kidney cancer.

Embracing that strategy, Linehan's team began looking at patients with an illness called von Hippel-Lindau syndrome (VHL). It predisposes individuals to nearly 600 types of tumors in multiple organs, including a form of kidney cancer called clear cell renal cell carcinoma (CCRCC). This form of cancer accounts for 80 percent of people with renal cell carcinomas, which in turn represents more than 90 percent of all malignant kidney tumors. The hope was that any breakthrough in von Hippel-Lindau would apply to CCRCC, which is not inherited.

After nearly ten years of performing genetic analyses, the researchers in 1993

W. Marston Linehan, M.D., Chief of CCR's Urologic Oncology Branch, has spent 25 years crafting better approaches to treating kidney cancers.

Unfortunately, the CCR team soon learned that the faulty *VHL* genes, while clearly the cause of von Hippel-Lindau and most cases of CCRCC, are not the instigators of all types of kidney cancer.

The researchers needed to broaden their focus.

More Cancers, More Genes

Individuals with hereditary papillary renal cell carcinoma (HPRCC) are plagued with multiple tumors in both kidneys. Papillary kidney cancer represents 5 percent to 10 percent of all cases of renal carcinoma but, more importantly, can run in families. Thus, the Linehan team again conducted a thorough genetic analysis, this time in families affected by HPRCC.

In 1997, after seven years of research with Zbar and Urologic Oncology staff scientist Laura Schmidt, Ph.D., the culprit emerged: the *c-Met* oncogene. This gene does the opposite of *VHL*—it triggers cells to divide.

The discovery prompted a thorough biochemical search for the molecules that *c-Met* and its protein product interact with in order to accomplish its pro-cell division function. Today, investigators are now engaged in “a very intense effort” to develop agents that block the oncogene, said Linehan. Clinicians and scientists are searching for an agent like imatinib mesylate (Gleevec®)—a Novartis drug first developed as a treatment for one form of leukemia and later soft tissue cancer—characterized by



(Photo: R. Baer)

finally narrowed their search to one area of chromosome 3. Later, the team pinpointed within that region a mutant gene, called *VHL*, which normally functions as a tumor suppressor, a molecular brake on cell division.

VHL was the sixth gene discovered with links to human cancer, but the first associated specifically with kidney cancer. When *VHL* is mutated, it cannot do its normal job of holding cellular reproduction in check, including in the cells that line the tubules within the kidney (from which carcinomas arise). This finding allowed the CCR researchers, and many others working around the world, to work out the biochemical details of how the *VHL* gene encodes a protein that forms a complex that keeps normal cells from dividing.

The finding also held clinical benefit. Drug developers later targeted the *VHL* pathway and came up with agents such as

sunitinib maleate (Sutent®; Pfizer) that appear to work in keeping tumor growth at bay. Linehan's own laboratory recently launched a Phase II clinical trial of an anti-biotic derivative called 17-AAG (pioneered by Leonard Neckers, Ph.D., Senior Investigator in the UOB). It is hoped that this type of therapy will advance the treatment of kidney tumors in patients with *VHL* and, in the future, help others such as Jeanne McCoy and Alice Coday (see “Patients: Part of the Team”) who, thanks to early detection and treatment, are currently cancer-free, despite having the associated *VHL* mutation.

VHL was the sixth gene discovered with links to human cancer, but the first associated specifically with kidney cancer.

mutation in the gene *c-Kit*—that can target the *c-Met* gene pathway. An agent like this should have therapeutic benefit for individuals with papillary kidney cancer as well as HPRCC.

One More Time

Individuals with Birt-Hogg-Dubé syndrome (BHD) have tumors in many tissues, including benign skin bumps arising from the hair follicles. While that manifestation might seem relatively trivial, 35 percent of these individuals also develop kidney cancer. And since BHD runs in families, it provided an opportunity for researchers at CCR to apply their strategy of gene discovery yet a third time.

In 2002, after seven years of scrutiny, Linehan, Zbar, Schmidt, and colleagues discovered what they called the *BHD* gene. It makes a protein that the team dubbed folliculin, a reference back to the benign hair follicle tumors.

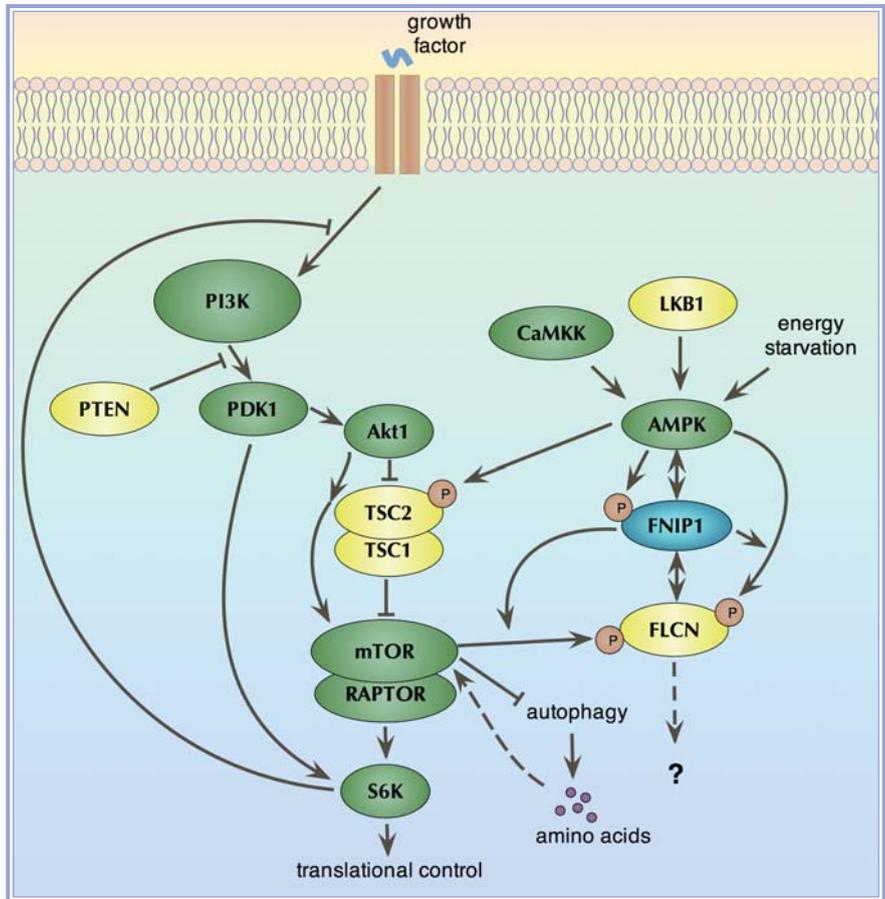
The gene was novel, with no known function. Linehan’s team set out to discover that function, hoping it would lead to more clues and ultimately therapies for BHD syndrome and the cancers linked to it. Using a mix of state-of-the-art genomic technologies and traditional biochemistry, the CCR team has unraveled the folliculin pathway and, with that knowledge, identified potential drugs (see “The BHD Mystery Solved”).

Collaboration

And still the kidney cancer story continues. The fourth chapter, and probably not the last, involves collaboration, a trademark of the environment at CCR.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a hamartoma syndrome, a condition in which affected individuals readily develop skin bumps called myomas. In women, these can also appear in the uterus as fibroids. About 20 percent of HLRCC patients develop a very aggressive type of kidney cancer.

In 2002, Ian P. Tomlinson, Ph.D., and colleagues at Cancer Research UK in



(Graphic: Feinstein Kean Healthcare)

Possible placement of FNIPI in the AMPK and mTOR growth signal transduction pathways. Proteins indicated in yellow are those that are known to be defective in hamartoma syndromes. The dotted lines indicate functional interactions that are not yet clarified. FNIPI (blue) is regulated by AMPK through phosphorylation (P). FNIPI is believed to facilitate folliculin (FLCN) phosphorylation by mTOR and AMPK signaling. The target(s) of FLCN remains unknown.

London showed that HLRCC is caused by mutations in an enzyme called fumarate hydratase, which normally acts in a fundamental metabolic pathway called the Krebs cycle. Although Tomlinson’s group knew that the enzyme could function as a tumor suppressor, they did not know how.

Linehan, working with senior investigator Maria Merino, M.D., of CCR’s Laboratory of Pathology, showed that the tumors taken from patients with HLRCC produce a very high level of a protein called hypoxia inducible factor (HIF). The protein normally senses low oxygen in tissues and, in response, spurs new blood vessel growth, an increase in glucose transport, and the secretion of growth factors. Linehan surmised that, in tumors, excess HIF might pro-

mote growth and sustenance, making the molecule or its activity a good target for an anti-cancer drug.

Still, Linehan did not have an answer to the question: what does a mutation in a Krebs cycle enzyme have to do with an upsurge in HIF? In a late-night conversation in the hallway outside his office, Linehan turned to Neckers. With the help of a chemistry fellow in his lab, Neckers offered the idea that excess fumarate might compete with the co-enzyme of another molecule, prolyl hydroxylase, which is critical for binding and regulating HIF. This was an “ah-ha” moment for Linehan, because his team had already implicated the VHL/HIF pathway in CCRCC. Since one of VHL’s functions is to

(continued on page 24)

A Team with a Patient Focus

Although Linehan's research team in the Urologic Oncology Branch is diverse, they share two common motivations: patients and a desire for performing in-depth basic research. At CCR, while people with kidney cancer seek help at the NIH Clinical Center, researchers are cloning, mapping, and probing DNA two floors below. This close juxtaposition of clinical and basic research is rare and exactly the reason that lab members have traveled to work here from around the world.

Donald P. Bottaro, Ph.D., Senior Scientist

Before joining the Linehan lab, cell biologist Don Bottaro's world fit on a microscopic slide. He spent two fruitful decades at NCI's Laboratory of Cellular and Molecular Biology, studying how molecules such as hepatic growth factor and a gene called *MET* signaled to each other in cells. Then, in 1999, he felt compelled to expand his borders.

"I wanted to use what we do in basic science to help patients with cancer," Bottaro said.

Thus, he left NCI for a two-year stint at a biotech company called Entremed, dedicated to discovering anti-cancer drugs. Bottaro then returned to join Linehan's multitalented, clinical and research-focused team. For three years, Bottaro attended surgeries and grand rounds each week, in addition to working long hours in the lab.

His heroic efforts to bridge "the very big gap" between basic research and clinical treatment is paying off. He has used the tumors from the patients seen upstairs to decipher how the *VHL* gene might cause the renegade invasiveness of cancer cells.

Masaya Baba, M.D., Ph.D., Postdoctoral Fellow

On his first day as a urologic surgeon in Japan, Masaya Baba developed a passion for kidney cancer. He examined a mother of three who had the disease, and who died within five years of diagnosis.

"That motivated me," Baba said. "I saw that many patients survive through surgery. But I thought that more research was necessary."

Thus, after establishing a clinical practice and later studying von Hippel-Lindau syndrome during graduate school in Japan, Baba wanted to push the frontier of cancer treatment still further. He wanted to work with a group that had molecular biology and genetic technology expertise, as well as access to many patients with rare, inherited forms of kidney cancers.

Enter the Linehan team, which Baba joined in 2003. Already he has uncovered the molecular underpinnings of folliculin.

Sunil Sudarshan, M.D., Clinical Fellow

Synergy drew Sunil Sudarshan to the Linehan lab in 2005. From the first day he studied biochemistry as an undergraduate to his residency in urology at the Medical University of South Carolina in Charleston, Sudarshan wanted to work both with patients and with their genes.

Sudarshan walked through the doors of the Linehan lab just as the researchers, in collaboration with Len Neckers, had made a breakthrough connection between the rare hereditary leiomyomatosis and renal cell cancer (HLRCC) and fumarate hydratase, which normally functions in the Krebs cycle.

Immediately, Sudarshan jumped on board. He now applies this molecular knowledge directly in patients with HLRCC, seeing them in the clinic as he studies their DNA in the laboratory.

"That is what CCR is set up for," Sudarshan said. "It is rare that a researcher gets to do both clinical and basic research on essentially the same thing."



(Photo: R. Baer)

Donald Bottaro, Ph.D.



(Photo: R. Baer)

Masaya Baba, M.D., Ph.D.



(Photo: R. Baer)

Sunil Sudarshan, M.D.

“After working on the complexities of biochemical mechanisms, it really brings us back to earth to see these patients.”

degrade HIF after oxygen concentrations reach adequate levels, the idea provided a possible connection between the two seemingly disparate forms of cancer through one biochemical pathway.

The next step was to prove the connection, which Linehan and Neckers both did. The researchers published the connection between fumarate hydratase and VHL in the August, 2005, issue of *Cancer Cell*. With the assistance of urologic surgeon Sunil Sudarshan, M.D., (see “A Team with a Patient Focus”) these investigators are working to

turn these findings into a targeted therapeutic approach for patients with HLRCC-associated kidney cancer. For example, Linehan is planning a trial to evaluate whether or not targeting HIF will have an effect on HLRCC as well. A drug called Avastin® (bevacizumab), which can inhibit new blood vessel growth, might reverse some of the damage caused by excess HIF.

The CCR researchers are in a perfect position to investigate the promise of Avastin and other therapies on patients because of the close association of lab

and clinic in CCR—the laboratories and clinic are housed two floors apart in the NIH Clinical Center. Researchers join clinicians on the Urologic Oncology Branch’s grand rounds, while clinicians work as fellows in the labs. It is a unique collaborative environment destined to advance the innovative basic research that translates to better therapies.

“After working on the complexities of biochemical mechanisms, it really brings us to back to earth to see these patients,” Linehan said. “We never lose that focus.”

The BHD Mystery Solved?

Individuals with Birt-Hogg-Dubé syndrome (BHD) have a mysterious condition, characterized by tumors in follicle cells, as well as those of the kidney, in some cases. To unravel the mystery, W. Marston Linehan, M.D., Laura Schmidt, Ph.D., and colleagues employed cutting-edge genetic mapping and linkage studies, thereby discovering the previously unknown gene folliculin.

But in order to find out how that genetic defect can lead to cancer, the CCR team had to map folliculin’s molecular interactions. The investigators used a technique called co-immunoprecipitation, in which one protein linked to a bead or antibody is used to fish out another from a mix of cellular contents. With that, the CCR team pulled out folliculin’s partner, a protein that functions in an energy/nutrition sensing pathway in the cell.

What Does Energy Regulation Have to Do with Follicle Tumors?

It turns out that folliculin-interacting protein-1, (FNIP1), as the CCR team named it, binds to

folliculin and so puts the brakes on cell division. Thus, this protein pair functions in a tumor suppressing pathway that appears to be controlled by the loss or gain of a phosphate group. And that group associates with an enzyme called 5'-AMP-activated protein kinase (AMP-K, a key energy sensing molecule within the cell).

Because the discovery of the folliculin-FNIP1-AMP-K relationship is so new—it was just published in 2006—Linehan, Schmidt, and colleagues can only speculate on how problems with an energy sensing pathway might be linked to renegade cell division. It could be that an energy deficit or stress triggers AMP-K, which works through another pathway involving a protein called mTOR. The kinase, when activated, appears to add a phosphate group to both FNIP1 and folliculin and so could potentially trigger a braking action within cells. This system likely evolved as a mechanism to keep cells from reproducing in times of energy scarcity.

Cells should release this brake when energy is plentiful, for example letting follicle cells divide and hair grow. But individuals with BHD make faulty folliculin proteins and so may lack this particular braking system. Thus, the follicle cells divide regardless of whether energy levels are high or low, fueling the benign skin bumps. These same mechanisms may allow the kidney cells in BHD patients to grow, although with more malignant consequences.

While further delving into how that putative pathway might work, Linehan’s team has already begun searching for clinical agents that block this pathway. Promising leads involve other drugs known to target the mTOR pathway. Linehan says investigators are studying this lead “very aggressively,” but cautions that the studies are preliminary.

Patients: Part of the Team

It ran in the family. Jeanne McCoy's grandmother and mother both suffered from von Hippel-Lindau syndrome (VHL), which manifests as a combination of one or more of nearly 600 types of tumors in multiple organs.

For McCoy's grandmother, the tumors appeared first on her retina (she went blind at the age of 30) and also on her brain stem. Eventually the cancer invaded her kidneys. Meanwhile McCoy's mother, who was told at a young age that she did not have the same diagnosis, was shocked to learn that she had already progressed to late-stage kidney cancer. The double diagnosis—combined with her family history—led McCoy to search the Internet, learn of the then recently-identified *VHL* gene, and seek out the NIH and Linehan.

Because of the late stage of McCoy's mother's cancer, she was quickly accepted into the clinical program at the NIH. There, she tested positive for the *VHL* mutation. Several months later, McCoy sent in her own blood sample and records to the NIH—just in case. The results confirmed McCoy's suspicions: she too, carried the *VHL* mutation, as did her grandmother.

But McCoy had no symptoms of kidney cancer. In fact, at age 34, she had just given birth only a year previously to the youngest of her three children. Except for faint back pain, something all new mothers experience, McCoy felt fine.

But she also knew that the *VHL* mutation foretold her fate. And she already had a benign tumor on the endolymphatic sac of her inner ear—a common symptom of VHL syndrome. So McCoy consulted her local oncologist, who advised her to undergo an ultrasound and CT-scan in a hospital near her Greenville, South Carolina, home. Within hours, she learned that both her kidneys were riddled with cysts and tumors that, if untreated, could eventually kill her.

Panicked, she called the NIH. Within two weeks, she was headed for Bethesda for more testing and consultation. There, she embarked upon an odyssey of surgeries, first to remove the tumor in her ear and then those in her kidneys. It was during the second of her kidney surgeries, just before Christmas 2003, that

McCoy first met Linehan, who came to see her in the surgical intensive care unit at the NIH.

"He sat down and talked to me and my husband," she recalled. "And suddenly, he put a real personal face on the research; that it wasn't these scientists lost in a lab; that this was about people, and this was about early diagnosis and detection, and this was about finding a cure."

In that interaction, and all of the subsequent ones over the next four years, McCoy moved from what she calls "survival mode" toward a more altruistic focus. She is part of "something larger," research that might help others today, as well as in the future.

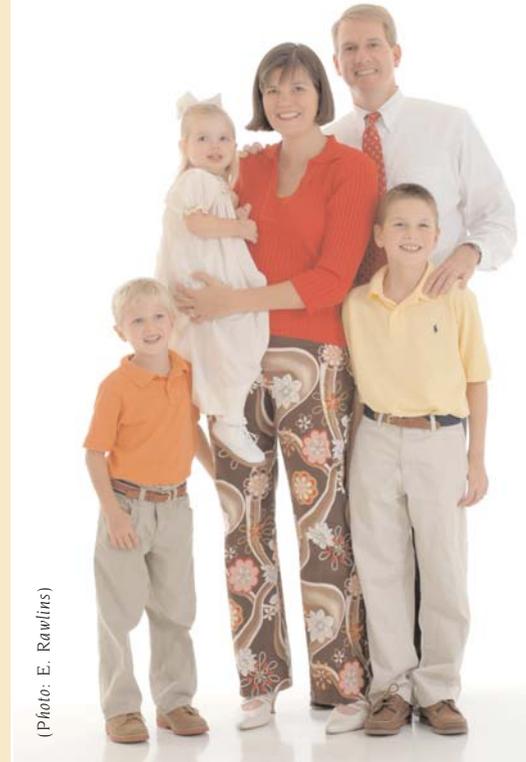
The same epiphany occurred for Alice Coday. The 52-year-old watched as physicians diagnosed her father, brother, and sister with VHL. All three had tumors on their brain stems. Coday did not have that kind of tumor, convincing her she had been spared the mutation.

But she had been diagnosed instead with Ménière's disease, characterized by tumors on the endolymphatic duct of the inner ear (just like McCoy). She suffered severe vertigo and, in 1988, lost hearing in her right ear. But she experienced nothing of the brain or kidney tumors that eventually plagued her family members.

Not until Coday had a happenstance conversation with a member of the Von Hippel-Lindau Family Alliance did she suspect a possible mistake in her diagnosis. She contacted the NIH and traveled from her home in Seattle for testing. Only then did she learn that she not only carried the *VHL* mutation but also had developed tumors in both of her kidneys and cysts in her pancreas.

In 1997, surgeons from CCR removed part of her left kidney and a year later, the right. She has been traveling to the NIH at least twice a year ever since.

She knows that if she had had the surgeries locally, she would have lost both of her kidneys entirely and probably would have needed dialysis. She knows that she could have died if the tumors had remained undetected. But she does not come to the NIH today for either of these reasons. Coday is cancer-free thanks to the discovery of the *VHL*



(Photo: E. Rawlins)

Patients like Jeanne McCoy (above with son Ford, daughter Mary Ellison, husband Ellison, and son Brooks) and Alice Coday (below, with her dog Clancy) are also Linehan's partners in kidney cancer research.



(Photo: A. Davison)

gene and the efforts of the Linehan group and others to foster early detection. She now comes to the NIH because she sees her larger purpose.

"When you go to a traditional doctor, you think of a doctor-patient relationship," she said. "The NIH is something more. You feel like you are participating. Like you are part of a team, making history."

A CCR in Belfast

The year I joined CCR—1987—more than 160,000 patients in the U.S. were diagnosed with colorectal cancer. Oncologists had only one drug to treat the disease, 5-fluorouracil (5-FU). And while that drug did show some effectiveness, particularly in cancer that was localized to the bowel, still nearly 60,000 patients either would not respond or would eventually develop resistance to the drug. Within five years of diagnosis, 60 to 65 percent would die. I found these statistics unacceptable.

Therefore, my first goal at CCR was to discover a way to predict patient response to 5-FU-based therapy and hopefully spare many patients the side effects of a treatment that would otherwise offer no benefit. I developed the first monoclonal antibodies to the enzyme human thymidylate synthase (TS), and a number of immunological and tissue-based assays for quantifying the enzyme. With my colleagues, Carmen Allegra, M.D., and Bruce Chabner, M.D., I was able to show, in the laboratory, that levels of TS could predict drug response: low levels of the enzyme forecast a good drug response, while high levels suggested a poor response. We went on to show that our TS findings translated to actual patients with metastatic colorectal cancer, whose disease was spreading outside the bowel.

When we used the same assay in patients with earlier stage colorectal cancer (stage II and III non-metastatic colorectal cancer), however, we found something surprising. The TS levels of early-stage patients failed to predict response, but could predict whether a patient's tumor would behave more benignly or aggressively after treatment. High TS levels correlated with a shorter time to relapse and shorter survival, and low levels a longer time for both parameters.

While heartening, this finding also posed a significant problem. We had learned that simply because an individual with a given diagnosis had a poor prognosis, he or she would not necessarily have the best response to a particular chemotherapeutic agent. Therefore, simply administering the most aggressive treatments to the patients with the worst potential outcomes leaves them open to the disturbing possibility of excessive—and unnecessary—pain and suffering. We would

do better to match prognosis, therapy, and response, delivering only those treatments that are likely to provide benefit.

This dilemma remains one of the major challenges for modern oncology today. With the introduction of high-throughput technologies, we are now just beginning to meet this challenge, thanks to the growing arsenal of genomic tools with the potential to refine both diagnosis and the prescription of care.

Lessons from CCR

The word “cancer” essentially means that important molecules in tumor cells have become abnormal, due either to mutated genes inherited at birth or alterations caused later in life because of environmental or lifestyle factors. At the same time that I was pondering questions of tumor behavior and response at CCR, the Human Genome Project (HGP) was fast uncovering genes that might be relevant to cancer. Completed in 2003, the HGP gave a more sophisticated answer to a long standing question in oncology: Why do all patients with the same diagnosis not respond to a specific treatment in exactly the same way? The HGP offered multiple genetic reasons for this disparity, and also underlined the naïveté of our classification schemes for cancer (naming and treating tumors based on the organ in which they first appear). For example, a single tumor in the colon can be linked to a large number of mutations in a host of different genes. Further, these mutations occur in combinations that may differ between neighboring cells, even those within that same tumor. Thus, colon cancer is not really cancer of the colon; rather, it is a combination and accumulation of genetic mistakes that happen to occur within colonic epithelial cells.

We would do better to match prognosis, therapy, and response, delivering only those treatments that are likely to provide benefit.

I understood that in order to accurately predict prognosis with certainty, we needed to examine both a tumor's genome and anatomic location. In the same vein, individual responses to chemotherapy agents also vary between patients, even those with the same diagnosis, due to the unique nature of each individual's genetic make-up, apart from that of their tumor. In other words, an individual's normal genes can also help forecast drug response.

Moving Forward—and Overseas

I carried this thinking back to Belfast in 1997, at a time when researchers had just invented gene expression microarrays. Using this tool, we could measure gene expression in colorectal tumors and compare it to healthy tissue. Instead of one gene, such as *TS*, we now had hundreds, perhaps thousands, of genes that together formed a genetic “signature” that might herald prognosis or response.

In Ireland, we and other investigators went on to use microarrays and other genomic technologies to search for genetic signatures that marked good and poor prognosis in colorectal cancer. Thus far, we have successfully



The organization and structure of Johnston's Centre for Cancer Research and Cell Biology at Queen's University in Belfast, Northern Ireland, is modeled on CCR.

identified numerous genes involved in cell replication, tumor suppression, and the expression of specific molecules that mark a tumor as cancerous. We also developed the first disease-specific, transcriptome-based arrays, tools that can measure gene expression in tissues fixed in formalin and embedded in paraffin (earlier technologies could not work with tissues preserved in this way, though the lion's share of pathology samples archived around the globe are saved in this format). We are now working with U.S. and European clinical cooperative groups to develop clinical classifiers of disease prognosis and chemotherapeutic response in patients with colorectal cancer based on this unique technology.

Prognosis Versus Response: A Current Debate

But this same genomic evidence also has its limitations—namely, that the same signatures that tell us whether a tumor will behave aggressively or benignly do not necessarily tell us whether it will respond to chemotherapy. For instance, 40 percent of stage II and III colorectal cancer patients will suffer a relapse within five years. Of that group, only a third will derive any benefit from chemotherapy. As with the TS assays, molecularly determining whether a patient's tumor is more benign or aggressive does not necessarily translate to whether or not that tumor will respond to chemotherapy.

Therefore, genomic technology, for all its value, brings us back to the same debate: who will benefit? For a given diagnosis and

therapeutic standard, do we treat everyone the same and expose those who would not benefit to undue side effects, or do we treat no one and risk the chance that those who might benefit would die eventually for lack of treatment?

While physicians continue to discuss and debate the best course of clinical treatment for such patient groups, our future research directions are clear. We must use emerging technologies to measure and predict drug responses as well as disease outcomes. Toward that end, we at the Centre for Cancer Research and Cell Biology at Queen's University, Belfast, are now working toward identifying exactly those kinds of predictive markers. For example, we have already identified numerous genes (e.g., spermine/spermidine acetyl transferase, annexin II, thymosin-beta-10, chaperonin-10, *MAT-8*) in the laboratory whose expression in colorectal cancer cells is altered by 5-FU treatment and which may serve as biomarkers for drug resistance.

The work is indeed challenging. Today, in colorectal cancer, we have six chemotherapeutic agents at our disposal instead of one, which can be used in various combinations and doses. Therefore, we have many, many variables to study.

To investigate them with any meaningful statistical power, we need ever larger population sets in order to accrue ever larger numbers of tumor samples. To draw useful conclusions from the reams of data produced by genomic studies, we need to analyze the data using complex bioinformatic techniques. As

such, I am grateful to my collaborators at NCI and U.S. cooperative groups supported by NCI, who have given us access to their collections of tumor biopsies. Collaborations like these are also helping us access the significant computing power needed to analyze our biological results. These partnerships would not have been possible had I not spent nine years at CCR making contacts and garnering scientific collaborations.

A New Infrastructure

The good news is that our Centre for Cancer Research and Cell Biology in Belfast now has the facilities and resources to meet these challenges. Last year, we opened a new \$120 million clinical center—modeled on the U.S. CCR—and further plan to unveil a \$50 million research center in June of this year. My experience and training in the intramural program at CCR in Bethesda taught me the importance of clinicians becoming scientists and working hand in hand with basic researchers to devise clinical experiments that are meaningful to patient treatment and the understanding of disease.

The legacy of CCR is greater than the education of this medical oncologist/molecular pharmacologist. It provides an environment where one can be mentored and further develop one's medical, scientific, and analytical skills to make fundamental discoveries that ultimately will benefit patients across the globe. I have embraced the opportunity afforded me and tried to establish the same culture and environment here in Belfast. By working together to solve the challenge of cancer, I believe we can make important strides in our understanding of cancer and in advancing cancer treatment for the benefit of patients.

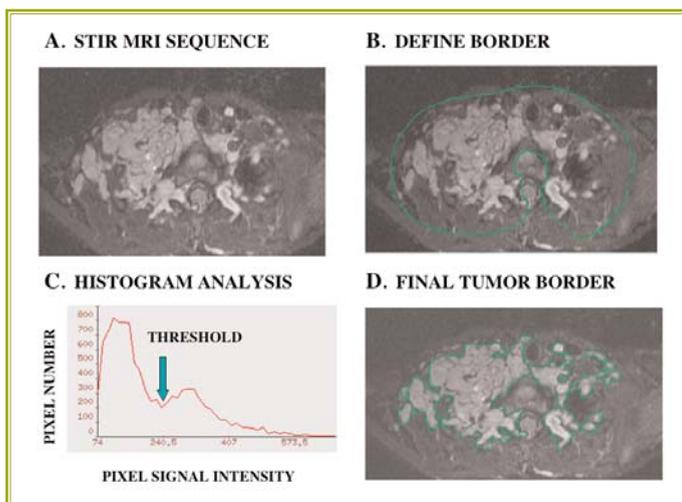


Patrick Johnston M.D., Ph.D.
Department of Oncology
Centre for Cancer Research and Cell Biology, Queen's University Belfast, Northern Ireland

(Photo: Centre for Cancer Research and Cell Biology)

CCR Team Tackles Neurofibromatosis

When Brigitte Widemann, M.D., and her colleagues Frank Balis, M.D., Elizabeth Fox, M.D., Eva Dombi, M.D., and research nurse Andy Gillespie in the Pharmacology and Experimental Therapeutics Section of CCR's Pediatric Oncology Branch treat their patients, they deal with more than their disease or the genetic mutations that cause it. The researchers study neurofibromatosis type 1 (NF1), a disorder that occurs in 1 out of every 3,500 individuals.



(Graphic: B. Widemann, CCR)

Figure 1: Medical Numerics' MEDx program helps doctors take accurate measurements of irregularly shaped and slow growing NF1 tumors.

does a physician deal with severe cosmetic disfigurement in a teen, who is trying to develop a healthy self-image? How does a pediatrician relieve the pain of a six-year-old child who has a tumor compressing her spinal cord?

Ultimately, we scale what we do know to the level of the children. We are honest, telling them that we currently do not have any medicines that will make the tumors go away, but if we can keep the tumors from growing while the children grow, then they will have fewer problems. Reaching that understanding with a patient is my goal and my colleagues' as well. We are working to learn more about the natural history of this disorder so that we can better test potential drugs that might reverse tumor growth, or at least halt it.

A Tumor Oddity

NF1 is a daunting disease to study, largely because of its unpredictable nature and multiple manifestations involving essentially every organ system. PN tumors follow the paths of nerves. They grow slowly, ten times slower than typical tumors of the breast or lung. Although that may be good news for patients (their life expectancy is only 15 years less than normal), slow-growing tumors mean years, even decades, of waiting just to observe tumor development—or the progression of NF1's natural history. Thus,

The genetic culprit behind neurofibromatosis type 1 (NF1) is a defect in a gene aptly named neurofibromin 1 (*NFI*). The product of this gene normally suppresses tumor growth (see "The Culprit: the *NFI* Gene"). About 25 percent of those afflicted develop tumors in the cells that make up nerve fibers. These tumors—called "plexiform neurofibromas" (PN)—are usually benign and slow-growing. Nonetheless, they can wreak great havoc.

NFI-associated PN tumors cause severe facial and body disfigurement, pain, and neurological impairment. In addition, individuals with NF1 develop coffee-colored "café-au-lait" spots all over their skin and distinctive freckles on their armpits and

groins. Some develop tumors on their optic nerves. Others sprout tiny nodules on their irises (the colored portion of the eye). The most serious complication—accounting for roughly 5 percent of the total—is the progression of PN toward aggressive malignancy (see "Turning Cancerous").

There is no effective therapy except for surgery—which is feasible in only a subset of patients.

Perhaps the greatest challenge we face is the young age of our patients: their median age is eight years. How does a doctor talk to a child about a disorder for which there is no cure, no working treatment, and a paucity of knowledge about tumor development and progression? How

(left to right) Brigitte Widemann, M.D., nurse Andy Gillespie, and Elizabeth Fox, M.D., work as a team to help children with NF1.

(Photo: R. Baer)

testing a potential therapy means we have to wait even longer before we can see any evidence of its effects.

There are additional hurdles as well. Because these tumors snake the length of the nerves, they take on strange shapes, like stretched-out, wadded-up chewing gum, rather than the perfect spheres or ellipses that characterize many other solid tumors. They can elongate from spinal cord to upper leg, for example. Therefore, they are often impossible to remove surgically.

NF1 tumors can also grow very large. Patients less than three years of age can appear in our clinic with masses in their legs that account for up to 20 percent of their body weight, and there are no drugs to date that can reverse the course of growth. In fact, until recently, we did not even have an accurate way to measure the growth of these tumors in order to track their development and progression. Oncologists who study other kinds of solid tumors can track their growth by measuring the change in the length of the longest segment or the diameter of the fattest portion of the tumor over

time. While this method works for fast growing, solid cancers, with a round or oval shape, it is not sensitive enough to pick up changes in an irregularly-shaped, slow-growing tumor like PN. We needed something better.

Virtual Measurement

Looking for a way to measure the ungainly tumors of NF1, we made use of an image analysis resource available to researchers at the NCI through a small company called Sensor Systems (now Medical Numerics, in

Germantown, Md.). In particular, we worked with a colleague there named Jeffrey Solomon, a physicist with a strong interest in applying physics and engineering to medicine. Solomon had developed software for “multimodality image processing,” a process for combining the best of different forms of imaging, including MRI scans and positron emission technology (PET) scans, which can help determine the size and shape of a tumor as it grows over time.

Rather than relying on diameter or length estimation, Solomon’s program, developed on a platform called MEDx, makes use of contrast and brightness. PN tissues tend to be brighter than their healthy neighbors on specific types of MRI scans. The MEDx program searches for high “intensity gradients,” quick drop-offs in brightness where the border of a tumor meets healthy tissue. The program then decides what is tumor and what is not, adds up all tissue deemed “tumor,” and compares it to the sum of that designated as “healthy.” In this way, Solomon’s program segments tumor from tissue and comes up with a

Perhaps the greatest challenge we face is the median age of our patients: eight years old.

number and a corresponding picture of the tumor ringed by an outline (Figure 1). The area of tumor is calculated for each MRI slice containing tumor, and then summed to calculate a final PN volume. While this method is labor intensive, it is reliable and reproducible, and it can detect volume changes as small as 10 percent, much smaller changes than what could be detected with standard solid tumor measurements.

Launching Clinical Trials

Having this powerful new imaging capability allowed us to launch our NF1 clinical trials program. In 2001, we began recruiting patients into a trial for an experimental drug called tipifarnib (Zarnestra™). Previously studied in patients with acute myeloid leukemia (AML) and other types of cancer, the drug blocks a molecular switch called Ras, which functions in a biochemical pathway that controls cell reproduction. Ras

normally activates neurofibromin (see “The Culprit: The *NF1* Gene”), making the drug a potentially good candidate for NF1 patients.

Enrollment in the trial requires demonstrating that the tumors are growing by about 20 percent a year, which allows us to track whether the drug is capable of slowing or stopping PN growth. This tracking is now possible with the improved imaging and analysis capabilities provided by our collaboration with Solomon and Medical Numerics.

Over the course of the last six years, the length of this first trial, we have learned that the younger the child, the faster the tumors tend to grow. We also learned that while PN tumor growth rates may differ among patients, they are fairly constant within patients, not erratic as was previously thought. This realization was important, and it raised the urgency for early detection and treatment intervention.

At the same time, this trial has made inroads with its unique design. Initially, we randomly divided 59 patients (the goal is 60, so enrollment is still open) at the start of the study into two groups, drug or placebo. After documenting PN tumor growth, the groups were switched. In addition to giving us critical information on the effects and effectiveness of tipifarnib, this study also gave us the opportunity to take a first systematic look at PN’s time to progression and tumor behavior; we had no knowledge about either characteristic before this trial.

We now know from the first treatment phase that the median time it takes for PN tumors to progress is 20 months. But more data will come. We have not seen any magic response to any drug, but we now have a good assessment of tumor growth and behavior that will be extremely beneficial for future work.

In addition to the tipifarnib trial, we

Beyond Skin Bumps

Plexiform neurofibromas have yet another cousin. Called NF1 dermal neurofibromas, these benign tumors appear as skin bumps that can cover the entire body. While not fatal, these tumors are horribly disfiguring. Imagine bumps the size of peas all over one’s face. They begin to develop just before adolescence, adding insult to injury, as youngsters grapple with normal self-esteem issues amid an outburst of what appears to be super-intense acne.

And, as with other NF disorders, researchers know very little about the natural history of the disease, except that hormonal changes, such as those of puberty, might exacerbate the illness.

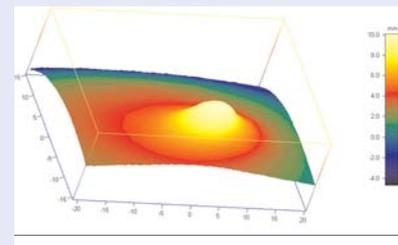
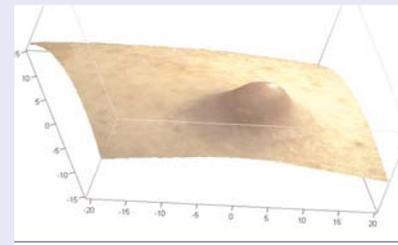
Enter a technique called “volume photography,” being applied by researchers at CCR. A camera takes a three-dimensional picture of a patient’s skin. Researchers like pediatrician Eva Dombi, M.D., then use a computer program to measure and tabulate the volume of each and every bump. In this way, investigators can measure and clock the progression of the disease. What do they do to help the young patient besides collect data on the size of the bumps?

At the same time, Brigitte Widemann,

M.D.’s team has also partnered up with medical geneticist Douglas Stewart, M.D., at the National Human Genome Research Institute. He is searching for more genetic culprits in the illness. Widemann and Stewart have won an NIH-sponsored bench-to-bedside award for their proposal to combine laboratory research on NF1 dermal neurofibromas with clinical treatment.

At the bench, Stewart is analyzing biopsies and blood samples from patients and their family members. Using DNA microarrays—tools that can measure the expression of hundreds of genes simultaneously—he is hunting for genetic differences between the two NF1 conditions that might flag individuals who will progress to more serious forms of the illness. The telltale genes could then be combined into a “signature,” which would go a long way toward helping researchers such as Widemann predict which individuals they should monitor most closely. And the genetic fingerprints might highlight promising targets for new NF1 drugs.

“We have so many resources at the NIH,” says Widemann. “If we can collaborate, we can make the most of every clinical trial.”



(Graphics: B. Widemann, CCR)

To understand the progression of dermal neurofibromas, the Widemann team takes 3D pictures of a patient’s bumps (top) and digitally measures their volume (above), a procedure termed “volume photography.”

Turning Cancerous

have five other trials ongoing as well, including a Phase II trial of an experimental-drug called pifrenidone (made by InterMune) in partnership with Roger Packer, M.D., from Children's National Medical Center in Washington, D.C., and Dusica Babovic-Vuksanovic, M.D., from the Mayo Clinic in Rochester, Minn. (Figure 2). All of these trials have multiple participating sites in order to ensure that enough participants enroll to complete the trials in a timely fashion. To help coordinate these myriad efforts, the Department of Defense has taken a lead role in formulating an infrastructure for NF1 clinical trials, including support of an NF1 consortium that includes CCR as a key site. For all of these trials, my CCR colleague, Eva Dombi, has coordinated and performed tumor volume analyses, ensuring that the measurements are consistent across trials and that large data sets are accumulated from each study—critical information for understanding the natural history of PN in NF1 as well as defining the benefits of new agents.

Although drugs such as tipifarnib and pifrenidone were developed to treat other diseases, including multiple myeloma and pulmonary fibrosis, they influence biochemical targets that relate to NF1, making them good potential candidates for PN tumors. Finally, we are also collaborating in three additional clinical trials of patients with other diseases in which the *NF1* gene also plays a role (see “Turning Cancerous” and “Beyond Skin Bumps”).

The Special Case of Children

As we design trial protocols, we never lose sight of the special considerations of our patients. Children, diagnosed with NF1 as young as 18 months, are still developing physically and emotionally. Thus, we have to be very careful about the toxicity of potential treatments, so as to not impact the children's normal growth.

For example, one of the new drugs we will soon be evaluating in PN patients, sorafenib (Nexavar®, Onyx Pharmaceuticals) blocks the growth of new blood vessels, those that could potentially feed a growing tumor. But the drug may potentially stunt

While the tumors that mark plexiform neurofibromatosis (PN) are benign and slow-growing, they can evolve into something more alarming. About 5 percent of patients with PN develop malignant peripheral nerve sheath tumors (MPNSTs), which, unlike their benign PN counterparts, are fast-growing, aggressive, and deadly.

While MPNSTs can arise in people without defects in the *NF1* gene, such tumors are very rare (0.001 percent of the general population). On the other hand, individuals with *NF1* mutations develop the deadly tumors at a lifetime rate of 8 percent to 13 percent. In addition,

those with the mutations may not respond as well to aggressive chemotherapy and, therefore, do not survive as long as those without the mutations.

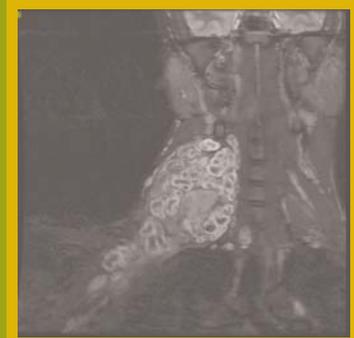
To make matters worse, the cancerous tumors are extremely tough to diagnose. They start as a tiny nub buried within an enormous and ungainly PN tumor. Thus, if a surgeon samples the “wrong” section of a PN tumor when taking a biopsy or image, he or she will miss the tumor-within-a-tumor entirely. And the cancer can spread so aggressively that if misdiagnosed, it will grow too quickly for even intense chemotherapy to stop its progression.

But the CCR team is working hard to change that outcome. They are testing a monitoring method in which they administer a radiolabeled compound called ¹⁸F fluorodeoxyglucose (FDG) to patients. Active cells in the body require glucose for energy. And so the most active cells, those in a fast-growing tumor, for example, metabolize the glucose the quickest. The radiolabel tag then lights up on a positron emission tomography (FDG-PET) scan. This method can now detect benign, but relatively rapidly growing tumors in PN patients. CCR researchers are now testing whether FDG-PET can pick out cancerous MPNSTs.

In addition, the CCR team is collaborating with the Sarcoma Alliance for Research through Collaboration (SARC) in Ann Arbor, Mich., to lead a pilot therapeutic study for MPNST patients. The study will test a combination of three chemotherapy agents (doxorubicin, ifosfamide, etoposide) that together are standard therapy for other cancers called soft tissue sarcomas—rare tumors that arise in connective tissues (e.g., muscle, fat, nerves, blood vessels, bone, cartilage). MPNSTs comprise about 10 percent of all soft tissue sarcomas, making SARC a logical partner for collaboration.

Upon entry to the new trial, patients will have baseline MRI and FDG-PET scans followed by four cycles of chemotherapy, as well as any needed surgery or radiation to relieve pain or decrease tumor size. Widemann and her team will then watch and wait.

Widemann says her team would equate success with a 40 percent rate of patient response (as evidenced by substantial tumor shrinkage). If they reach that number, her team will have a “platform study” with which to guide future trials. This designation means that in the future, clinicians can use the standard chemotherapy (as the platform) and add in more targeted agents, such as imatinib mesylate (Gleevec®, Novartis), a targeted treatment used to combat soft tissue sarcomas in the gastrointestinal tract.



A malignant MPNST tumor forms within benign PN growths.

(Photo: B. Widemann, CCR)

(Graphic: B. Widemann, CCR)

NF1 clinical trials			
Trial focus	Tumor	Phase	Coordinating site
Tipifarnib	PN	I, II	NCI
Pirfenidone	PN	I, II	NCI
PEG-Intron®	PN	I, II	Pittsburgh
Sorafenib	PN	I (opening soon)	NCI/ NF Consortium
Chemotherapy	MPNST	II	SARC
Basic biology and natural history	Dermal neurofibromas	n/a	NHGRI / NCI

Figure 2. Widemann’s team—and their collaborators—are conducting a host of clinical trials on different aspects of NF1.

skeletal formation. Thus, we had to build into our trial protocol regular monitoring of the so-called “growth plate,” the space where bone cells lay down new material to lengthen a child’s growing bones. In addition, consent, compliance, and managing expectations are issues for preteens and teenagers, who have lived years with this disorder without hope of a cure.

Because half of the cases involve a gene being passed down from parent to child, parents are often afflicted themselves. The disorder has become a lifestyle in these families, and this fact has implications, including deep feelings of guilt on the parents’ parts. For example, a parent with a milder form of NF1 might choose to bear children. On average, half of the couple’s children will get the faulty gene. If a child inherits that mutation and ends up with a more severe form of the disease, the parents may feel intensely guilty. Thus, our team includes well-trained research nurses, social workers, psychologists, and psychotherapists who can help parents deal with these and other emotions tied to their experience and their child’s. Those same counselors help children and rebellious teens to follow their regimens—taking pills, writing symptoms in a diary, and coming in for regular MRI scans.

Meanwhile, our clinic itself is focused on being “kid-friendly.” Children walk into a space of bright colors and crayoned pictures, typical of pediatric wards, and play together while waiting for their appointments. Two guinea pigs, named Chocolate and Buttercup, scurry about, ready for anyone to pet.

The CCR program caters to families of any socioeconomic background. Those who want to join in a trial need only pay the transportation costs for their first visit to the clinic. Subsequent visits are paid for if they are enrolled in a trial, as are stays at the nearby Children’s Inn. It is a home away from home for many, and a place to meet others with a similar disease. Indeed, many families who connect here start timing their visits to meet up with one another. Frequently, the first time a person with NF1 meets someone else with the same disorder is when they come to our clinic, giving them a new feeling that they are not alone and helping them deal with the complications of NF1. This kind of mutual support can do wonders, and it continues to give us hope that we will soon find effective new ways to stop this terrible disease.

The Culprit: The *NF1* Gene

Although Frederick von Recklinghausen first described neurofibromatosis 1 (NF1) in 1882, it was not until a century later that National Human Genome Research Institute director Francis S. Collins, M.D., Ph.D., (then at the University of Michigan) and the University of Utah’s Ray White, Ph.D., simultaneously but separately cloned the actual gene involved in the illness. The gene encodes a protein called neurofibromin that suppresses tumors.

Neurofibromin is expressed in most cells of the body, but those of the peripheral and central nervous system seem to produce the most. It normally works within a pathway involving a protein called Ras, a signaling molecule that controls a cell’s reproduction. When mutated, *NF1* genes cause misregulation in Ras signaling, potentially one cause of the tumors in NF1. Therefore, clinicians are now testing drugs in NF1 patients, compounds that act upon Ras-related targets, such as a molecule called mTOR.

Meanwhile, cancer researchers are also working hard to determine whether or not Ras regulation is the primary and/or only function of neurofibromin. If other molecules are involved, those, too, could become eventual drug targets for novel NF1 therapies.

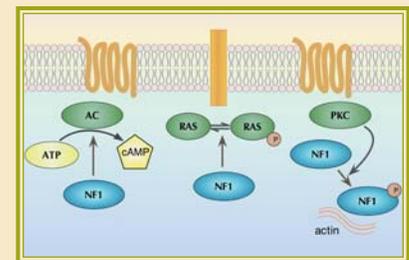


Illustration showing the multiple functions of NF1. Recent studies have demonstrated that NF1 is involved in several critical cellular pathways by acting directly on RAS, protein kinase C (PKC) and cyclic AMP (cAMP). By modulating these intracellular messengers, NF1 can affect several processes related to cellular growth and division. Recently, it has also been suggested that NF1 can interact with actin, affecting cellular structure and motility.

(Graphic: Feinstein Kean Healthcare)

Web Sites with More Information about CCR

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

Office of the Director
<http://ccr.cancer.gov/about/default.asp>

Office of the Clinical Director
http://ccr.cancer.gov/trials/clinical_director.asp

Office of Communications
<http://ccr.cancer.gov/news/ooc.asp>

Office of Science and Technology Partnerships
<http://ccr.cancer.gov/research/ostp>

Office of Training and Education
http://ccr.nci.nih.gov/careers/office_training_education.asp

Patient Information on Cancer and Clinical Trials

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

How to Refer a Patient
<http://bethesdatrials.cancer.gov/professionals/refer.asp>

NCI Cancer Information Service
<http://cis.nci.nih.gov>
1-800-4-CANCER (1-800-422-6237)

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

Clinical Studies Support Center (CSSC)
<http://ccr.cancer.gov/trials/cssc/staff/services.asp>

Additional Links

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

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

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



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