CCR frontiers IN SCIENCE

November 2002, Volume 1 Published by the Center for Cancer Research, National Cancer Institute

Contents

NATIONAL CANCER INSTITUTE

A New 3D Multi-functional Microscope

Currently specific molecular species must be fluorescence labeled to be detected with optical microscopy. However, in general only large (macro) molecules can be labeled without perturbing function, and only two to four different species can be labeled in the same cell. These limitations can make it difficult to study complex molecular processes within cells, where more than a few molecular species are often involved and where small molecules and subcellular structures have a role.

To acquire more information from live cell specimens, the Image Analysis Laboratory at NCI-Frederick, in collaboration with Pacific Northwest National Laboratory, will recruit a postdoctoral fellow to help develop a multi-functional 3D microscope (MFM) consisting of fluorescence, second harmonic imaging (SHIM), and coherent anti-Stokes Raman scattering (CARS) spectroscopy imaging.

SHIM detects a scattered, frequency-doubled signal from unlabeled polymer-like structures in cells that are composed of the ordered arrangements of molecular units (e.g., the cell membrane, cytoskeletal elements), thus providing structural information (Campagnola PJ, et al., *Biophys J* 77: 3341-9, 1999).

CARS detects chemical bonds (e.g., –CH, –NH, and –SH bonds) via their vibrational (Raman) spectra with the spatial resolution of a confocal microscope and high sensitivity (Holtom GR, et al., *Traffic* 2: 781-8, 2001). For example, when CARS is tuned to a vibrational line of the –CH bond, it will produce an image showing high concentrations of these bonds (e.g., lipid-rich vesicles in cells). CARS can detect specific molecular species of choice when hydrogen atoms in the specific molecules have been replaced with deuterium, because the substitution shifts the spectra to a frequency where naturally occurring bonds do not vibrate (Figure 1). This technique has two practical applications: specific molecules—either small or large—can be detected without perturbing function, and deuterium labeling does not suffer photodamage, which is an inherent problem with fluorescent labels.

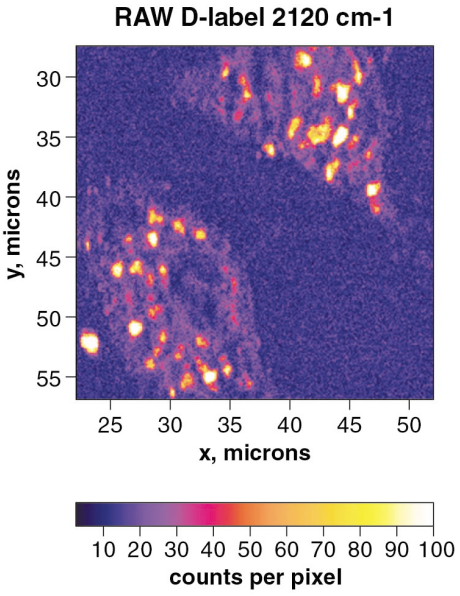

Figure 1. Images of living RAW cells that have been exposed to deuterium-labeled lipids (Holtom GR, et al., *Traffic* 2: 781-8, 2001).

Pacific Northwest National Laboratory researchers Gary Holtom, Ph.D., and Steve Colson, Ph.D., are designing and building a prototype MFM, while the postdoctoral fellow in the laboratory of Stephen Lockett, Ph.D. (Image Analysis Laboratory,

CCR frontiers IN SCIENCE

If you have scientific news of interest to the CCR research community, please contact the **scientific advisor** responsible for your area of research, Tracy Thompson, or Sue Fox.

Tracy Thompson, *Editor-in-Chief*
thompstr@mail.nih.gov
Tel: 301-594-9979

Sue Fox, *Managing Editor*
smfox@mail.ncifcrf.gov
Tel: 301-846-1923

SCIENTIFIC ADVISORY COMMITTEE

Biotechnology Resources
David J. Goldstein, Ph.D.
goldsted@mail.ncifcrf.gov
Tel: 301-846-1108

Clinical Research
Greg Curt, M.D.
curtg@nih.gov
Tel: 301-496-4251

Retroviruses
Vinay K. Pathak, Ph.D.
vpathak@mail.ncifcrf.gov
Tel: 301-846-1710

Carcinogenesis, Cancer and Cell Biology, Tumor Biology
Joseph DiPaolo, Ph.D.
dipaoloj@dc37a.nci.nih.gov
Tel: 301-496-6441

Stuart H. Yuspa, M.D.
yuspas@dc37a.nci.nih.gov
Tel: 301-496-2162

Structural Biology, Chemistry
Christopher J. Michejda, Ph.D.
michejda@mail.ncifcrf.gov
Tel: 301-846-1216

Molecular Biology
Jeffrey N. Strathern, Ph.D.
strather@ncifcrf.gov
Tel: 301-846-1274

Translational Research
Stuart H. Yuspa, M.D.
yuspas@dc37a.nci.nih.gov
Tel: 301-496-2162

Immunology
Jonathan Ashwell, M.D.
jda@box-j.nih.gov
Tel: 301-496-4931

Jay Berzofsky, M.D., Ph.D.
berzofsk@helix.nih.gov
Tel: 301-496-6874

NCI-Frederick) will assess the feasibility of and define areas of improvement in multi-functional microscopy for investigating molecular mechanisms within live cells. It is intended that the MFM will become available at NCI-Frederick.

Collaborators at NCI-Frederick are 1) Nancy Colburn, Ph.D., who is studying how arachidonic acid metabolism contributes to the transformation response of JB6 mouse epidermal cells. The MFM should enable simultaneous following of the cytosolic redistribution of green fluorescent protein-tagged protein kinase C after activation by 12-*O*-tetradecanoylphorbol-13-acetate, the redistribution of deuterated arachidonic acid from the perinuclear regions to the cell membrane, and ruffling of the cell membrane caused by protein kinase C phosphorylation of cytoskeletal elements. 2) Ji Ming Wang, M.D., Ph.D., who is interested in the internalization and trafficking of the FPRL1/amyloid β complex, which plays a key role in the pro-inflammatory response of brains damaged by Alzheimer's disease. The MFM should determine whether the internalization of the fluorescence-labeled cell surface receptor, FPRL1, is via lipid-rich vesicles. 3) Chris Michejda, Ph.D., who designs drugs against cancer and AIDS. His studies are limited because some potential drugs cannot be fluorescence labeled, making it difficult to quantify and localize them in cells. This limitation might be overcome if drugs or peptides were labeled with deuterium and imaged using CARS microscopy. Specifically, Dr. Michejda hopes to use the MFM to determine the trafficking and cellular processing of the deuterated peptidic drugs such as dolostatins, hemiasterlins, and other non-fluorescent drug candidates.

For further information on the MFM, please contact Dr. Stephen Lockett.

■ **Stephen J. Lockett, Ph.D.**
Director, NCI/SAIC Image Analysis Laboratory
NCI-Frederick, Bldg. 538/Rm. 157
Tel: 301-846-5515
Fax: 301-846-6552
E-mail: slockett@ncifcrf.gov

■ FROM THE DIRECTOR

Concept-based Clinical Trials: The Interface Between Laboratory and Clinic

This is the first part of a two-part series focusing on concept-based clinical trials. The second part will be published in the next issue (January 2003).

The NCI's Center for Cancer Research (CCR) places emphasis on early-phase clinical trials as a direct result of its extraordinary commitment to accelerating the translation of novel technologies and treatment modalities from the laboratory into the clinic. Most of the Center's early-phase protocols are designed to establish specific scientific concepts derived from basic, pre-clinical, and/or clinical observations applicable to the treatment of many cancer types. High priority is given to trials that entail the use of technologies yielding new molecular or biological insights into disease or draw upon the unique strengths, resources, and patient populations available within the CCR. Trials addressing high-priority, programmatic needs of the Institute or focusing on understudied or refractory diseases are also strongly supported. Various types of concept-based trials are currently taking place, including multiple approaches to immunotherapy; technologies that can be employed in the clinical setting to improve early detection, diagnosis, and patient prognosis; molecular profiling at the genomic and proteomic levels; and molecularly targeted agents developed

in the CCR that are often tested in the context of innovative combination treatment regimens.

Establishing the principal that immunotherapy can be an effective approach to treating cancer is an area in which the NCI's Intramural Program has excelled for more than a decade, exerting a leadership role that has profoundly influenced the direction of cancer research worldwide. Until the 1990s, little scientific credence was given to the concept that the immune system could be harnessed to fight cancer because malignant cells are, at best, only weakly immunogenic. Now, however, immunotherapeutic approaches represent one of the most rapidly expanding and innovative areas under basic and clinical investigation within the CCR. A Vaccine Working Group has been established to facilitate efforts to develop novel vaccines for cancer and HIV immunotherapy by bringing together investigators with diverse scientific backgrounds from across the Institute, including CCR branches and laboratories placing particular emphasis on this field. These include the Surgery, Metabolism, Pediatric Oncology, Urologic Oncology, and HIV/AIDS Malignancy Branches and the Laboratories of Molecular Biology, Cellular Oncology, and Tumor Immunology and Biology.

Multiple immunotherapeutic modalities are now being examined in early-phase trials, including adoptive cell transfer, vaccines, cytokine modulation, recombinant immunotoxins, and targeted monoclonal antibodies. Although adoptive cell transfer has long appeared attractive in the laboratory, it has proved disappointing in clinical trials because cell populations stimulated with tumor-specific antigens have failed to proliferate and persist after being infused in patients. Recently, however, investigators in the Surgery Branch completed a trial confirming their hypothesis that treatment with non-myeloablative lymphocyte-depleting agents immediately prior to transfer of tumor-infiltrating lymphocytes sensitized to a specific tumor antigen would permit the cells to persist long enough to achieve a clinically significant effect. This trial is the first to demonstrate that transferred cells can proliferate rapidly *in vivo*, repopulate patients' immune systems, traffic to tumor sites, and stably persist as the major T-cell component. Based on this proof of concept, a Phase II study has been launched examining the effects of adoptively transferred lymphocytes on patients with metastatic melanoma.

This major advance in adoptive cell therapy is complemented by advances in many other immunotherapeutic modalities now in early-phase trials. Investigators in the Pediatric Oncology Branch have demonstrated that cytokine-based therapy utilizing interleukin 12/pulsed interleukin 2 inhibits neovascularization and induces local expression of anti-angiogenic chemokines. They have now launched a series of hypothesis-driven Phase I investigations in adults with solid tumors to elucidate the precise mechanisms underlying the anti-angiogenic effects of this therapy *in vivo*.

In the area of recombinant vaccine development, researchers in the Laboratory of Tumor Immunology and Biology have established that inserting three co-stimulatory molecules into recombinant avipox vectors carrying transgenes for tumor-associated antigens enhances T-cell responses to levels far greater than those achieved by the use of any one or two co-stimulatory molecules. They are now conducting two Phase II trials of a recombinant vaccine carrying the transgene for the carcinoembryonic (CEA) antigen coupled with three co-stimulatory molecules in patients with advanced CEA-expressing colorectal, pancreatic, or lung carcinomas. The objective of these trials is to elucidate mechanisms underlying the synergy observed between T-cell receptor signaling and co-stimulatory signaling in the induction of anti-tumor responses.

A pioneer in the development of recombinant immunotoxins to selectively kill metastatic tumors, the Laboratory of Molecular Biology has designed non-immunogenic immunotoxins that have the ability to penetrate tumors and remain stable enough to be highly active for several hours. Investigators are currently conducting clinical trials of several novel agents, including LMB-9 for colon, breast, and other epithelial cancers; SS1P for mesothelioma, ovarian, and pancreatic cancers; and B122 for B-cell leukemias and lymphomas. B122, which is directed against the CD22 antigen, produced many remissions in patients with drug-resistant hairy cell leukemia in a now-completed Phase I trial.

During development, many novel agents require the expenditure of a vast array of resources and expertise on a scale attainable by very few research centers outside the NIH. Now undergoing early trials are three novel monoclonal antibodies developed in the Metabolism Branch that are armed with radionuclides to treat patients with relapsed or metastatic breast cancer, Tac-expressing T-cell leukemia, or other Tac-expressing hematologic malignancies. Developing these agents has necessitated the use of such rarely available resources as the cyclotron and extensive multidisciplinary collaborations among investigators with the necessary expertise in synthetic chemistry, radiochemistry, systemic radiotherapy, radiation oncology, immunology, and molecular biology to generate the antibodies, develop α- and β-emitting nuclides, and design the chemical linkers needed to join the radionuclides to the antibodies.

During the past several years, the concept-based approach to clinical trial design has proved enormously fruitful and will undoubtedly prove even more valuable in the future, as the revolutionary advances made possible through basic research are increasingly used to guide clinical practice.

▪ Carl Barrett, Ph.D.

The p53-effector Gene *Gadd45a* Is an Autoimmune Disease Suppressor Gene

Salvador JM, Hollander MC, Nguyen AT, Kopp JB, Barisoni L, Moore JK, Ashwell JD, and Fornace AJ Jr. Mice lacking the p53-effector gene *Gadd45a* develop a lupus-like syndrome. *Immunity* 16: 499-508, 2002.

The Gadd45 (growth arrest and DNA damage-inducible) protein family has pivotal roles in regulating cell growth and apoptosis. Only one gene member, *Gadd45a*, is transcriptionally regulated by the p53 tumor suppressor. To determine whether this gene has a role in controlling proliferation and apoptosis in the immune system, our laboratory has employed a genetic approach involving mice lacking *Gadd45a, p21*, or both genes. In the case of *p21* (*Cdkn1a*), the product of this gene has a well-known role in controlling cellular proliferation because it is a potent Cdk inhibitor with a major role in G1 checkpoint activation. Our report indicates that both of these p53-effector genes modulate the immune response to prevent autoimmunity. Lack of Gadd45a leads to the development of an autoimmune disease remarkably similar to human systemic lupus erythematosus (SLE), which is a frequent autoimmune disease often affecting women of childbearing age. SLE can affect many organs in the body, causing arthritis, leukopenia, heart disease, osteoporosis, and kidney failure. The etiology of this disease is still poorly understood.

The main findings of our study are that the lack of Gadd45a is sufficient for the development of autoimmune disease and that the lack of both Gadd45a and p21 dramatically accelerates the development of this disease in mice. These observations suggest that the *Gadd45a* and *p21* genes have different roles in controlling immune regulation. *Gadd45a*$^{-/-}$ mice spontaneously develop high titers of anti-DNA autoantibodies, hematological disorders, and kidney disease (autoimmune glomerulonephritis) leading to premature death. To investigate the cellular roles of Gadd45a and p21 in immune function, we determined whether the lack of either gene can affect T-cell or B-cell proliferation and apoptosis. Interestingly, *Gadd45a*$^{-/-}$ T-cells showed a lower threshold of activation, but no abnormalities in cytokine-induced proliferation or in T-cell receptor–independent activation. In contrast, *p21*$^{-/-}$ T cells had a hyperresponsive phenotype only after sustained cytokine stimulation, which is consistent with previous results (Balomenos D, et al., *Nat Med* 6: 176-6, 2000). Surprisingly, the lack of Gadd45a and p21 did not affect B-cell proliferation or T-cell and B-cell apoptosis, indicating specific roles for both genes in the immune system as negative regulators of T-cell proliferation. These findings demonstrate that the p53-effector genes *Gadd45a* and *p21* have different and perhaps complementary roles in controlling immune responses: Gadd45a is a negative regulator of primary T-cell activation (T-cell receptor dependent), whereas p21 controls T-cell proliferation after sustained cytokine activation.

It is well established that T-cell activation has an important role in murine and human autoimmunity. Although the causes of the loss of tolerance (the ability to distinguish self proteins and other macromolecules from foreign antigens) are unknown, evidence is increasing that abnormalities in T-cell proliferation can contribute to this process. Our results support the idea that the hyperproliferative response in *Gadd45a*$^{-/-}$ CD4^{+} cells together with the lower threshold for T-cell activation are sufficient to provoke loss of tolerance. Thus, the *Gadd45a*$^{-/-}$ mouse model may prove useful in dissecting some of the molecular mechanisms involved in immune tolerance. Results with this mouse model also raise the possibility that abnormalities in the expression or regulation of these p53-regulated genes and perhaps p53 itself could contribute to the development of human SLE. For example, a variety of viral factors, as well as p53 mutations or increased expression of cellular p53-inhibitory proteins like Mdm2, are known to interfere with normal p53 function. If such events occur in T cells *in vivo*, then expression of both Gadd45a and p21 would be expected to be compromised.

The lack of Gadd45a is sufficient for the development of autoimmune disease and… the lack of both Gadd45a *and* p21 *dramatically accelerates the development of this disease in mice. These observations suggest that the* Gadd45a *and* p21 *genes have different roles in controlling immune regulation.*

■ **Jesús M. Salvador, Ph.D.**
Visiting Fellow
■ **Albert J. Fornace Jr., M.D.**
Chief, Gene Response Section
Basic Research Laboratory
NCI-Bethesda, Bldg. 37/Rm. 6144
Tel: 301-594-7535
Fax: 301-480-2514
E-mail: salvadoj@dino.nci.nih.gov

Adoptive Transfer of Tumor-infiltrating Lymphocytes Effective in Treating Patients with Refractory Metastatic Melanoma

Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, and Rosenberg SA. Cancer regression and autoimmunity following clonal repopulation with anti-tumor lymphocytes and non-myeloablative conditioning. Published online in *Science Express*, http://www.sciencemag.org/sciencexpress/recent.shtml September 19, 2002.

Mark Dudley, Ph.D., Steve Rosenberg, M.D., Ph.D., and their colleagues in the Surgery Branch have demonstrated that the adoptive transfer of tumor-infiltrating lymphocytes (TILs) combined with high-dose interleukin-2 (IL-2) therapy can induce metastatic melanoma regression when preceded by non-myeloablative lymphodepleting chemotherapy. Thirteen human leukocyte antigen $A2^+$ patients with metastatic melanoma refractory to standard therapies, including IL-2 therapy, were enrolled in the study. Six of the 13 demonstrated objective clinical responses to treatment, and 4 more showed mixed responses with significant shrinkage of one or more metastatic deposits.

Immunotherapy of patients with cancer requires large numbers of highly selective TILs capable of overcoming mechanisms of tolerance and persisting long enough to sustain a clinically significant anti-tumor response. Although adoptive cell transfer therapies involving the selection and *ex vivo* activation of T cell subpopulations have long appeared promising, clinical trials prior to this study uniformly failed to demonstrate the engraftment, proliferation, and persistence of such populations. On the basis of animal studies showing that lymphodepletion markedly improves the efficacy of adoptive transfer therapy, Dr. Rosenberg and his colleagues hypothesized that administering non-myeloablative lymphodepleting chemotherapy immediately before TIL infusion could improve cell proliferation and persistence *in vivo*.

The investigators harvested TILs reactive to melanoma antigens from each patient and induced the cells to proliferate rapidly *in vitro*. The cells proved highly reactive when stimulated with a human leukocyte antigen A2-matched melanoma or an autologous melanoma cell line. All patients received cyclophosphamide and fludarabine for 7 days

Dr. Rosenberg and his colleagues hypothesized that administering non-myeloablative lymphodepleting chemotherapy immediately before TIL infusion could improve cell proliferation and persistence in vivo.

before adoptive cell transfer and concomitant treatment with IL-2. Beginning 1 day after chemotherapy ended, the patients received, on average, 7.8×10^{10} cells and nine doses of IL-2. Tumor regression became apparent in the lung, liver, and lymph nodes as well as at cutaneous and subcutaneous sites; intraperitoneal masses also exhibited regression. Some patients developed side effects from autoimmune melanocyte destruction, including vitiligo and uveitis.

Follow-up studies at 1 week and approximately 1 month after cell transfer demonstrated that clonal populations reactive to the MART-1 melanoma-melanocyte antigen proliferated rapidly *in vivo*, repopulated the patients' immune systems, and stably persisted as the major T-cell component, sometimes comprising more than 80 percent of a patient's $CD8^+$ lymphocytes. Histologic comparisons of tumor specimens before and after treatment revealed that the transferred cells were capable of trafficking to and infiltrating tumor deposits. Whereas pre-treatment samples showed little or no lymphocytic infiltration, post-treatment samples exhibited areas of dense, diffused lymphocytic infiltrates and large areas of necrotic tissue.

Although the underlying mechanisms remain to be elucidated, Dr. Rosenberg and his colleagues speculate that the lymphodepletive regimen may have eliminated regulatory cells or altered homeostatic mechanisms that limit lymphocyte numbers, allowing the transferred cells to grow rapidly and persist *in vivo*. In summary, adoptive transfer can effectively treat patients with refractory metastatic melanoma, and normally expressed "self-antigens" can act as useful targets for human tumor immunotherapy. The authors believe that a similar approach could be applied to other cancer types and to infectious diseases for which functionally active lymphocytes can be selected *in vitro*, such as human immunodeficiency virus-associated AIDS.

- **Mark Dudley, Ph.D.**
  Research Fellow
- **Steven A. Rosenberg, M.D., Ph.D.**
  Chief, Surgery Branch
  NCI-Bethesda, Bldg. 10/Rm. 2B42
  Tel: 301-496-4164
  Fax: 301-402-1738
  E-mail: sar@nih.gov

Getting a "Grip" on Reverse Transcription: Initiation of DNA Synthesis and RNASE H Cleavage Specificity *In Vivo*

This is the first in a series of three articles focusing on the role of RNase H primer grip region in HIV reverse transcription.

Julias JG, McWilliams MJ, Sarafianos SG, Arnold E, and Hughes S. Mutations in the RNase H domain of HIV-1 reverse transcriptase affect the initiation of DNA synthesis and the specificity of RNase H cleavage *in vivo*. *Proc Natl Acad Sci U S A* 99: 9515-20, 2002.

The genome of the human immunodeficiency virus type 1 (HIV-1), which causes AIDS, is single-stranded RNA. Upon entering a host cell, the viral enzyme reverse transcriptase (RT) converts the single-stranded viral RNA into double-stranded DNA, which is then inserted into the genome of the host cell by the viral enzyme integrase. RT has a DNA polymerase domain that can copy either RNA or DNA, and an RNase H domain that can cleave RNA only if it is part of an RNA/DNA duplex. Both enzymatic activities are essential for viral replication: RNase H is required for the degradation of the RNA template after it has been copied into DNA, which permits the synthesis of the second DNA strand by DNA polymerase.

Like many other DNA polymerases, RT cannot initiate *de novo* DNA synthesis; both a template and a primer are required. The primer for the minus-strand DNA is a host tRNA ($tRNA^{Lys3}$) that base pairs to the viral RNA genome near the 5' end, and the primer for the plus-strand DNA is a small purine-rich segment near the 3' end of the viral genome called the polypurine tract (PPT). Although most of the cleavages that RNase H makes are non-specific, the generation and removal of the PPT primer and the removal of the $tRNA^{Lys3}$ primer are precise. This precision is essential because removal of the primers defines the ends of the viral DNA, which are the substrates for integrase. Recently, the RNase H primer grip region was identified as a structural feature of HIV-1 RT in complex with an RNA/DNA template-primer duplex (Sarafianos SG, et al., *EMBO J* 20: 1449-61, 2001) (Figure 1). The RNase H primer grip region is a series of amino acids in the connection and RNase H domains that interact with the DNA primer hypothesized to position the DNA primer strand near the RNase H active site and play an important role in the RNase H activity and cleavage specificity.

To determine the role of RNase H primer grip *in vivo* (the corresponding *in vitro* analyses were done by Rausch JW, et al., *Biochemistry* 41: 4856-65, 2002), the authors generated a series of mutants in which single amino acids of the HIV-1 RNase H primer grip region were substituted with alanine. Most mutations (R448A, N474A, K476A, Q500A, and I505A) had a less than twofold effect on the viral titer, but two single-amino acid mutations (Q475A and Y501A) and one double-amino acid mutation (N474A + Q475A) exhibited 5- to 10-fold reductions in viral titer in single-cycle assays. Quantitative real-time PCR analysis of viral DNA products indicated that the defect in viral replication could be accounted for by a defect in initiation of minus-strand DNA synthesis using the $tRNA^{Lys3}$ primer, indicating that mutations in the RNase H domain could influence the *in vivo* activity of the polymerase.

To analyze the effects of RNase H primer grip mutations on the specificity of RNA cleavage, the authors analyzed the sequences of 2-long terminal repeat

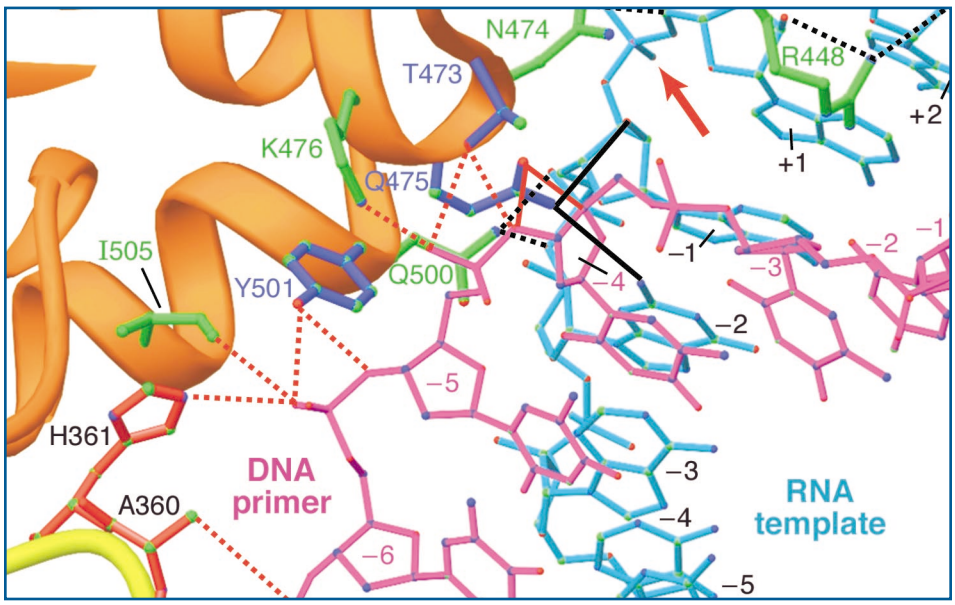

Figure 1. The structure of HIV-1 reverse transcriptase in complex with an RNA/DNA template-primer duplex derived from the polypurine tract. Orange indicates a portion of the RNase H domain, yellow a portion of the connection subdomain, blue the RNA template strand, and pink the DNA primer strand. The scissile phosphate is designated by a red arrow. The amino acids forming the RNase H primer grip and the amino acids in the connection subdomain that contact the DNA primer are labeled; contact points are indicated by dashed lines and solid lines (black for RNA template, red for DNA primer). This network of amino acids helps position the nucleic acid in the vicinity of the RNase H active site, which allows specific cleavages to occur. (Prepared by Stefan Sarafianos at the Center for Advanced Biotechnology and Medicine, Rutgers University.)

To determine the role of RNase H primer grip in vivo *the authors generated a series of mutants in which single amino acids of the HIV-1 RNase H primer grip region were substituted with alanine.*

(LTR) circle junctions that are formed in cells by ligating the ends of a small fraction of viral DNA. Aberrant RNase H cleavage at viral DNA ends could result in the insertion of sequences from the $tRNA^{Lys3}$ and/or PPT primers at the LTR junctions; they could also result in deletions in the LTR sequences at the junctions. Analysis of LTR circle junctions indicated that compared with wild-type RT, the RNase H primer grip Y501A mutation and N474A + Q475A double mutation had substantially higher frequencies of PPT insertions and LTR sequence deletions. These mutations indeed appeared to substantially alter the specificity of RNase H cleavages that resulted in the generation and deletion of the PPT primer.

The results of these *in vivo* studies indicate that mutations in the RNase H primer grip region that contact the DNA primer can influence the initiation of DNA synthesis at the polymerase active site as well as the specificity of RNase H cleavage at the RNase H active site. The results also lend support to the authors' earlier conjecture that proper positioning of the nucleic acid is essential for both the polymerase and RNase H activity and define which amino acids in the RNase H domain are important for the proper positioning.

- **John G. Julias, Ph.D.**
  Research Fellow
- **Stephen D. Hughes, Ph.D.**
  Chief, Retroviral Replication Laboratory
  HIV Drug Resistance Program
  NCI-Frederick, Bldg. 539/Rm. 116
  Tel: 301-846-1611
  Fax: 301-846-6966
  E-mail: jjulias@ncifcrf.gov

***CCR Frontiers in Science*—Staff**

Center for Cancer Research
J. Carl Barrett, Ph.D., *Director*
Greg Curt, M.D., *Clinical Director*

Deputy Directors
C. Norman Coleman, M.D.
Lee Helman, M.D.
Douglas R. Lowy, M.D.
Robert H. Wiltrout, Ph.D.
Stuart H. Yuspa, M.D.

Associate Directors
Beverly A. Mock, Ph.D., Scientific Policy and Planning
Ron Thayer, Clinical Affairs

Scientific Advisory Committee
Jonathan Ashwell, M.D.
Jay Berzofsky, M.D., Ph.D.
Greg Curt, M.D.
Joseph DiPaolo, Ph.D.
David J. Goldstein, Ph.D.
Christopher J. Michejda, Ph.D.
Vinay K. Pathak, Ph.D.
Jeffrey N. Strathern, Ph.D.
Stuart H. Yuspa, M.D.

Editorial Staff
Office of the Director, CCR
Tracy Thompson, *Editor-in-Chief*
Sue Fox, *Managing Editor*
Ave Cline, *Assistant Editor*
Kay Fleming, *Writer*
Deborah Pearson, *Writer*

Palladian Partners, Inc.
Rob Wald, *Publications Manager*
Lizzie Hess, ELS(D), *Scientific Editor*
Meryl Thomas, *Graphic Artist*

CLINICAL RESEARCH

Support Center Launches Enhanced Web Site

People with cancer, their friends and families, health care professionals, CCR staff, and the general public can now access expanded information about the Clinical Studies Support Center (CSSC) online at http://bethesdatrials.nci.nih.gov. The revamped site, which is a component of NCI's official cancer site (http://ccr.cancer.gov), provides detail on 150 cancer clinical studies taking place on NCI's Bethesda campus.

The site now includes:

- **Frequently asked questions** about cancer clinical studies, including risks and benefits, treatments, and facts about placebos;
- **Specific study information,** including title, protocol number, eligibility criteria, treatment plan, and contact information; and
- **Information on how to participate,** from contacting CSSC to scheduling a screening visit to deciding whether to enroll.

Fact sheets and study matrices are available for download, and links connect Web users with a wealth of information about various cancers. In addition, an improved search function allows users to enter unique criteria to customize searches. Interested individuals are encouraged to visit the site and call CSSC to speak with an information specialist, who will describe available studies, their objectives and methods of treatment, inclusion and exclusion criteria, and how to participate. The site is also rich in content for community-based health care professionals, who can register to receive regular CSSC mailings, find out how to make referrals, and communicate with NCI study teams about their patients' progress.

Since its inception in 1997, CSSC has served as a repository for comprehensive information about Bethesda-based cancer clinical trials. It aims to provide the latest information through a toll-free telephone service and community outreach. It also aims to enhance relationships between NCI and referring physicians, other health care professionals, clinical researchers, and patient advocates. For more information, call CSSC toll-free at 1-888-NCI-1937.

ON THE TENURE TRACK

David Symer, M.D., Ph.D.

David Symer, M.D., Ph.D., joined CCR's Laboratory of Immunobiology in April 2002 as a new tenure-track principal investigator. He graduated from Dartmouth College in 1984 (with honors in mathematics) and from the Medical Scientist Training Program at the Johns Hopkins University School of Medicine with M.D. and Ph.D. degrees in 1993. While at Johns Hopkins, he studied T-cell regulation in Dr. Howard Dintzis' lab using defined, soluble antigen arrays. He received the W. Barry Wood student research award for this work. Dr. Symer subsequently completed his clinical residency in internal medicine at Brigham and Women's Hospital and clinical fellowships in hematology and oncology both there and at Johns Hopkins. He is board certified in internal medicine and in medical oncology.

Starting in 1997, he joined Dr. Jef Boeke's lab at Johns Hopkins as a senior clinical and postdoctoral research fellow. That year he was awarded a Howard Hughes Medical Institute physician postdoctoral fellowship supporting his project, "Modeling the molecular determinants of *p53* mutagenesis in 'humanized' yeast." This postdoctoral project led Dr. Symer to develop interests in transposable elements and the new field of epigenetics. The latter term refers to heritable processes that are encoded not by primary DNA sequences, but by reversible chemical marks on the DNA and the proteins that package DNA in cells. These epigenetic marks appear to be fundamentally important in normal human development and to be altered very frequently in diseases such as cancer.

At CCR, Dr. Symer's new group seeks to address the following questions:

- What role is played by certain molecular factors that shape the pattern of cytosine methylation and chromatin modifications in human development and disease?
- Is there a connection between these normal or abnormal epigenetic processes and the control of transposable elements in the genome?
- Have epigenetic marks changed through the course of human evolution?

At this time, Dr. Symer has chosen to focus on basic research problems, but he fondly recalls his clinical training in the care of cancer patients. For now, as he establishes his own independent research group at NCI-Frederick, he is relishing the opportunity to synthesize scientific, administrative, and mentoring skills accrued over several decades of learning.

His wife, Dr. Maura Gillison, is an assistant professor of oncology at Johns Hopkins, where she sees patients with head and neck cancer and works on virus-associated malignancies. They live in Baltimore and have a wonderful two-year-old daughter. Dr. Symer formerly played bassoon and practiced Shotokan karate, but now has no spare time.

Dr. Symer

ADMINISTRATIVE LINKS

New Policy on Refreshments at Meetings Supported by Contracts

Effective August 2002, funds from Consolidated Support Services (CSS) contracts may not be used for light refreshments and meals at meetings, seminars, retreats, conferences, etc. Entertainment funds must be requested and approved before meals and refreshments can be authorized at a CSS-supported event. If an event does not meet the circumstances that permit the use of appropriated or gift funds, you must notify the CSS contract that money must be collected from all attendees to cover meals and light refreshments. Please see your administrative officer for guidelines and exceptions (e.g., Government Employee Training Act) on the use of appropriated funds to cover meals and light refreshments.

NIH Title 42 Pay Model

A consolidated Title 42 Pay Model has been established for NIH. The intent of this model is to provide a flexible and consistent framework to establish policy and procedures for determining initial pay and performance-based pay adjustments for employees appointed under Title 42. The goals are to (1) provide a flexible salary system to support scientific research and research management by scientists, (2) allow salary comparability with the private sector, and (3) establish consistency in payment of similarly qualified scientists performing similar work. Please see your administrative officer for further information on Title 42 appointments, pay adjustments, and extensions.

CCR Frontiers in Science: Online and Hard Copies

Current and archived issues of *CCR Frontiers in Science* are now available online at http://ccr.cancer.gov/news/newsletter.asp. Please contact Sue Fox at 301-846-1923 or smfox@mail.ncifcrf.gov if you would like to receive additional hard copies.