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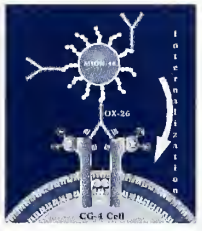
DYE AND LET LIVE: MAGTAGS EMERGING AS CELLULAR COMPASS

by Celia Hooper

The Clinical Center's Laboratory of Diagnostic Radiology Research (LDRR) is on to something big, and the excitement surfaces quickly when you talk to lab chief Joe Frank or staff scientist Jeff Bulte. Along with colleagues at NIH and at the University of Wisconsin, Bulte and Frank have developed an elegant New Age version of what old-school microscopists called a vital dye—that is, a substance that marks cells without damaging them.



Magtag "magic"—see page 10

The New Age twist is that the "dye" in this case is a magnetic label that allows researchers to image tagged cells in living animals, noninvasively, without radioiosotopes, at a microscopic level, using magnetic resonance imaging (MRI).

If LDRR's work pans out, it could yield the label of choice for tracking cells in vivo. Beyond the tracking of oligodendrocyte progenitor cells injected into the rat—LDRR's starting point—Frank envisions the technique being used to trace a variety of stem cells and transgenic cells that have been harvested, cultured, or manipulated in some way outside the body and then injected back in. This could include "any stem cell, tumor cell, or other transplantable cells—for instance, islet cells or specific subpopulations of T cells to evaluate temporal-spatial

continued on page 10

GENE THERAPY TRIAL AND ERRORS RAISE SCIENTIFIC, ETHICAL, AND OVERSIGHT QUESTIONS

by Fran Pollner

Current Event

Nearly a decade after it permitted NIH intramural researchers to pioneer this country's first two human gene therapy trials—and some 350 diverse human gene therapy protocols later—the NIH Recombinant DNA Advisory Committee (RAC) held a three-day assembly centered on the first reported death of a gene therapy research patient attributed to the therapy itself.

The death last September of Jesse Gelsinger, an 18-year-old patient with partial ornithine transcarbamylase (OTC) deficiency, injected a sobering pause in a field rushing toward the 21st century's promise of an avalanche of gene therapy applications inspired by the completion of the Human Genome Project and related discoveries.

The death also precipitated an FDA freeze on the clinical trial in which Gelsinger was enrolled—a safety and biological efficacy study of the recombinant adenoviral vector–OTC gene delivered to the liver via the intrahepatic artery. Gelsinger was the scheduled penultimate patient in what was to have been an 18-patient study and the second one to receive the highest protocol-defined dose in this dose-escalation trial that was begun in 1997 at the University of Pennsylvania in Philadelphia.

The pivotal speakers at the RAC proceeding, held December 8 through 10, 1999, on the NIH campus and under bright media lights, were the OTC study investigators: veteran gene therapy researcher James Wilson, director of the Institute for Human Gene Therapy, and surgeon Steven Raper, both of UPenn,

Fran Pollner

University of Pennsylvania gene therapy researcher James Wilson reads a prepared statement at a press briefing after the first day of the RAC proceedings in December. The RAC meeting was convened to review the track record of adenoviral gene therapy vectors and the conduct of the UPenn protocol that resulted in the death of patient Jesse Gelsinger. Wilson was introduced by Paul Gelsinger (right), Jesse Gelsinger's father, shown exiting after delivering his own statement and answering reporters' questions. Wilson declined to entertain questions.

continued on page 4

Harold Varmus on the road again. See p. 8.

CONTENTS

Improving the Postdoctoral Experience at NIH: Two Words to the Wise

Michael Gottesman

Postdocs are critical to NIH. They have a hand in most NIH intramural research and are our largest group of trainees. And critical to postdocs is mentoring. A previous column on the expectations that a postdoc should have about training at NIH led to the "Guide to Mentoring and Training in the Intramural Program." This booklet, which I hope you have all read carefully, provides an outline of the responsibilities of both mentor and trainee at NIH. This column targets what I consider to be the most basic first step in mentoring: Whether you are a supervisor or mentor—or both—I encourage you early on, to approach your fellows and begin a dialogue that opens with a simple invitation:

"Let's talk."

Starting shortly after a postdoc arrives at a lab, and then continuing on a regular basis, the point of discussions between mentor and fellow should be to set goals and assess progress toward them, provide a framework for the training experience at NIH, clarify expectations on both sides, and provide career advice for the fellow.

These discussions should be frank and fair, or they will be of little value. If necessary, discussions can be initiated by the fellow; but, in any case, they should occur. Where it is helpful, meeting details could be put in writing.

These discussions [between mentor and fellow] should be frank and fair, or they will be of little value. If necessary, discussions can be initiated by the fellow; but, in any case, they should occur. Where it is helpful, meeting details could be put in writing.

When a fellow enters a lab, his or her supervisor should spend some time discussing the research project or projects currently underway in the lab; the role of the fellow in each project; expectations about independence, authorship (including the likelihood of there being publishable work from a project), and collaborations; any prior agreements that could affect the fellow's work; and any rules that govern conduct in the lab.

A first meeting is also a time to provide and discuss the "Guidelines for the Conduct of Science in the NIH Intramural Program" and the "Guide to Mentoring and Training." There should be explicit mention of the duration of the appointment, the experience of previous fellows in finding jobs, and what the expectations of fellow and mentor are with respect to careers in biomedical research. In a recent survey conducted by the NIH Fellows' Committee, about one-quarter of the fellows who responded were unable to identify their mentors. (See *The NIH Catalyst*, March–April 1999, pages 4–5.) The first meeting is an excellent time for the supervisor to offer to be a mentor and to suggest other scientists who could also offer useful advice to the fellow.

Beyond this first talk, there should be regular meetings to discuss science, conduct of science, and career-related issues. Data sessions should be held frequently—perhaps weekly—especially early in the training of a postdoc. The same Fellows' survey suggested that about 20 percent of the fellows who responded meet less than once a month with their supervisors.

There should also be an annual meeting specifically to evaluate the work of the fellow and indicate whether he or she is on track to achieve research and career goals. This is an important opportunity to begin a discussion about jobs after the postdoctoral experience, to indicate that some goals are impractical, and to help a fellow choose a reasonable course of action.

In the year before a fellow leaves, more specific career planning has to take place. Is another postdoc a reasonable option? To what jobs should a fellow apply? What scientific work can be taken from the lab and what should stay? It is very helpful to fellows to have someone critique their cover letters, their CVs, and their applications to specific jobs. Will the mentor be willing to make some telephone calls on behalf of the fellow?

Finally, the fellow should have an exit interview with the mentor to outline what went well, what could use improvement, and what should be avoided in the future. These kinds of discussion are difficult, but very important if we want to fulfill our duties as mentors and improve the postdoctoral experience at NIH.

—Michael Gottesman
Deputy Director for Intramural Research

A MATTER OF INTEGRITY: FABRICATION IN RESEARCH AND ADMINISTRATIVE RECORDS

by Joan P. Schwartz, Ph.D., NINDS Assistant Director, OIR

Science is built on trust. Everyone expects that data published in the literature are *real* data, and we base new experiments on that assumption. But occasionally an instance of misconduct is uncovered in which an author of a scientific paper has falsified or fabricated the reported results. A proposed new definition of research misconduct—which will apply government-wide for the first time—was published for comment in the *Federal Register* [**64** (198): 55722–55725, October 14, 1999]. The definition is:

Research misconduct is defined as fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research reports.

- **Fabrication is making up results and recording or reporting them.**
- **Falsification is manipulating research materials, or changing or omitting data or results such that the research is not accurately represented in the research record.**
- **Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate credit, including those obtained through confidential review of others' research proposals and manuscripts.**
- **Research misconduct does not include honest error or honest differences of opinion.**

Research is defined as all basic, applied, and demonstration research in all fields of science, engineering, and mathematics. The research record is defined as the record of data or results that embody the facts resulting from scientific inquiry, and includes, for example, laboratory records, both physical and electronic, research proposals, progress reports, abstracts, theses, oral presentations, internal reports, and journal articles.

How NIH handles allegations of scientific misconduct within the intramural program will also be modified. The largest change is that NIH will carry out its own investigations, rather than turning them over to the Office of Research Integrity (ORI). The NIH Committee on Scientific Conduct and Ethics is currently rewriting the Guidelines that describe NIH Policies and Procedures for Investigation of Scientific Misconduct—a subject for a future *Catalyst* column.

Over the past few months, instances of falsification have turned up at NIH involving radiation contamination survey data, animal weight records, and publications listed in the bibliography of a scientist's CV. In pondering the ramifications of these events, the specific offices involved, as well as the NIH Committee on Scientific Conduct and Ethics, first considered whether the material in question constituted a part of the *research record* as defined above, in which case the falsification or fabrication would be deemed scientific misconduct. In each case, it was concluded that, technically, no scientific misconduct had been committed but the scientist involved had behaved unacceptably and deserved some type of sanction.

Joan P. Schwartz

Radiation contamination surveys must be done monthly in any laboratory that uses radioisotopes. The Nuclear Regulatory Commission (NRC) requires them as a condition of the NIH's license because they ensure that no staff are exposed to radiation of which they are unaware. Twice recently, NIH lab staff members fabricated surveys in response to notifications that survey results for certain months were missing. Because such records are not a part of the *research record* per se and are never published as research results, this fabrication does not reach the bar for scientific misconduct. Rather, surveys of this sort might fall into a category of administrative records. Nevertheless, such fabrication is a violation of NRC regulations and the individual(s) involved could be subject to criminal penalties. The "authorized user" responsible for the laboratory could be barred from using NRC-licensed radioactive materials for up to 3 years, and NIH could deliver an official reprimand that becomes a part of the individual's personnel record. As all NIH staff were recently notified in a desk-to-desk memo, such falsification of radiation contamination surveys is unacceptable.

The second incident involved fabrication of weights for a research animal that had been placed on a restricted diet—instead of weighing the animal and recording the actual weight weekly. As a consequence, the animal lost significantly more weight than acceptable, thereby endangering its health. The weight data were used neither in a scientific publication nor in a specific research protocol and, again, could be considered an administrative record. However, these actions clearly violated the NIH Guidelines for Animal Care, and the scientist involved therefore received an official reprimand and was prohibited from participating in animal protocols for the duration of his appointment at NIH.

The third example involved the identification of a series of irregularities in five references included in the bibliography of a researcher's CV. For two of them, actual references existed in the literature, but the first author's name had been replaced with the name of the person accused of the falsification; the year had also been changed. Two other references cited papers that do not exist; the fifth had the correct authors and title but the wrong journal. A CV is also a type of administrative document, used for applications for jobs and grants. If this CV had been used to apply for a federal job, the falsification would have been a criminal act. If used in a grant application, it would have constituted research misconduct. In this case, the NIH appointment of the errant CV author was ending and was simply not renewed. A major issue, however, was what a supervisor should say if asked for a letter of recommendation for this individual. It is ultimately a decision each supervisor must make, but NIH has recommended that the fact be disclosed that a CV was received from the individual in which certain bibliographic citations proved to be erroneous. Such a statement advises the next employer of potential problems with the applicant.

Too often, when a supervisor encounters such behaviors, he or she is eager to get the offender out of the laboratory but avoids coming to grips directly with the issues of fabrication and falsification. But we cannot turn our backs on the integrity of either the research record or the administrative record that supports it. The public trust—reflected in our licenses to conduct research with radioisotopes, animals, and human subjects, as well as the tax dollars that support NIH—is contingent on our responsible behavior as a community. ■

Gene Therapy Trial
continued from page 1

and pediatrician Mark Batshaw, chief academic officer at the Children's National Medical Center in Washington. All three apologized for communication gaps with oversight agencies and lapses in complying with adverse event reporting requirements, which, had they been adhered to, might have led to a protocol modification or a reevaluation of Gelsinger's candidacy for trial participation. Gelsinger's precipitous and ultimately irreversible response to the experimental therapy included signs of disseminated intravascular coagulation (DIC), massive cytokine release, adult respiratory distress syndrome (ARDS), and multiple organ failure.

The purpose of the meeting, federal officials and RAC officers emphasized, was largely educational: to pool information on the biology and toxicity of adenoviral vectors; to scrutinize the conduct of the OTC trial with a view toward identifying red flags and improving gene therapy trial design in general; and to revisit federal reporting requirements, interagency communications, and the role of the RAC in gene therapy oversight. Patient advocates, however, voiced worries that gene therapy research might be stopped or curtailed; industry representatives cautioned against increased public scrutiny that might bare trade secrets or misleading information; and reporters wanted to know the specifics of protocol violations and any other problems in the OTC study, as well as steps planned by overseers to enforce compliance with scientific and ethical guidelines in the conduct of human gene therapy research.

RAC chair Claudia Mickelson, a biosafety officer at the Massachusetts Institute of Technology in Cambridge, and molecular biologist and former RAC chair Inder Verma, of the Salk Institute for Biological Studies Laboratory of Genetics in San Diego, served as moderators.

Adenoviral Vector Experience

According to Amy Patterson, director of the NIH Office of Biotechnology Activities (OBA, formerly the Office of Recombinant DNA Activities), about 4,000 patients have participated in about 350 gene therapy trials in the past decade; adenoviral vectors have been used in about one-quarter. Immediately after OTC investigators notified OBA of Gelsinger's death, Patterson said, OBA notified the more than 70 other researchers currently using similar methods in their own gene transfer studies.

After Gelsinger's death, the FDA conducted a database search of all gene therapy protocols with adenovirus vector and found no similar ARDS, aplastic anemia, or extensive DIC findings, an FDA official said.

A refresher course in adenovirus basics was presented by experts summoned to the RAC proceedings. Among the relevant facts noted were that adenovirus can cause fatal hepatitis in immunocompromised individuals; and that adenovirus attaches to the coxsackie-adenovirus receptor (CAR) in the liver, a receptor whose function and level of expression in different tissues are not well known and whose abundance in the liver varies among humans and animal models. To render adenovirus suitable as a gene vector, viral genes required for replication are deleted; thus enfeebled, adenovirus generally serves well as a vector. There are, however, some concerns regarding viral infection: In simian species, adenoviral replication is enhanced in the context of co-infection with another virus, such as SV40; and adenovirus can also function as a helper virus, for instance, potentiating the growth of parvovirus. Still to be understood is the ability of certain integrins that facilitate adenoviral integration into target cells to induce cytotoxic and apoptotic responses.

Verma recounted his own lab's adaptation of adenovirus for use as a gene vector, with the deletion of the E1a, E1b, and E4 adenoviral genes to blunt its immunogenicity and the summoning of cytotoxic T cell lymphocytes. The ease of disarming these replication components and of growing billions of virus particles quickly, as well as adenovirus infectivity of both dividing and nondividing cells, he said, make it an "attractive" vector.

University of California at San Francisco (UCSF) surgical oncologist Robert Warren offered observations from a trial involving adenovirally packaged p53 delivered via the intrahepatic artery to the liver of colorectal cancer patients with hepatocellular metastases. Postinfusion transaminase elevations invariably came down and were never dose limiting; hypotension, he said, was the dose-limiting toxicity, with liver toxicity "significantly less than expected." Commenting on questions later related to the possibility that high doses could mobilize proinflammatory cytokines and lead to intravascular coagulation, Warren said that duration of exposure to the vector in his trial may have been too short to observe adverse cytokine events. "We would expect adenovirus induction of cytokines in the liver," he said.

Margaret Rick, hematology chief in the NIH Clinical Center pathology department, noted that acute hepatic failure is among possible triggers of DIC. She suggested that patients be screened for he-

OTCD in General and in the Case of Jesse Gelsinger

Ornithine transcarbamylase deficiency (OTCD) is an inborn error of metabolism in which the second enzyme in the urea cycle is missing; it affects one in 40,000 to 80,000 children. The inability to excrete urea can precipitate an acute rise in ammonia concentrations, brain damage, coma, and death. Neonatal mortality within the first month is 50 percent, and 50 percent of early survivors die within the first five years. Without a liver transplant, 75 percent of survivors will sustain severe neurological dysfunction.

Patients with partial OTCD have some residual enzyme activity and can be managed on a strict low-protein diet and the "alternative pathway" regimen (intravenous sodium benzoate, sodium acetate, and arginine hydrochloride), co-developed by Mark Batshaw, principle investigator in the UPenn study. They are always at risk of coma, however, and the therapy, says Batshaw, is "incomplete at best."

Jesse Gelsinger's partial OTCD was diagnosed at 33 months, when he presented lethargic, incoherent, and with elevated ammonia and glutamine concentrations. He was hospitalized at age 10 and again in December 1998. In June 1999, he met screening criteria for the UPenn trial, but his ammonia concentration the day before actual dosing three months later exceeded the cutoff for entry eligibility. He was placed on the alternative pathway regimen, hydrated, and the next day, September 13, dosed as per protocol. He died on September 17. Though the relationship of his death to his liver function at dosing had not been established, co-investigator Steve Raper asserted that "We agree that future inclusion criteria" should address standards of patient status directly before dosing. ■

Gene Therapy Oversight

Fran Pollner

Overseers Answer to the Press *(left to right): Amy Patterson, director NIH Office of Biotechnology Activities; Lana Skirboll, director, NIH Office of Science Policy; Phil Noguchi, director, FDA Division of Cellular and Gene Therapies; and Kathryn Zoon, director, FDA Center for Biologics Evaluation and Research, respond to questions during a news briefing after the first day of the RAC meeting. Nearly all questions centered on FDA's oversight role in the OTC study, protocol violations, and FDA's steps to uncover, punish, and prevent irregularities in gene therapy clinical trials.*

The rules and roles of federal agencies involved in overseeing gene therapy experiments are undergoing review and may be revised in the wake of Jesse Gelsinger's death. Specifically, the role of the NIH Recombinant DNA Advisory Committee (RAC) is being revisited, and proposed amendments to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* have been published. The harmony—or lack of it—between NIH and FDA adverse event reporting requirements in the conduct of clinical trials is also being scrutinized.

Revisiting the RAC. Before vacating the office of NIH director, Harold Varmus announced the formation of a subcommittee of the director's advisory group to recommend further actions NIH might take to minimize adverse events in gene therapy trials. Although the group's charge also places the role of the RAC back on the table, Varmus stands by his actions in 1995 to recast the RAC from a quasi-regulatory body with approval authority over every gene therapy application.to a public policy forum on novel methods in gene therapy clinical trials and thorny issues related to gene therapy (see interview, p. 10).

NIH Guidelines. NIH clarified the definition of adverse events and investigators' reporting obligations in a proposed action to amend the *NIH Guidelines*, published in the *Federal Register* November 22, 1999.

The proposal reaffirms that investigators must report serious adverse events immediately to NIH, so NIH may rapidly notify the RAC and others involved in gene transfer studies. "Immediately" is defined as no later than 15 days from the event. A "serious adverse event" is defined as any "expected or unexpected adverse event, related or unrelated to the intervention, occurring at any dose" that results in death, a life-threatening event, hospital admission or prolonged stay, or disability.

It also rejects recent claims by some gene therapy investigators and sponsors that human gene transfer protocols and serious adverse event reports are trade secrets. Informed consent documents would reflect the necessarily public nature of RAC discussions of adverse events.

The *NIH Guidelines* require the principal investigator to report serious adverse events to local review bodies and the FDA as well as to NIH and the federal Office of Protection from Research Risks. FDA reporting requirements require the study sponsor (who has presumably been informed by the investigator) to immediately report serious and *unexpected* adverse events to FDA. NIH proceedings are public; FDA proceedings are often closed.

During the RAC proceedings, industry representatives accepted the need for immediate reporting only of "related and unexpected" serious adverse events, and some RAC members voiced skepticism that they could deal meaningfully with reports of all adverse events. ■

—F.P.

patic vulnerabilities to tissue factor upregulation due to infection- or cytokine-induced endothelial cell damage.

RAC member Estuardo Aguilar-Cordova, of the Baylor College of Medicine in Houston, suggested that dose-related adenoviral toxicity may not be linear. "Perhaps there is a threshold effect at a certain point where only a slight increase in dose results in a great increase in toxicity," he said, noting that different species exhibit different levels of inflammatory response to the equivalent dose and, to make matters more complicated, titer and dose specifications are not standardized. "It's not clear we are all even talking in the same language," he commented, a lament later answered by Savio Woo, president of the American Society for Gene Therapy, who offered his group's services in developing standards for vector quantification.

Phase 1 adenoviral vector–gene therapy studies have been ongoing at the University of Pennsylvania for the past five years and have involved 95 patients, 18 of whom participated in the OTC study. Prior to his detailed account of that trial, Wilson summarized findings among the 77 other gene therapy patients enrolled in trials addressing cystic fibrosis and various types of malignancy. "In general," he said, "toxicities seem to be dose-dependent, time-limited, and confined to the target site." However, he added, there is a suggestion of "broader cytokine release" in the presence of fever.

Ron Crystal, chief of pulmonary and critical care medicine at New York's Cornell Medical Center, summarized safety data from gene therapy studies involving E1/E3-deleted adenoviral vectors of three different transgenes. Among 90 patients with cystic fibrosis, metastatic colon cancer, or coronary or peripheral vascular conditions that would benefit from angiogenesis, there have been more than 140 gene administrations, more than 44,000 follow-up days, and 13 deaths—all related to the patients' disease and unrelated to therapy dosage. One serious adverse event in a cystic fibrosis patient was linked to bronchoscopic delivery and has not recurred since a switch to aerosol spray, he said. Adverse pulmonary events were found to be dose related in animal studies conducted by the Genzyme Corporation (Cambridge, Mass.).

The OTC Trial

The prelude, conduct, and aftermath of the OTC trial were presented by Batshaw, Raper, and Wilson. Using the sparse fur mouse as a model, they delivered what would have been a fatal ammonia dose to animals pretreated with the adenoviral OTC gene vector. The ensuing rapid drops in glutamine and ammonia concentrations matched the speed the investigators sought to prevent the irreversible brain damage sustained by children who manage to survive coma longer than 72 hours. The adenoviral vector evolved from an E1-deleted to an E1-deleted/E2-mutated and, finally, to an E1/E4-deleted construct.

Although newborns with full-blown OTCD were the population for whom the gene therapy was ultimately targeted, it was decided that the study cohort for this first experiment to determine safety would be stable adults with partial disease who could give informed consent.

The investigators submitted their protocol application to the RAC in March 1994, when the RAC still had approval authority, along with the FDA, for all gene therapy protocols. In 1995, the RAC approved the protocol contingent on what members concluded would be a safer route of administration: intravenous, rather than intrahepatic. The FDA, however, later opted for intrahepatic delivery, in part to lessen the possibility of unintended delivery of the transgene to the gonads. The RAC was never informed of the protocol modification.

"We recognize that we probably should have gone back to the RAC to discuss this," Batshaw said, "but RAC's responsibilities were changing. . . . We apologize." Wilson issued the same apology during his presentation later that day.

FDA approved the protocol—with the intrahepatic artery route—in 1996; the first patient was dosed in April 1997; and Jesse Gelsinger, the last patient, was dosed in September 1999.

All told, four men and 14 women participated in the trial. High fever postinfusion was frequent, as were backache and nausea. Drops in platelet counts and phosphates were dose related and increased over the course of the cohorts (the protocol called for six cohorts, with three patients in each—the first two women and the last a man—to receive doses escalating in half-log increments beginning with 2 times 9^9 particles/kg and ending with 6 times 10^{11}). Similarly, transaminase elevations appeared to be dose related, but not consistently.

An odd and surprising finding related to antibody response to the vector: adenovirus-naive patients developed an expected lymphoproliferative response (LPR), but those who had antibodies to begin with experienced a "transient LPR loss," a phenomenon some panelists viewed as a safety concern in the context of inadvertent exposure to adenovirus infection during LPR downtime. This loss of T cell ability to respond to adenovirus, Wilson said later that day, was not seen in any other clinical or animal trials.

In general, the team was concerned about platelet lowering and its relation to vector dose. In collaboration with FDA, the team began DIC monitoring with the second cohort, tracking platelet counts and fibrin split products before, during, and after infusion and keeping an eye out for the presence of vector in the systemic circulation.

But it was not until the fourth cohort that there were recurring problems that the investigators were required by protocol to report to FDA before proceeding with subsequent dosing. These involved marked elevations in liver function assays, two of which were reported in a timely manner and two of which were not. Raper acknowledged these lapses: "We should have called FDA before dosing patient 14, and we should have made another call before proceeding to the next dose level," he said.

Other irregularities mentioned by FDA officials during the meeting but not addressed publicly by the investigators included the investigators' failure to get FDA permission to have a man (Jesse Gelsinger) replace a woman as the second patient in his cohort and that information in the consent form submitted to FDA—that primates had died on high doses in preclinical studies—was removed from the final consent document.

When Raper turned to the specifics of Jesse Gelsinger's clinical course, he did not address a question raised during the meeting: whether the investigators should have proceeded to infuse Gelsinger in the face of abnormal liver function tests that required alternative pathway therapy prior to dosing. He later implied that patient status before infusion should be rethought.

Fran Pollner

James Wilson details gene therapy trial events to RAC members, scientists, and the public.

Gelsinger's Course

Gelsinger's status eight hours postinfusion was not unlike that of other study participants. Raper said: he had a fever, normal liver function tests, and no evidence of DIC; he was given intravenous potassium phosphate for low phosphate concentrations. The morning after, however, nurses noticed an altered mental state; his ammonia concentration had risen, and he was jaundiced and tachycardic. A coagulation workup showed increased prothrombin time and fibrin split products and decreased platelets.

He became "progressively obtunded" and by the second evening he was comatose. Chest X-ray showed infiltrates suggestive of ARDS. Raper described a series of developments in Gelsinger and actions taken by medical teams to offset or reverse what eventually became what he called a "desperate lung situation." These measures did not save the patient.

Commenting generally on Gelsinger's clinical course and the efforts to save his life, UCSF's Warren later observed that "each intervention seemed logical and appropriate" but did not prevail against the "cascade of irreversible events."

With the Gelsinger family permission, Raper said, a postmortem examination was performed. There was little vector-induced hepatitis and no significant signs of DIC. Jesse Gelsinger died of intractable ARDS, with anoxia evident in the liver, kidney, brain, and spleen. The team speculated that ARDS was secondary to "secondary inflammatory response syndrome," referred to by Raper as "immune revolt."

Bone marrow yielded "perhaps the most unexpected findings: an absence of erythroid precursors and mature granulocytes, suggesting acute insult."

The team speculated that the anemia could be an idiosyncratic reaction to the medications or a sign of human parvovirus infection. One RAC consultant noted during discussions later that the one disease associated with such acute and striking bone marrow aplasia is parvovirus infection.

Questions

"We in no way expected to see what we saw in Jesse Gelsinger. Our animal studies never demonstrated these pulmonary events," said Wilson, who presented a chronology of the team's vector manipulations and animal studies—including mice, newborn and adult rhesus monkeys, and baboons—and ruminated on the cytokine findings in Gelsinger's case and the questions remaining to be answered.

Test animals, he said, had demonstrated self-limited, dose-dependent hepatitis, a transient decline in platelets, and transient transaminase elevations, with an apparently biphasic dose-toxicity relationship. Dose-limiting toxicities were liver damage and DIC at significantly higher doses, he said, than that received by Gelsinger. UCSF's Warren commented: "We have not seen the pulmonary dysfunction in our 60 patients that you saw with Jesse Gelsinger."

Wilson elaborated on Gelsinger's immune response to the vector, as well as vector biodistribution on autopsy. At the higher vector doses received by patients in the last two study cohorts, there were rapid, dramatic cytokine increases, specifically of interleukin-6 (IL-6) and IL-10. Recovery followed in all cases but Gelsinger's, whose IL-6 trajectory never

returned to base line. "Maybe we are activating some aspect of the immune system," Wilson said, suggesting that some "inciting event of acute cytokine release" from macrophages and monocytes had occurred. "What was different about this patient?" he asked—and listed hemodialysis, intubation, and external ventilation among the atypical procedures Gelsinger had undergone.

The investigators used PCR to track the vector after hepatic artery infusion. The highest DNA concentration was found in the liver—first in the macrophages (Kupffer cells) and then the hepatocytes—with significant amounts also appearing in the spleen, lymph node, and bone marrow. Biodistribution, Wilson remarked, "was not as hepatospecific as I had hoped." RAC co-chair Verma later noted that animal models are generally not good enough to pinpoint biodistribution. He cited the fact that rhesus monkeys and humans do not have the same CAR distribution.

After the death, the team studied the specific vector lot Gelsinger had received, as well as the equivalent of the preceding lot, and tested them at the same doses in rhesus monkeys. "Clinically, the animals did fine," Wilson said.

Among issues to be investigated, he said, are what role, if any, Gelsinger's underlying condition and medications played in his outcome; whether he had a genetic predisposition to such an outcome; why the vector distributed beyond the liver and whether this affected outcome; and what stimulated cytokine release and, again, whether this affected outcome. "We don't know the roles of IL-6 and IL-10," he emphasized.

Asked what he would have done differently, Wilson replied, "Including not having done this at all, the trial could have been designed differently [with respect to] the half-log increments. The increments should be smaller." He noted that the dose-toxicity relationship appeared to be "elbow shaped" and that at the half-log increment the difference in dosage between the later cohorts is significantly greater than that between the earlier ones and "may be breaching that elbow." A RAC member agreed that there appeared to be a "narrow window between early and severe toxicity, requiring meticulous measures."

FDA's investigation into the conduct of the UPenn trial was ongoing at *Catalyst* press time. ■

Gene Therapy: Preclinical Work at 5 Research Court


Fran Pollner

Robert Donahue

My purpose here," says Robert Donahue, in his headquarters at 5 Research Court in Rockville, Md., "is to demonstrate that novel and potentially therapeutic vectors are both safe and efficacious"—after they have been evaluated in small animals and before they are tested in human gene therapy trials. "It's important to make our program visible," Donahue said in an interview. "Preclinical testing enables us to predict whether a vector has the possibility of being pathogenic in humans."

Donahue directs the NHLBI program on gene transfer and bone marrow transplantation in nonhuman primates and, with NHGRI collaborators Rick Morgan and Jay Lozier, has been exploring the potential of an adenoviral vector for human coagulation factor IX as a therapeutic agent in hemophilia B. He is more enthusiastic about other viral candidates than adenovirus as a vector for gene transfer in the treatment of chronic deficiency disorders.

Retroviral vectors of murine origin and weakened or "gutless" human lentiviral vectors appear more promising as vectors that can safely target multiple cell types, he said. He and his team currently have six gene therapy projects underway, with a variety of collaborators, pursuing these vectors as conduits for gene expression in multiple lineages of hematopoietic cells. They're currently looking at green fluorescent marker genes, provided by jellyfish, that enable them to track both the gene and the protein product. They anticipate moving on to genes for drug resistance, factor IX, chronic granulomatous disease, the gamma chain for the T cell receptor in severe combined immunodeficiency disease, and proteins that could inhibit replication of such viruses as HIV.

The work with factor IX packaged in an adenoviral vector was originally a project in Morgan's lab, where Lozier developed the vector; it was brought to 5 Research Court for evaluation in nonhuman primates, Donahue recounted. Delivered alone, the factor IX protein was not immunogenic; delivered in the adenoviral package, however, both the protein and the vector were immunogenic. This suggests, he said, that an adenoviral vector could be quite useful in a vaccine construct, enhancing the immunogenicity of an antigen, but less as a therapeutic agent for delivery of a protein on a chronic basis. The team determined—and Lozier reported more than a year ago at a meeting of the American Society of Hematology and again in June 1999 at an American Society of Gene Therapy meeting—that expression of the gene for human coagulation factor IX could be achieved in rhesus monkeys using an E1/E3-deleted adenoviral vector. But the price was acute liver toxicity and coagulopathy.

The study dose ranged from 1 times 10^{10} to 1 times 10^{11} plaque-forming units (pfu)/kg. At the low doses, there was no gene expression; at the high doses, gene expression was accompanied by severe liver pathology in response to the vector. Among the findings were increased interleukin-6, decreased serum iron, and significant derangments in liver enzyme, albumin, and bilirubin concentrations and clotting time. These findings were formally published in the December 15, 1999, issue of the journal of the American Society of Hematology (Jay Lozier, Mark Metzger, Robert Donahue, and Richard Morgan. "Adenovirus-mediated expression of human coagulation factor IX in the rhesus macaque is associated with dose-limiting toxicity." *Blood* **94**:3968–3975, 1999). They were also cited during the meeting of the NIH Recombinant DNA Advisory Committee to review adenoviral vectors and the death of a patient in a University of Pennsylvania gene therapy trial (see "Gene Therapy Trial and Errors," p. 1).

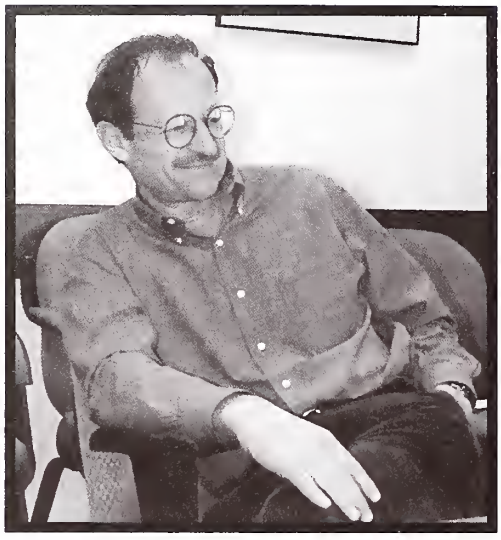
"In our study," Donahue said, "administration was intravenous; in the Pennsylvania study, a higher dose was administered directly to the liver, which led to even more damage." He speculated that vector distribution beyond the liver in that study could be attributed to the high dose and noted, too, that the liver's Kupffer cells are a type of macrophage that processes antigen and releases hematopoietic growth factors that activate an immune response. "There's a very narrow window between efficacy and toxicity," he observed, noting that converting animal to human dosages in this context demands precision and must take surface area into account. Formulas for computing comparable doses in rodents, dogs, monkeys, and humans can be found in an article by former NCI director Vincent DeVita in the text *Cancer: Principles and Practice of Oncology*, he said. ■

—Fran Pollner

Harold Varmus: Like a Rolling Stone (with Receptors)

by Fran Pollner

In the last month of the twentieth century, Harold Varmus took his leave of NIH. He offered "valedictory thoughts" to his scientific advisory committee at his final meeting with them; he bid fond adieu to the NIH community gathered in Masur Auditorium to laugh away the pangs of parting (see "Coda," page 9); and he obliged reporters from *The NIH Catalyst*, the *NIH Record*, *Nature*, *Science & Government Reports*, *Washington Fax*, and the *Blue Sheet* with a farewell interview.



Fran Pollner

Harold Varmus: "I'm going from one very good job to another very good job."

Time To Go

Why are you leaving? asked a reporter. "It began to feel repetitive," Varmus said, especially the annual appropriations process. And, he added, there was also the matter of "timing": "To have gone deeply into my sixties would have reduced the chances of getting another really good job—and this particular job became available now," he said of the opportunity to head the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City. "In an ideal world, I probably would have left [NIH] one year after the next administration came in"—in which case he would have gone through two more of those appropriations shuffles he does not relish but which he counts among the priorities for the NIH leadership.

It's up to NIH leaders to chart and promote the "right flight path" for future budgets, he said. It's also up to them to recognize that technologic advances and the "genomic revolution" could drive "deeper divisions in access," in which case, he told his advisory group, "we won't have achieved the NIH mission of improving the public health and we will look like an institution that serves the elite." Similarly, he told reporters of his concern that "we're going to cut a significant proportion of the population out of the benefits of certain approaches to health that are paid for with public money and that ought to be publicly accessible." Current NIH initiatives related to research into health disparities should help, as should tapping the wisdom of the NIH director's Council of Public Representatives (COPR), an activity his successor would be well-advised to engage in seriously and often, he said.

"I was slow to appreciate the importance of a nonsectarian council like COPR, a good sounding board," he said, for scientific, political, and ethical matters, especially equity and health disparity issues.

Infrastructure

Varmus repeated his suggestion that NIH be reconfigured into perhaps five "clusters" that would logically organize existing centers and institutes and discourage add-ons, but such a proposal from him or any NIH official, he said, would appear self-serving. Rather, he hopes Congress or the National Academy of Sciences will some day arrive at a similar conclusion based on independent study, and then such a reorganization could be accepted and implemented. His own dim view of proliferating NIH institutes is well known, he noted. Far better, from both scientific and administrative viewpoints, are NIH's new interdisciplinary Neuroscience Center and the new transinstitute Office of Bioimaging and Bioengineering (asked for and received in the appropriations bill).

But there are still some components of the intramural research program that "are too far removed from the centers of activity. These [geographically] outlying groups are a worry," he said.

Nearly out the NIH door, Varmus nonetheless used the word "we" frequently in addressing future courses of action for NIH. It was also clear, however, that *his* future at the biomedical complex on New York's East Side was close upon him and he was eager for it.

The Varmus Lab

"I will be at a place," he said, "where cancer research and cancer treatment coexist—at a strong institution, with interesting neighbors (New York University–Cornell Medical Center, Rockefeller University) and many good friends and colleagues." And, of course, he'll be taking his lab with him—a lab, he proudly said as the interview opened, that had "just received a quite favorable review" from NCI. He noted with bemusement that someone had asked, "Who's going to run the Varmus lab now?" as if it were a permanent entity at NIH and not the work-in-progress of the person who brought it to NIH and would be taking it with him when he left.

Fran Pollner

"When recombinant DNA came to the table 25 years ago, society was shocked; no one had thought much about ethical issues in biology. In general, we've become much better at this."

Indeed, at the MSKCC, Varmus will have more time to spend in that lab than he did at NIH, where, he said, he really couldn't "settle in" in the hour or so a day and off-hours that he had there. Half his time in New York, he said, will be in his hospital executive office and the other half will be in his lab. The administrative aspects of the new job may actually be more onerous than his counterpart tasks as NIH director, which dealt less with administrative details than with the broader policy issues he enjoys grappling with. Nonetheless, it's the science that's most compelling, and the science in the Varmus lab, he said, has been centered on "two major themes":

■ Getting better models for studying human cancer in the mouse. No longer confined to making transgenics and crossing them with knockouts, the Varmus team has been using viral vectors to deliver conditional genes to organs expressing target receptors. The repertoire has included oncogenes, tumor-suppressor genes, marker genes, and recombinases, or genes programmed to be rearranged. They've been working with tumors characteristically hard to grow in cell culture, such as gliomas and breast tumors, and are crafting models for lung, pancreatic, and ovarian cancer. Ultimately, knowledge of gene abnormalities in human cancer should yield therapeutic and preventive interventions that are less toxic and more effective than currently available agents. It should also yield a more profound understanding of metastasis.

■ Understanding how growth and differentiation factors (such as WNT proteins, fibroblast growth factors, and hedgehog and TGF-β genes) signal the cell nucleus to alter the way the cell behaves. Varmus's interest in signaling was "activated" about 20 years ago, he said, when he and his colleagues discovered the first *WNT* gene and traced the role of *WNT1* in cancer development after the insertion of viral DNA from the mouse mammary tumor virus.

The World at Large

Asked if he would miss his role in the international arena, working on such problems as malaria, he replied: "I'd miss it if I lost it, but I don't think I will." He'll likely be a consultant to the World Health Organization and will soon be traveling to India; he'll also continue his involvement in PubMed Central, which, he said, is an "important issue in international health and the support of science in the developing world."

"I plan to make a tape [of some of the 'goodbye party'; see below] to show at Memorial Sloan-Kettering, so people can see how we have fun here and how I plan to interact. . . ."

Beyond that, he'll be rejoining the steering committee of the American Society for Cell Biology, from which, he said, he was apparently granted a "six-year leave of absence." And he's been asked to join the "p53 Club" in New York, as well as many other clubs, athletic and scientific, that he will consider once he figures out how much time his job leaves him for other pursuits. Among other things he looks forward to are New York's artistic offerings, having to answer to "fewer bosses," and "more flexible interactions with the private sector," although he intends to "serve only on nonprofits."

Recollections

But he will leave behind the NIH intramural program, which "from the first day I arrived here, I've really enjoyed"— his scientific collaborations, the seminars, the interest groups, the "esprit," and the campus building boom. He lauded the speed with which NIH was able to respond to a perceived national need—both physically and intellectually—with the building of the Vaccine Research Center and recruitment of Gary Nabel

CODA

Fran Pollner

The Party's Over: *As the NIH troops filed out, a few remained for parting photos (left to right): HHS secretary Donna Shalala, Tracey Rouault (NICHD, keyboardist), now acting NIH director Ruth Kirschstein, Chuck Allerson (NICHD, drums), NIAID director Tony Fauci, Francis Collins (NHGRI director, "lead guitar"), Harold Varmus, Connie Casey, Richard Klausner (NCI director, "lead guitar"), Steve Katz (NIAMS director, "lead guitar"), and John O'Shea (NIAMS, bass, "the only one who knows how to play a guitar").*

—band hierarchy provided by Richard Klausner

Celebrating Harold Varmus and, at the same time, saying goodbye to him is what "bittersweet" is all about, said Tony Fauci, NIAID director and emcee at a farewell sendoff for Harold Varmus in the month before the NIH director would officially leave his position to head to New York City and the Memorial Sloan-Kettering Cancer Center.

"It's been "six years and 23 days and counting," Fauci said, as if when that ball dropped 15 days later—at midnight in Times Square—the roar from the crowd would not be "Happy New Year" but "Welcome, Harold!"

Paeans mixed with jokes for a couple of hours as farewell messages rained down upon Harold Varmus. HHS Secretary Donna Shalala was on hand to voice her deep respect for Varmus' brilliance—and his unique sense of fashion.

And a videotaped "documentary" from the "troops" showed that however much Varmus may inspire awe from the world at large, he's more than fair game for the inspired wit of his NIH colleagues, about a dozen of whom deadpanned hilarious answers to such questions as: "Why did Harold Varmus hire you?"

The event rocked to a close with a stellar performance by "The Directors," NIH's unparalleled rock band, and their doctored lyrics of sixties hits that ended with the exhortation "Oh, won't you stay . . . just a little bit longer . . . . please, please, please, please . . . say-ay you-ou will. . . ."

But he wouldn't. ■

—F.P.

as its director.

For none of these aspects of NIH for which he expressed appreciation did Varmus take credit. He even punctuated his praise of the Clinical Center's activity level and clinical research on campus in general with an aside that though he himself did not contribute to the clinical side of NIH studies, his research had benefited from its resources, such as the small animal imaging facility.

But he had some good things to say for his handling of several matters as NIH director.

On patents: "I chose not to pursue intellectual property rights on anonymous cDNAs, and I think that was the right decision. Why prosecute a claim you don't think should be made? I'm pleased the Patent Trademark Office is closer to our position now: that the specific utility must be known in order to patent a squence."

On material transfer agreements: "We're not trying to lay down a strict law but to move the scientific community to a more generous mode of behavior. We had a major success with Cre-Lox technology, and although our hold is less secure with corporate entities that don't have NIH funding, the movement is in the direction of greater sharing."

On PubMed Central: "Once it's unveiled, the public interest will soar."

On stem cell research: "This issue will continue to heat up. I look back with a sense of pride at the Human Embryo Research Report of 1994, which was prescient [regarding issues raised in 1999]."

On his past actions to recast the role of the NIH Recombinant DNA Advisory Committee (RAC): "I have no objection to its being put on the table [that RAC again approve gene therapy protocols], but I think it's the wrong way to go. Jesse Gelsinger's death would not have been prevented if the RAC had approval authority, and NIH is not a regulatory agency [see *Catalyst* coverage of the RAC meeting in December on these matters, page 1]. I don't think we should respond to this unfortunate episode by returning RAC to what was a somewhat dysfunctional state. Now it's working well."

Asked if he had any disappointments, he said he rued his lack of visible success in increasing the numbers of investigators from minority backgrounds. "I haven't seen major changes. . . . Perhaps you couldn't in just six years." ■

MAGTAGS

continued from page 1

migration in autoimmune disease," Frank suggests.

Many types of cells, he says, will take up the nanoparticles of oxidized iron in LDRR's magnetic tagging system. If the appropriate number of cells are then injected back into a living body and then home in on any soft tissue, Bulte and Frank predict they will generate detectable signal changes on MRI.

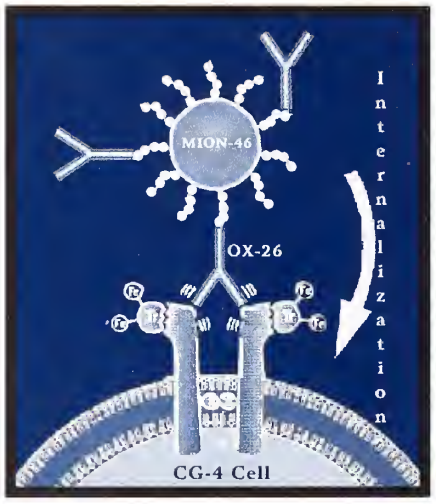
"People have wanted to do this for a long time," says Bulte, and he himself could barely believe it when the research team first injected and imaged magnetically tagged cells in an animal.

"I thought I was looking at charcoal particles" that had been used to mark the injection site "or some other artifact," he recalls. The key to LDRR's success, according to Frank, was getting a high degree of magnetic labeling of cells.

The LDRR group constructed their first-generation magtag by attaching a nanoparticle of maghemite to a monoclonal antibody to the rat's transferrin receptor—a receptor found on almost all cells in all species of animals. The cell surface receptor binds the antibody and then rapidly internalizes the iron, stashing it away in endosomes. The iron oxide in the tag is superparamagnetic, the LDRR scientists explain. This means that cells loaded with the tag are magnetized only when they are in the strong magnetic field of an MR scanner. Tagged cells retain no magnetic memory after being scanned and will not clump with one another or with metallic materials.

The researchers believe the nano-particles of iron eventually get used by the cell and then recycle through the animal's iron pool. The team has been able to follow magtagged oligodendrocyte progenitor cells for 42 days after transplantation into the rat. They have watched the cells slowly migrate from the injection site.

Bulte says there is almost no chance of overloading an experimental subject with iron. Humans, for example, typically have 4 grams of iron in their bodies, and experimental dosing would be in the microgram range. Bulte found that the iron tag did not impair the migration or other functions of labeled cells. In fact, he says, "you can take these cells back and put them in culture" and they will thrive. He points out that birds, butterflies, bacteria, and other organisms with innate directional sense all use nanoparticles of iron in their internal compasses.

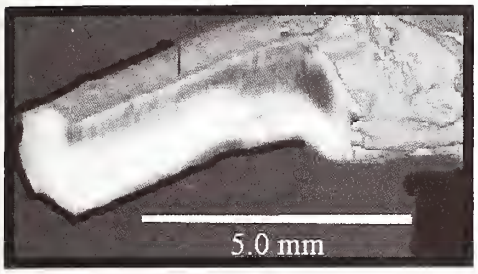


Original magnetic tagging system developed by the LDRR team and their colleagues shuttles nanoparticles of iron oxide (MION-46L) into cells via a monoclonal antibody (OX-26) to the cell's transferrin receptor. Cells internalize the superparamagnetic nanoparticles, ultimately leading to the visible marking of the cells during magnetic resonance imaging.

In their initial experiments, Frank, Bulte, and Peter van Gelderen of the NIH In Vivo NMR Center viewed the magtagged cells with a 4.7 Tesla MR instrument. But they have since shown that they can follow injected cells with a 1.5 Tesla instrument—a field strength that can be used clinically in humans. This field strength showed signal changes from a few millimeters of rat spinal cord bearing 50,000 or so tagged cells injected during the group's experiments.

These days, Frank says, clinical MRI has a resolution of 1 mm in the human brain, and the team expects to be able to reach this level of resolution, or better, when magtagged cells make their way into clinical applications.

For animal imaging, the potential resolution could be much greater, however, with the higher field strength MR scanners now being installed in the In Vivo NMR Center. With the current 4.7 Tesla instrument, the team can resolve patches



Three-dimensional reconstruction magnetic resonance image of rat spinal cord shows distribution of magnetically tagged cells 10 days after the tagged oligodendrocyte progenitor cells were injected into a myelin-deficient animal. This image was captured by Peter van Gelderen of the NIH In Vivo NMR Center using a 4.7 Tesla MR imager.

as small as 78 micrometers. At least in animal models, they expect to be able to resolve as few as five to 100 tagged cells in living animals, depending on the location of the cells within tissue. Bulte says one factor boosting the system's sensitivity is a "blooming" or magnetic susceptibility effect—an amplification of signal from surrounding water molecules that occurs when tagged cells are excited in the MR field.

Fittingly, the first research employing the new technique involved a central nervous system study that appeared in the final issue of the *Proceedings of the National Academy of Science* in the Decade of the Brain [*PNAS* **96**:15256–15261, 1999]. The work sprang from LDRR's research on repair of dysmyelination and demyelination—damage or loss of the myelin sheath around axons seen in multiple sclerosis and other neurological diseases.

Su-Chun Zhang and Ian Duncan of the University of Wisconsin's School of Veterinary Medicine injected oligodendrocyte precursor (CG-4) cells into the spinal cord of myelin-deficient rats. Because the injected cells had been magtagged, the researchers were, for the first time, able to follow the migration and integration of the cells into the nervous system noninvasively.

The distribution of magtagged cells on their three-dimensional MR images correlated closely with myelination in the rats, shown subsequently by sacrificing the rats, dissecting the spinal cord, and looking at the distribution of the cells with traditional histopathological techniques 14 days post-transplantation. Bulte says the high resolution, MRI-computer-generated sections of tissue were actually more helpful in interpreting the traditional anatomic sections, rather than vice versa.

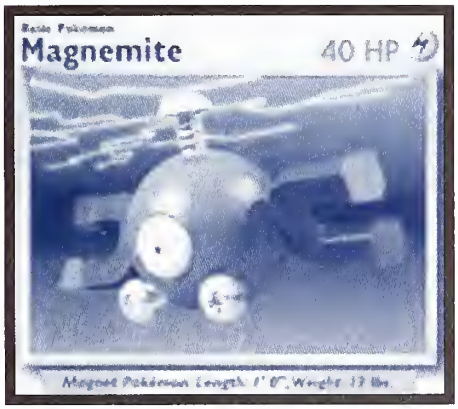
Since establishing the efficacy of their technique, the LDRR scientists have gone on to document the migration of oligodendrocyte precursors in young normal rats. Along with their collaborators, they are planning studies to track migration and remyelination of stem-cell derived oligospheres injected into a myelin-deficient dog developed by the Wisconsin group.

Frank says he foresees no major technical barriers that would block use of magnetic tags in humans. "It's just a matter of time." Tracking neural stem cells inserted in the brain or spinal cord to repair neurodegeneration or trauma-related injury would be natural extensions of LDRR's magtag work.

In tandem with their substantive research, LDRR scientists have also been improving upon and developing the original magtag technique, for which they filed a patent application. They now have a second-generation magtag that is not dependent on species-specific monoclonal antibodies or even on the transferrin receptor. Like the first-generation tag, the new marker—a coated polymeric iron compound—shows high affinity for cell membranes and will work on any cell type.

Although the magtags have been developed to be used on a broad range of cells and to applied ex vivo, Frank says it should be possible to adapt the tagging system to target individual cell types via specific receptors and to label these cell populations in vivo. But targeted delivery of magtags would be another frontier with additional hurdles and probably couldn't be explored for a few years—at least until the researchers have mapped the research continent they just discovered.

Bulte observes that the group is not the first to use magnetism in navigating uncharted territory: "Human explorers made their greatest discoveries only after the compass was invented." ■



Staff scientist Jeff Bulte showed this picture of one of his daughter's Pokémon game cards at a recent professional meeting, but strenuously denies that the Pokémon character was the inspiration for LDRR's magbemite cell-tagging system.

Averting Violence

CIVIL, a newly established coordinated NIH resource, is now available to help the NIH community prevent and respond to workplace threats and violence.

If you feel there is **IMMEDIATE DANGER:**

Dial 911, if on campus
Dial 9-911, if off-campus

Call CIVIL if:

- You need help assessing the potential seriousness of a threatening situation;
- You are experiencing a threatening situation at work and need intervention from trained staff;
- You become aware of a workplace situation involving intimidating, harassing, or other unproductive/dangerous behaviors and need consultation; or
- A situation involving threats or aggressive acts already has occurred and you need assistance managing the aftermath and its effect on staff.

Anyone can call CIVIL directly by dialing C-I-V-I-L or 402-4845.

Call for Abstracts

The NIH Bioengineering Consortium (BECON) symposium on "Nanoscience and Nanotechnology" is set for June 25–26. For info, contact

<http://grants.nih.gov/grants/becon/symposium2000.htm>.

Recently Tenured

Henry Levin *received his Ph.D. in the lab of Howard Schachman at the University of California, Berkeley, in 1987. He did his postdoctoral work at Johns Hopkins Medical School before joining the NICHD Laboratory of Molecular Genetics in 1993. He is now a senior investigator in the Laboratory of Eukaryotic Gene Regulation.*

Fran Pollner

Henry Levin

My interests lie in the proliferation of retroelements, the most medically important class of which are the retroviruses. The genetic complexity of the host vertebrates for retroviruses, however, complicates the study of particle assembly, reverse transcription, nuclear entry, and integration into chromosome.

Our approach to understanding these events is to study retrotransposons, a family of elements closely related to retroviruses that offer a significant advantage: They exist in yeast, a host that can be studied using the powerful techniques of molecular genetics.

The retrotransposon we study is the Tf1 element of fission yeast, *Schizosaccharomyces pombe*. Like retroviruses, Tf1 encodes a protease, reverse transcriptase, and integrase. We have developed a genetic assay for Tf1 transposition that has allowed us to study several aspects of its propagation.

One of our early results was perhaps the most surprising. Although all long terminal repeat retroelements were thought to initiate reverse transcription from specific tRNA primers, we showed that tRNAs are not essential primers of reverse transcription. We found that Tf1 undergoes an unusual mechanism of self-primed reverse transcription that defines a new family of retroelements. In place of a tRNA primer, the first 11 bases of the Tf1 mRNA anneal to the primer binding site, and a nucleolytic cleavage at the 12th base allows the first 11 ribonucleotides to prime reverse transcription.

A series of genetic and biochemical experiments identified a complex structure in the Tf1 mRNA that is essential for priming. Despite the novelty of this mechanism, we identified structural features within the self-priming mRNA that bear surprising similarity to sequences in the mRNA of retroviruses. The similarities of the self-priming mRNA to RNAs of many other retroelements have motivated us to continue our analysis of the self-priming mechanism.

One of the principal goals of our research is to identify host functions that are necessary for retrotransposition. As we characterize these basic features of cellular biology, we can simultaneously identify potential targets for antiviral therapies. We screened randomly mutagenized strains of *S. pombe* and identified genes that are necessary for transposition. Much of our effort is focused on Nup124p, a nuclear pore factor that is required for the nuclear import of Tf1. Strains with mutations in *nup124* showed normal growth rates and exhibited no defects in the nuclear import of other nuclear proteins or in the nuclear export of poly(A) mRNA.

The specificity of this import pathway for Tf1 is likely due to the direct interaction between Nup124p and Gag that we detected. The contribution of Nup124p to the nuclear import of Tf1 bears significant similarity to the nuclear transport of HIV in that the Vpr protein of HIV mediates the import of HIV via an interaction with specific nuclear pore factors. This particular similarity suggests there may be common properties of large viral complexes that require a specialized form of nuclear import, and we are continuing our analysis of Tf1 import to test this possibility.

In addition, we are exploring our collection of mutant strains for evidence of other genes that contribute to the import of Tf1.

Charles Rabkin *received his M.D. from Brown University (Providence, R.I.) in 1981 and his M.Sc. in epidemiology from the London School of Hygiene and Tropical Medicine in 1988. He trained in internal medicine at the University of Colorado in Denver. He was an Epidemic Intelligence Service officer and medical epidemiologist at the Centers for Disease Control and Prevention before joining NCI in 1989 and is now a senior investigator in the Viral Epidemiology Branch, NCI.*

Charles Rabkin

My research combines epidemiologic approaches to cancer in human populations with the evolving tools of molecular genetics. My primary interest is the molecular mechanisms of HIV-associated cancers, focusing on non-Hodgkin's lymphoma and Kaposi's sarcoma. AIDS-related cancers are an important model for investigating the role of specific mutations in carcinogenesis.

With colleagues in NCI's Laboratory of Genetics, we demonstrated that HIV-infected-but-lymphoma-free individuals frequently harbored circulating lymphocytes with the t(8;14) chromosomal translocation characteristic of Burkitt's lymphoma. Translocation prevalence increased with duration of HIV infection, and aberrant clones could persist for many years without evolution into non-Hodgkin's lymphoma. We also found that these individuals did not have increased prevalence of follicular (that is, non-AIDS-related) lymphoma-associated t(14;18) translocations, demonstrating the specificity of HIV's effect.

Having identified somatic mutation as an early event, I am now concentrating on abnormalities in advanced HIV infection that may control the rate of tumor development.

With collaborators in the NCI-Frederick Cancer Research and Development Center, we have also examined germ-line mutations and recently found that a common polymorphism in the *SDF-1* chemokine gene strongly increases lymphoma risk in AIDS, whereas a variant of the chemokine receptor gene *CCR5* is highly protective. Because the *SDF-1* variant is four times as common in whites as in blacks, these data may explain racial differences in AIDS-lymphoma risk that our group and others previously reported.

My Kaposi's sarcoma studies have examined the neoplastic nature of this disorder. In collaborations with several laboratories, we found that Kaposi's sarcoma tumors appear to derive from clonal replication of a single cell. By combining

microdissection with the clonality assay, we then found that spindle cells from multiple Kaposi's sarcoma lesions from the same patient appeared to be clonally related, suggesting the disease derived and disseminated from a single cell. These experiments have been based on the X-linked androgen receptor (HUMARA) clonality assay, and I am currently attempting to validate and extend these findings with more robust techniques.

My other major interest is in infectious mechanisms in gastrointestinal cancers. *Helicobacter pylori* infection is associated with both gastric cancer and nonmalignant duodenal ulcer disease. The reasons for these divergent clinical outcomes are not clear, but the gastric physiological response is influenced by the severity and anatomical distribution of *H. pylori*–induced gastritis. Our group is focusing on polymorphisms in the genes regulating inflammation and immunity as possible determinants of gastric cancer and its precursors.

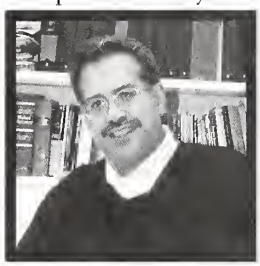
Another endeavor is to better determine the long-term prognosis of hepatitis C infection—currently a difficult proposition. The risk of liver cancer in particular is controversial. In collaboration with NIDDK, we ascertained the health outcomes after 45 years' follow-up of initially healthy hepatitis C–seropositive young men. Liver-related morbidity and mortality were low, suggesting that the risk of progressive disease may be less than is currently perceived.

Heavy use of alcohol and other substances may alter this natural history, however, and we are extending these studies with a unique collection of sera and liver biopsy specimens of hepatitis C–seropositive injection drug users followed for as long as 25 years.

Infection-related cancers are fruitful areas for research of fundamental mechanisms of carcinogenesis. Better understanding of these processes should help in the development of targeted interventions for cancer prevention.

Juan Rivera *received his Ph.D. from the Catholic University of America (Washington, D.C.)in 1990 and did his postdoctoral work in the Arthritis and Rheumatism Branch of NIAMS, where he is now a senior investigator and head of the Signal Transduction Group.*

My interests are in the area of receptors that bind the crystallizable fragment of an antibody (Fc receptors) and their role in inflammation, with a focus on how these receptors transduce signals that result in gene expression. The underlying objective is the discovery of the receptor-proximal molecules that link the immune complex activation of Fc receptors to expression of particular cytokine genes. I see this as an important step in understanding how Fc receptors contribute to the process of inflammation in health and disease. Hopefully, these studies will facilitate development of better therapeutics that target those mediators that contribute to inflammatory disease, without affecting the production of others that may be beneficial for recovery.



Fran Pollner

Juan Rivera

Fc receptors can either activate or inhibit cell effector functions. The subfamily of activating receptors share in common the Fc receptor gamma signaling subunit. My colleagues and I study the molecular signals initiated by the activating Fc receptor with high affinity for immunoglobulin E (IgE) that is expressed on mast cells and is involved in allergy and inflammation. We focus on identifying IgE Fc receptor–activated signaling molecules that could modulate cytokine gene expression.

We found that selected members of the protein kinase C family participate in IgE Fc receptor induction of the early response genes c-*fos* and c-*jun* and cause a selective induction of IL-2 and IL-6. In addition, we discovered that another protein kinase C isoform selectively phosphorylates the IgE Fc receptor and that this event is important in creating an appropriate surface for the binding and activation of Syk, the kinase critical to propagating signaling and mast cell effector function. We observed that in the absence of this protein kinase C–mediated phosphorylation there is a loss of cytokine production. These studies demonstrated that Fc receptors use different members of the protein kinase C family to either selectively or generically influence the production of cytokines.

We are now investigating how the formation of macromolecular signaling complexes contributes to the regulation and specificity of Fc receptor cytokine gene expression. Much of our effort is focused on Vav, a guanine nucleotide exchange factor that is selectively expressed in hematopoietic cells. Our progress in this area relied on the development of a versatile gene expression system based on the Semliki Forest virus that can be used to overexpress or restore proteins of interest in most primary and immortalized cell lines tested. We found that Vav modulates selected cytokine expression in mast cells. We also found that Vav moves from the cytosol to detergent-insoluble plasma membrane domains, or rafts, where activated IgE Fc receptors also reside. Inhibition of the redistribution of Vav results in inhibition of c-*jun* NH_2-terminal kinase activity, an activity required for expression of selected cytokines. In addition, we found that Vav co-immunoprecipitates with the raft-localized scaffold protein linker of activated T cells (LAT) in mast cells. In recent collaborative studies, we found that LAT-null mast cells show ablation or decreased production of most cytokines tested. Thus, macromolecular signaling complexes are seemingly comprised of constituents that individually influence selected genes but collectively affect many.

We are currently investigating how Fc receptors engage the macromolecular signaling complexes and are trying to determine the importance of each signaling complex constituent in cytokine gene expression. We expect to explore the role of the constituent proteins in inflammatory disease in animal models.

CATALYTIC REACTIONS

An anonymous reader responded to two questions posed in the last issue: 1) What's the biggest challenge for the next NIH director? and 2) Can you suggest any new rules as a basis for biomedical research for the next 100 years?

1) Living up to the high standard set by Dr. Varmus.

2) Requiring PIs to actually follow the guidelines for authorship set forth in the NIH Fellows handbook. It's a shame when one postdoc analyzes and writes another postdoc's paper. ■

People

Recently Tenured

Sandra Swain *received her M.D. from the University of Florida in Gainesville in 1980. She did an internal medicine residency at Vanderbilt University in Nashville, Tenn., and an oncology fellowship at NCI, where she supervised breast cancer clinical trials until 1988. After serving as director of the Comprehensive Breast Center at Georgetown University's Lombardi Cancer Center (Washington, D.C.)and then as medical director for Salick Health Care, Inc., she returned to NCI, where she is deputy branch chief of the Medicine Branch and a senior investigator in the Developmental Therapeutics Department.*

Fran Pollner

Sandra Swain

My primary area of interest is breast cancer clinical research. I have designed, implemented, participated in, analyzed, and published findings from numerous clinical trials, ranging from Phase 1 to large, multicenter Phase 3 trials. Crucial to my understanding of translational research issues was my work on a project in the laboratory of Marc Lippman that led to the discovery of pleiotropin, which is implicated in angiogenesis.

I have participated in several national cooperative group trials and am currently on the breast cancer committee of the National Surgical Breast and Bowel Project (NSABP). I am also national principal investigator for the node-positive NSABP clinical trial (NSABP B-30), which is accruing 4,000 patients at the rate of 105 patients a month. The trial has three treatment arms and is designed to determine the effect of docetaxel on survival and quality of life in women who are node-positive at breast cancer diagnosis. This study will capture the effect of menopause by including and following women who are premenopausal at diagnosis. As national PI, I review ongoing trials, with particular attention to toxicities.

Earlier in my career, I supervised an NCI study of the effect of chemotherapy before local therapy in patients with locally advanced breast cancer, a study whose results led to a larger version of the trial in the NSABP B-18. I plan to continue research in this area to evaluate new agents that could be used in the context of pre- and posttreatment biopsy for gene discovery and protein expression analysis.

The cardiotoxicity of some anticancer agents is another of my research interests. I participated in the design, implementation, conduct, and data analyses of two national, multicenter, placebo-controlled, randomized studies that showed that dexrazoxane, an iron-chelating agent, could decrease cardiotoxicity in breast cancer patients taking doxorubicin.

These findings were the basis for approval of the drug by the Food and Drug Administration in 1995, and dexrazoxane remains the only drug approved for cardioprotection in oncology. I plan to continue working in the area of cardiotoxicity.

My service on the FDA's Oncology Advisory Committee, as a member from 1994 to 1998 and continuing as a consultant, has provided invaluable understanding of the scientific and regulatory processes that underlie the determination of safety and efficacy of new therapeutic modalities. This experience will serve me well in my ongoing work in drug development and clinical trial design.

David Wink *received his Ph.D. in chemistry at the University of California, Santa Barbara in 1985. Following a postdoctoral fellowship in biochemistry as a National Research Service Award recipient at the Massachusetts Institute of Technology in Cambridge, he joined the Laboratory of Comparative Carcinogenesis at NCI-FCRDC as a staff fellow. In 1995, he joined NCI's Radiation Biology Branch, where he is now a senior investigator.*

Fran Pollner

David Wink

My general aim is to elucidate the chemical reactions of small redox molecules and their importance in different physiological and cellular mechanisms. Since coming to NCI, I have been particularly interested in the role of nitric oxide (NO) in regulatory mechanisms as well as in pathophysiological conditions. Appreciation of the importance of NO in biology has increased exponentially, making it one of the fastest growing fields in biomedical research.

My early work concentrated on elucidating different chemical mechanisms of NO and how these reactions might be involved in different pathophysiological conditions. We began to define the chemistry of NO in toxicological and carcinogenic mechanisms and to identify different molecular targets that are modified by NO and related chemical species. My current research interests center on the chemistry and biochemistry of free radicals in cellular and tissue damage and how these reactions can be modulated. We have explored some of the basic chemical mechanisms of reactive oxygen species (ROS) formation from metal-peroxide interactions as well as reactions involved with NO and the effect these reactions have on biological systems.

One important discovery was the selective inhibition of specific DNA repair enzymes by NO. These mechanisms were shown to prevent the repair of DNA lesions induced by alkylating agents as well as ROS. In these studies, we have catalogued and defined the effects of the interaction of different radicals on cytotoxic mechanisms. For instance, NO serves to protect mammalian cells from hydrogen peroxide and xanthine oxidase-mediated cytotoxicity. Yet in prokaroytes, NO in combination with peroxide and xanthine serves as an impressive bactericidal agent. Correlation of the different effects NO has on the cytotoxicity of other agents with the chemical mechanisms has been described in what I refer to as the "chemical biology of nitric oxide."

This concept has provided a guide to understanding the free radical chemistry of NO in biological systems (D.A. Wink and J.B. Mitchell. "The chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radic. Biol. Med.* **25**:434-456, 1998). We have collaborated with several groups to explore the role of NO in infectious diseases, ischemic reperfusion injury, and neurological disorders.

We became particularly interested in the effect of NO on increasing the toxicity of chemotherapeutic agents and radiation. This has resulted in a major new challenge—to apply what has been learned in the

chemical biology of NO to improving cancer treatment. We have investigated the use of free radicals to modulate modalities of cancer treatment. Nitric oxide can radiosensitize hypoxic cells. In addition, recent results show that NO dramatically enhances the cytotoxicity of some chemotherapeutic drugs including melphalan and cisplatin. A major objective is to selectively target tumor sites as opposed to normal tissue. We have taken two approaches. The first is the use of exogenous chemical agents that can specifically target tumors and deliver NO. Second, because there are reports that certain tumors may exhibit nitric oxide synthase (NOS) activity, we have begun to explore different strategies of inducing NOS directly in tumors, thereby providing a source of NO to the tumor directly.

Throughout this research, we have had to explore different methods to deliver the NO redox chemistry to cellular models as well as in vivo. This has required the development of an analytical repertoire and synthetic systems that mimic redox reactions. Using a variety of analytical methods, we can determine the production of NO, RNOS, and ROS derived from different stimuli of the immune system. We can then develop a corresponding chemical model that can be used to treat cells and probe different molecular aspects of redox chemistry. We have been mapping the chemical effects on molecular targets to understand in vivo mechanisms of NO and to define the redox chemistry required to improve therapeutic outcomes. ■

Going Places

The Office of Research Services announces that a new performance-based contract has been awarded to **WorldTravelService (WTS)**. The transition began November 15, with full implementation and performance standards effective January 18, 2000.

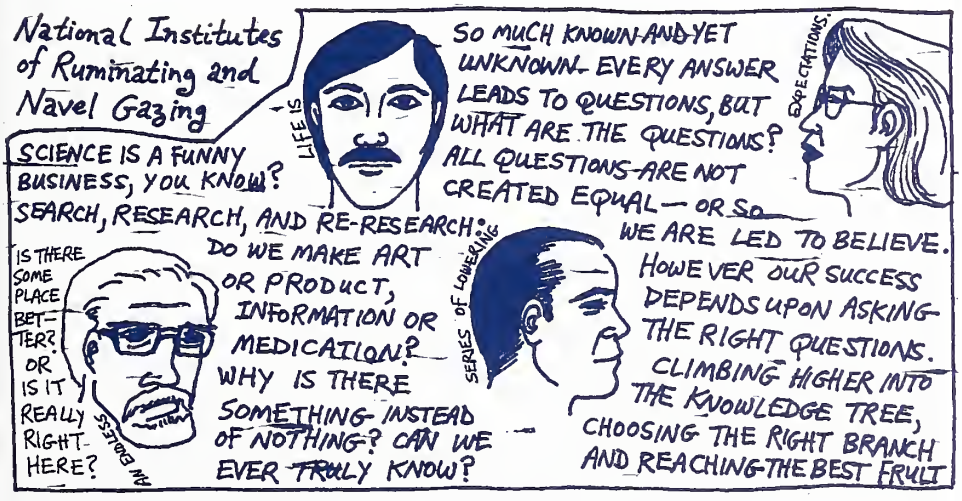
WTS bring NIH:

- Reservations via e-mail and fax
- Lowest fare guarantees
- Meeting planning services (for a fee)
- Upgraded telephone and fax system
- Web site dedicated to NIH travelers
- 24-hour emergency customer support
- Electronic booking (in the future)

A new requirement for this contract entails a **nonrefundable service fee,** which will be charged for each transaction that results in issuance of an airline or train ticket. A fee-for-service fact sheet and other information can be found at <**http://www.nih.gov/od/ors/dss/special/index.htm**>, or you may call 301-402-8180 to request copies.

WTS is in Building 10, Room 1C200, and is open between the hours of 8:00 a.m. and 5:30 p.m. In addition, WTS has agreed to staff the Executive Plaza South location, Room 150A, from 8:00 a.m. to 7:00 p.m. until they move into a permanent off-site location January 18. WTS can be contacted at **301-496-8900 (staff travel)** and **301-496-6676 (patient travel)**.

If you have any questions or comments, please contact the project officer, Tim Tosten, at 301-402-8180 or e-mail at <**tt17b@nih.gov**>.



Alex Dent

Life Phases Seminars

The NIH Work and Family Life Center (**http://wflc.od.nih.gov**) and the NIH Employee Assistance Program present the following seminars for Spring 2000.

Preregistration is requested; call WFLC at 301-435-1619. Sign language interpretation is available. For reasonable accommodations, call at least 48 hours in advance at 301-435-1619, TTY/TDD: 301-480-0690. Teleconferencing of all seminars is available to most locations upon request, and videotapes of all seminars can be checked out from the WFLC Resource Library.

January 4, 12–1 pm, 31/6C6. *Caught between Trains: Interruptions in the Mid-Life Journey*

January 11, 12–1:30 pm, 31/6C6. *Survival Tactics for New Parents*

January 18, 12–1 pm, 31/6C6. *Levels of Care for the Elderly*

January 19, 11–1 pm, 31/6C10. *Creating an Individual Development Plan*

January 25, 12–2 pm, 31/6C6. *Family Violence: What Every Employee & Manager Should Know*

February 1, 12–1:30 pm, 31/6C6. *Time Management: Concepts for Planning & Prioritizing*

February 8, 12–1:30 pm, 1/Wilson Hall. *How Your Baby Grows*

February 15, 12–1:30 pm, 31/6C6. *Paying for Care for Older Relatives: Medicare, Medicaid, & Insurance*

February 22, 12–1:30 pm, 31/6C6. *Depression in the Workplace*

February 29, 12–2 pm, 31/6C6. *Communicating Effectively: Starting from Scratch*

March 1, 12–1:30 pm, 31/6C6. *Overcoming Procrastination*

March 9, 12–1:30 pm, 31/6C6. *Summer Child Care Options*

March 15, 12–1:30 pm, 31/6C6. *Estate Planning*

March 28, 12–1:30 pm, 31/6C6. *University College (UMUC)—Graduate Program Options*

April 4, 12–1 pm, 31/6C6. *Transition Management: Coping with Workplace Change*

April 11, 12–1:30 pm, 31/6C6. *Parenting Styles that Work with Teens*

April 18, 12–1:30 pm, 31/6C6. *Understanding Alzheimer's Disease*

April 25, 11–1 pm, 31/6C6. *Preparing Federal Application Materials*

May 3, 12–1:30 pm, 31/6C6. *Relax Your Body, Clear Your Mind: Relaxation Techniques for Managing Stress*

May 9, 12–1:30 pm, 31/6C6. *Successful Step Families: Common Concerns, Practical Solutions*

May 16, 12–1:30 pm, 31/6C10. *Compassion Fatigue: Care for the Caregiver*

May 23, 11–1 pm, 1/Wilson Hall *Mentoring and Being Mentored in a Dynamic Workplace*

Call for Catalytic Reactions

In this issue, we are asking for your reactions in four areas: mentor-postdoc communication; falsifying records; challenges to intramural research; and *Catalyst* coverage.

Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 1, Room 209.

In Future Issues...

- Rotavirus Update
- Clinical Research Standards
- Write Right: It's the Law

1) What has been your experience—from either or both sides or as an observer—related to communication between mentor and postdoc at NIH?

2) What are some ways to deal with falsification in administrative records or other missteps that fall short of the definition of scientific misconduct?

3) What do you see as the greatest challenges to conducting intramural research at NIH today?

4) What subjects would you like to see covered in *The NIH Catalyst*?

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 209, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

U.S. Department of
Health and Human Services
Public Health Service
National Institutes of Health
Building 1, Room 209
Bethesda, Maryland 20892