# Annual Report on the Rare Diseases Research Activities at the National Institutes of Health

## FY 2005


**August 2006**

**Office of Rare Diseases**
**National Institutes of Health**
**Department of Health and Human Services**

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

AUG 24 2006

The Honorable Joe Barton
Chairman, Committee on Energy and Commerce
House of Representatives
Washington, D.C. 20515

Dear Mr. Chairman:

I am pleased to submit to you the National Institutes of Health (NIH) Annual Report on Rare Diseases Research Activities: FY 2005. Section 404F of the Public Health Service Act, as amended by Public Law 107-280, the Rare Diseases Act of 2002, requires an annual report to Congress on the activities that NIH conducted and supported through our Institutes and Centers with respect to rare diseases research.

This report presents the contributions and research advances of the NIH research programs and the Office of Rare Diseases (ORD). The basic, clinical, and research training programs contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases. Many advances presented in the report are the results of years of basic research sponsored by the NIH. Patients with rare diseases and their families continue to benefit from the treatment applications realized from the diverse nature of and emphasis placed on both basic and translational research by NIH.

Should you or your staff have any questions regarding the report, please feel free to contact Dr. Stephen Groft, Director of ORD, at 301-435-6041.

Sincerely,

Elias A. Zerhouni, M.D.
Director

Enclosure

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

AUG 24 2006

The Honorable John D. Dingell
Ranking Member
Committee on Energy and Commerce
House of Representatives
Washington, D.C. 20515

Dear Mr. Dingell:

I am pleased to submit to you the National Institutes of Health (NIH) Annual Report on Rare Diseases Research Activities: FY 2005. Section 404F of the Public Health Service Act, as amended by Public Law 107-280, the Rare Diseases Act of 2002, requires an annual report to Congress on the activities that NIH conducted and supported through our Institutes and Centers with respect to rare diseases research.

This report presents the contributions and research advances of the NIH research programs and the Office of Rare Diseases (ORD). The basic, clinical, and research training programs contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases. Many advances presented in the report are the results of years of basic research sponsored by the NIH. Patients with rare diseases and their families continue to benefit from the treatment applications realized from the diverse nature of and emphasis placed on both basic and translational research by NIH.

Should you or your staff have any questions regarding the report, please feel free to contact Dr. Stephen Groft, Director of ORD, at 301-435-6041.

Sincerely,

Elias A. Zerhouni, M.D.
Director

Enclosure

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

AUG 24 2006

The Honorable Michael B. Enzi
Chairman, Committee on Health,
Education, Labor and Pensions
United States Senate
Washington, D.C. 20510

Dear Mr. Chairman:

I am pleased to submit to you the National Institutes of Health (NIH) Annual Report on Rare Diseases Research Activities: FY 2005. Section 404F of the Public Health Service Act, as amended by Public Law 107-280, the Rare Diseases Act of 2002, requires an annual report to Congress on the activities that NIH conducted and supported through our Institutes and Centers with respect to rare diseases research.

This report presents the contributions and research advances of the NIH research programs and the Office of Rare Diseases (ORD). The basic, clinical, and research training programs contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases. Many advances presented in the report are the results of years of basic research sponsored by the NIH. Patients with rare diseases and their families continue to benefit from the treatment applications realized from the diverse nature of and emphasis placed on both basic and translational research by NIH.

Should you or your staff have any questions regarding the report, please feel free to contact Dr. Stephen Groft, Director of ORD, at 301-435-6041.

Sincerely,

Elias A. Zerhouni, M.D.
Director

Enclosure

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

AUG 24 2006

The Honorable Edward M. Kennedy
Ranking Member, Committee on Health,
Education, Labor and Pensions
United States Senate
Washington, D.C. 20510

Dear Senator Kennedy:

I am pleased to submit to you the National Institutes of Health (NIH) Annual Report on Rare Diseases Research Activities: FY 2005. Section 404F of the Public Health Service Act, as amended by Public Law 107-280, the Rare Diseases Act of 2002, requires an annual report to Congress on the activities that NIH conducted and supported through our Institutes and Centers with respect to rare diseases research.

This report presents the contributions and research advances of the NIH research programs and the Office of Rare Diseases (ORD). The basic, clinical, and research training programs contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases. Many advances presented in the report are the results of years of basic research sponsored by the NIH. Patients with rare diseases and their families continue to benefit from the treatment applications realized from the diverse nature of and emphasis placed on both basic and translational research by NIH.

Should you or your staff have any questions regarding the report, please feel free to contact Dr. Stephen Groft, Director of ORD, at 301-435-6041.

Sincerely,

Elias A. Zerhouni, M.D.
Director

Enclosure

**Office of Rare Diseases**

National Institutes of Health
6100 Executive Boulevard, 3B - 01
Bethesda, Maryland 20892 -7518
Telephone: (301) 402 - 4336
Fax: (301) 480 - 9655
E-mail: ord@od.nih.gov

Staff

Stephen C. Groft, Pharm.D.
Director

Mary Demory
Director, Public Outreach and Education Program

John H. Ferguson, M.D.
Medical Advisor

William Gahl, M.D., Ph.D.
Clinical Director, National Human Genome Research Institute, Intramural Research Program

Chris Griffin, B.A.
Program Analyst

Henrietta D. Hyatt-Knorr, M.A.
Director, Policy and Program Planning and Analysis

Sharon Macauley, B.A.
Program Assistant

Geraldine Pollen, M.A.
Consultant

Giovanna M. Spinella, M.D.
CDR, U.S. Public Health Service
Director, Extramural Research Program

Rashmi Gopal-Srivastava, Ph.D.
Health Scientist Administrator

Marita Eddy
Transportation Coordinator
Angel Flight/Mercy Medical Airlift

## Table of Contents

## Executive Summary[1]

The Rare Diseases Act of 2002, P.L. 107-280, instructs the Director of the Office of Rare Diseases, National Institutes of Health (NIH), to prepare the NIH Director's annual report to Congress on rare disease research activities. The annual report presents the contributions and research advances of the fiscal year (FY) 2005 NIH extramural and intramural research programs and of the Office of Rare Diseases (ORD) and other research offices.

Responses from the individual institutes and centers (ICs) provide an overview of ongoing rare diseases research activities, recent scientific advances in rare diseases research, new or planned rare diseases research initiatives, and rare disease-related activities such as scientific workshops and symposia, public and professional education and training, information dissemination, and other rare diseases research-related activities. Many advances presented are the direct result of years of rare diseases research sponsored by NIH in the past. Patients with rare diseases continue to benefit from the treatment applications realized by the emphasis NIH places on both basic and clinical intramural and extramural research programs. Many of the rare diseases activities conducted at the NIH Clinical Center have been reported by the Institutes/Centers (ICs) in their respective sections of the report. For example, the Bench-to-Bedside research program at the Clinical Center was reported by the Office of Rare Diseases.

This report uses the definition of rare diseases as set forth in the Amendments to the Orphan Drug Act as a disease or condition with a prevalence of fewer than 200,000 people in the United States. Prevalence refers to the number of individuals alive with the disease within a geographic parameter, i.e., the United States. There are approximately 6,800 known rare diseases in the United States (see the rare diseases terms at http://rarediseases.info.nih.gov/asp/diseases/diseases.asp). Rare diseases are thought to affect approximately 25 million people in the United States. (Rare Diseases Act of 2002, Section 2, Findings.)

Activities undertaken in FY 2005 by the NIH ICs and the ORD included

- Support of the Rare Diseases Clinical Research Network that includes 10 consortia and a data and technology coordination center.
- Support by the ORD of the NIDDK Biliary Atresia Research Consortium, which contains ten pediatric liver disease centers;
- Development of a model program [CETT (Collaboration, Education, Test Translation) pilot project for rare diseases] to make available to patients genetic tests through a network of Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories.
- Support of the ORD/NHGRI rare diseases intramural research program that promotes fellowship training in the areas of clinical and biochemical genetics focusing on rare diseases; fosters protocol-based initiatives into rare diseases not currently investigated in the intramural program; assists in the investigation of select, unique disorders of unknown

[1] In the text of this report, common diseases may be included when particular subpopulations are rare or treatments are under development that are not expected to be financially recoverable.

etiology; and provides overall research support for diagnostics including genetic testing and therapeutics of rare diseases;

- Support of Bench-to-Bedside Grants in the NIH Clinical Center. With matching support from the ICs;
- Cosponsorship by the ORD and NIH ICs of 112 scientific conferences in FY 2005 and to date in FY 2006, 61 scientific conferences; their topics are listed later in this report. The ORD scientific conferences program contributes to the establishment of research priorities; development of program announcements; establishment of diagnostic and monitoring criteria; initiation of the development of animal models; support of the development of patient and tissue registries, research protocols, and collaborative research arrangements; and dissemination workshop results through publications and other means to encourage new collaborations.

## OFFICE OF RARE DISEASES (ORD)

### Overview

The Office of Rare Diseases (ORD) was established in 1993 within the Office of the Director of the National Institutes of Health (NIH). On November 6, 2002, the President established the office through the Rare Diseases Act of 2002, Public Law 107-280. The Amendments to the Orphan Drug Act define a rare disease as a disease or condition affecting fewer than 200,000 persons in the United States, an estimate that 25 million people in the United States have a rare disease.

The goals of ORD are to stimulate and coordinate research on rare diseases and to support research to respond to the needs of patients who have one of the approximately 6,800 rare diseases known today. To leverage its resources, stimulate rare diseases research activities, and foster collaboration, ORD works with NIH institutes or centers (ICs) to support

- An extramural research program that includes a network of clinical research centers on rare diseases and the training of rare diseases researchers;
- An intramural research program for patients with rare conditions and programs to stimulate clinical research on rare diseases, including the Bench-to-Bedside research program, and the training of researchers interested in rare diseases and in clinical and biochemical genetics;
- A scientific conferences program in response to scientific opportunities or to stimulate research where little exists or where research progress may have stalled;
- An information center to supply reliable and valid information to the public, researchers, and health care providers, including various databases to provide access to information over the Web and a number of educational activities including regional workshops to assist national patient advocacy groups become partners with the NIH by developing better understanding of NIH research programs;
- Other seminal rare diseases research and education activities.

The Fogarty International Center (FIC) and the National Center on Minority Health and Health Disparities (NCMHD) did not have any rare diseases research activity to report.

### Scientific Advances

Extramural Research Program

*The Rare Diseases Clinical Research Network*

Since FY 2003, ORD and several NIH institutes and centers support the Rare Diseases Clinical Research Network. The network consists of 10 clinical research consortia with more than 70 sites, a data and technology-coordinating center (DTCC), and 30 patient advocacy groups. The network consortia conduct research on approximately 50 rare diseases. Collaborating NIH components include the Office of Rare Diseases, National Center for Research Resources,

National Institute of Child Health and Human Development, National Institute of Neurological Disorders and Stroke, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, and the National Heart, Lung, and Blood Institute.

At this time, 22 clinical research protocols have been approved, eight are being reviewed, and an additional 25 are expected to be developed totaling 55 clinical research studies. The vast distribution of research locations across the United States will make investigational treatments more accessible to patients with rare diseases. The network consortia systematically collect clinical information to develop biomarkers and new approaches to diagnosis, treatment, and prevention of rare diseases; to provide training of new clinical research investigators; and to support demonstration projects.

The 10 consortia, the disorders under investigation, and the DTCC comprise the network:

- Children's National Medical Center, Washington, DC —**Urea Cycle Disorders Consortium** — Dr. Mark L. Batshaw: Urea cycle disorders including citrullinemia, argininosuccinic aciduria, hyperargininemia, and others.
- Baylor College of Medicine, Houston, TX — **Angelman, Rett, and Prader-Willisyndromes Consortium** — Dr. Arthur L. Beaudet: Genetic developmental disorders, including Angelman syndrome, Rett syndrome, and Prader-Willi syndrome.
- Boston University School of Medicine, Boston, MA — **Vasculitis Clinical Research Consortium** — Dr. Peter A. Merkel: Vasculitides including Wegener's granulomatosis, Takayasu arteritis, and Churg-Strauss syndrome.
- Cleveland Clinic Foundation, Cleveland, OH — **Bone Marrow Failure Disease Consortium** — Dr. Jaroslaw P. Maciejewski: Bone marrow failure including aplastic anemia, myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria, and large granular lymphocyte leukemia.
- Mount Sinai School of Medicine, New York, NY — **Rare Genetic Steroid Disorders Consortium** — Dr. Maria I. New: Rare genetic defects in steroidogenesis leading to congenital adrenal hyperplasia, androgen receptor defects, and low renin hypertension.
- Children's Hospital Medical Center, Cincinnati, OH — **Rare Lung Diseases Consortium** — Dr. Bruce C. Trapnell: Rare lung diseases including lymphangioleiomyomatosis, alpha-1 antitrypsin deficiency, pulmonary alveolar proteinosis, and hereditary interstitial lung disease.
- University of Rochester, Rochester, NY — **Consortium for Clinical Investigations of Neurological Channelopathies (CINCH)** — Dr. Robert C. Griggs: Rare lung diseases including lymphangioleiomyomatosis, alpha-1 antitrypsin deficiency, pulmonary alveolar proteinosis, and hereditary interstitial lung disease.
- University of North Carolina at Chapel Hill, NC — **Genetic Diseases of Mucociliary Clearance Consortium** — Dr. Michael R. Knowles: Genetic impairments in mucociliary clearance including primary ciliary dyskinesia, cystic fibrosis, pseudohypoaldosteronism, and other chronic sinopulmonary diseases.
- Childrens Hospital, Denver, CO — **Cholestatic Liver Disease Consortium (CLiC)** — Dr. Ronald J. Sokol: Genetic causes of intrahepatic cholestasis including rare liver

diseases associated with alpha-1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis, bile acid synthesis defects, and mitochondrial hepatopathies.

- Duke University School of Medicine, Durham, NC — **Rare Thrombotic Diseases Consortium** — Dr. Thomas L. Ortel: Antiphospholipid antibody syndromes; heparin-induced thrombocytopenia; paroxysmal nocturnal hemoglobinuria; catastrophic antiphospholipid antibody syndrome (thrombotic storm); thrombotic thrombocytopenic purpura.
- Pediatrics Epidemiology Center, University of South Florida, Tampa, FL — **The Data and Technology Coordinating Center (DTCC)** — Dr. Jeffrey P. Krischer

Other Extramural Research Opportunities in Rare Diseases

In addition, ORD is cosponsoring with NIH institutes the following research activities:

- A program announcement with the NHLBI for pilot studies, demonstration projects, and/or exploratory research studies in rare heart, lung, and blood diseases;
- A request for applications published with the National Human Genome Research Institute (NHGRI) for applications for research training grants in genomics and proteomics;
- A program announcement with the NINDS to improve treatment outcomes for lysosomal storage disorders (LSDs). ORD cofunded one grant on LSDs developing a treatment strategy to overcome the blood-brain barrier;
- A joint program announcement with 11 ICs to provide support for the planning of phase III clinical trials;
- An announcement for scientific research conferences jointly with the NIH ICs;
- With the NIDDK, cofunding of the Biliary Atresia Research Consortium (BARC), a multi-center pediatric liver disease research consortium.

*Genetic Testing*

Since FY 2005, ORD, in collaboration with NIH ICs and other Federal organizations, has developed a pilot program to make available to patients genetic tests through a network of Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories. The tests were previously only available in research laboratories. Using the experience gained through the ORD intramural research program that expanded the development of genetic tests for rare diseases, ORD developed the CETT program (Collaboration, Education, Test Translation pilot project for rare diseases.)

With input from the Trans-NIH Rare Diseases Research Working Group, Federal agencies, professional associations, patient advocacy groups, and others, the CETT Program will develop models to facilitate the translation of genetic tests from research laboratories to clinical practice. Health outcomes for individuals can in many cases be greatly increased if rare diseases diagnostic tests from CLIA-certified laboratories exist. Furthermore, by making available tests that currently only exists in non-CLIA certified research laboratories, ease of access to tests is greatly improved. CLIA-certified laboratories also are required to have adequate follow-up

systems including genetic counselors, who may also provide education and support to physicians and patients after testing. The collaboration of patient advocacy, research laboratory, clinician and clinical (CLIA) lab will enable systematic collection of the clinical and genetic information as well as the identification of new genetic information and a better understanding of the spectrum of individual rare diseases.

At this time, the program is beginning the implementation of models to facilitate the translation of genetic tests from research laboratories to clinical practice. A collaborative group (i.e., a research laboratory, in collaboration with a clinician involved in the study of the disease, clinical laboratory, and patient advocacy group) will apply to ORD for funds to develop a new clinical test for a rare genetic disease. As experience is gained with the pilot program, ORD will evaluate progress and develop means by which this pilot program can be expanded into a comprehensive NIH program.

*Intramural Research Program*

Research Relating to Diagnosis, Evaluation, and Treatment of Rare Diseases

The Rare Diseases Intramural Research Program is a collaborative effort between ORD and the NHGRI at the NIH Clinical Center. Researchers diagnosed, cared for, and treated a number of patients with rare diseases. Also, a member of the intramural program of the ORD provided gynecology consultations for rare disease patients throughout the NIH Clinical Center as she continued her fellowship in the field of clinical biochemical genetics. In addition, the program is supporting the conduct of a number of clinical therapeutic trials (see Table 1.)

**Table 1. ORD Cosponsored Research Protocols in Collaboration with the National Human Genome Research Institute and the NIH Clinical Center**

- Use of Cysteamine in the Treatment of Cystinosis
- Diagnosis and Treatment of Patients with Inborn Errors of Metabolism
- Therapeutic Clinical Trial of Oral Pirfenidone for the Pulmonary Fibrosis of Hermansky-Pudlak Syndrome
- Clinical, Biochemical and Molecular Investigations into Alkaptonuria
- Clinical Investigations into Hutchinson-Gilford Progeria Syndrome
- Long-Term Clinical Trial of Nitisinone in Alkaptonuria
- Pilot Study of the Use of Intravenous Immune Globulin in Hereditary Inclusion Body Myopathy
- Clinical Investigations into Autosomal Recessive Polycystic Kidney Disease and Congenital Hepatic Fibrosis
- Genetic Analysis of Gray Platelet Syndrome
- Investigations of Megakaryocytes from Patients with Abnormal Platelet Vesicles

*Angel Flight America*

ORD and NHGRI jointly invited Angel Flight America to participate in the NIH Clinical Center efforts. This not-for-profit organization provides transportation free of charge to and from the Clinical Center for patients enrolled in rare disease research protocols. Since it began operations through the Office of the Clinical Director of the NHGRI in January 2004, Angel Flight America has flown 252 patients and family members to and from the NIH Clinical Center for treatment. The patients with a variety of diagnoses have been enrolled in protocols of the National Eye Institute (NEI), National Cancer Institute (NCI), NHGRI, and other institutes. Rare diseases included alkaptonuria, autosomal recessive polycystic kidney disease (ARPKD), cystinosis, leukemia, Hermansky-Pudlak syndrome (HPS), Pallister-Hall syndrome, periodic fever syndrome, and various types of cancers.

*Rare Diseases Research Initiative*

The Office of the Clinical Director of NHGRI continued to admit patients with rare, undiagnosed disorders under a protocol entitled Diagnosis and Treatment of Patients with Inborn Errors of Metabolism.

*Molecular Diagnostics of Rare Diseases*

In collaboration with the NHGRI, the intramural program of the ORD conducted a program to contract with Clinical Laboratory Improvement Act (CLIA)-certified laboratories to establish molecular diagnostic tests for specific rare diseases research. Thereafter, these tests are made available as genetic tests on a fee-for-service, insurance-reimbursable basis to the general public. In the past year, 16 genes responsible for disorders were contracted to have mutation analysis made available as genetic tests in a CLIA-certified laboratory (see Table 2.)

| Table 2. Genetic Tests under Development for the NHGRI/ORD Intramural Research Program | |
|---|---|
| Gene Symbol | Rare Disease |
| • ATP7A | Wilson disease |
| • GP1BB | Bernard-Soulier disease |
| • HALG6 | congenital disorder of glycosylation-Ic |
| • ARTEMIS | severe combined immune deficiency |
| • CASP10 | autoimmune lymphoproliferative disorder |
| • CASP8 | autoimmune lymphoproliferative disorder |
| • SNCA | early-onset Parkinson disease caused by mutations in the SNCA gene |
| • AAAS | Allgrove [achalasia-addisonianism-alacrima (AAA)] syndrome |
| • MMAA | methylmalonic acidemia A |
| • MMAB | methylmalonic acidemia B |
| • MYH8 | trismus-pseudocamptodactyly syndrome |
| • AIRE | autoimmune polyendocrinopathy |
| • XPA | xeroderma pigmentosum group A |
| • XPC | xeroderma pigmentosum group C |
| • CKN1 | Cockayne syndrome group A |
| • ERCC6 | Cockayne syndrome group B |

*Research Training: Biochemical Genetics*

One physician in the ORD/NHGRI Intramural Research Program was certified in clinical biochemical genetics and another two are clinical biochemical genetics fellows.

*Bench-to-Bedside Awards*

In FY 2005, ORD co-funded with NIH institutes and centers ten 2-year Bench-to-Bedside awards at the NIH Clinical Center (see Table 3). Intramural scientists at the NIH enter into basic science-clinical research collaboration with colleagues in other NIH laboratories with a focus on rare disease.

**Table 3. FY 2005/2006 Bench-to-Bedside Awards**

- Natural History, Biology, and Treatment of Dermal Neurofibromas in Neurofibromatosis Type 1 (NF1)
- Testing Treatment of Hutchinson-Gilford Progeria Syndrome with Farnesyl Transferase Inhibitors
- Analysis of Global Gene Expression Patterns and Mitochondrial DNA Damage in Lymphocytes of Friedreich's Ataxia Patients Undergoing Idebenone Treatment in a Phase II Double-Blind Placebo-Controlled Study
- Adoptive Cell Therapy for Ewing's Sarcoma Using Artificial Antigen Presenting Cells
- Ganaxolone Therapy for Niemann-Pick Type C
- UVA Sensitivity in Smith-Lemli-Opitz Syndrome: Possible Involvement of Cholesta-5,7,9(11)-trien-3β-ol
- Pre-Clinical and Clinical Investigations into the Mechanisms and Efficacy of Extracorporeal Photopheresis (ECP) in the Abrogation of Graft-versus-Host Disease
- Site-Selective cAMP Analogs for Treatment of Carney Complex
- Pathogenesis of and Risk Factors for Autoimmunity in the Wiskott-Aldrich Syndrome
- Development of a Specific Drug Treatment for WHIM Syndrome

For second-year Bench-to-Bedside awards see Table 4.

**Table 4. FY 2004/2005 Bench-to-Bedside Awards**

- Therapeutic Application of Intra-Vascular Nitrite for Sickle Cell Disease (CC/NHLBI)
- Molecular Profiling of Response to Proteasome Inhibition by Bortezomib (PS341) in a Clinical Trial of Mantle Cell Lymphoma (NHLBI/NCI)
- A Phase I/II Pilot Study to Evaluate the Treatment of Intraocular Lymphoma with the BL22 Immunotoxin (NEI/NCI)
- A Phase I Treatment Trial of the Circadian Sleep Disturbance in Smith-Magenis Syndrome (SMS) (NHGRI/CC-Pharmacy)
- Preclinical Nonhuman Primate Studies of an In Vivo Selectable Vector Intended for Use in a Planned Clinical Trial of Gene Therapy for Chronic Granulomatous Disease (NIAID/NHLBI)
- Isolation and Characterization of Circulating Endothelial Cells in Primary Pulmonary Hypertension: Implications for Early Diagnosis and Novel Therapeutic Targets (CC-CCMD/CIT)
- Evaluation of the Humanized MiK-â-1 Monoclonal Antibody Directed Toward the IL-2/IL-15Rβ Subunit (CD122) that Blocks IL-15 Action: Effect on IL-15 Induced Immunopathogenic Virus-Specific T Cells in Patients with HTLV-1-

Associated Neurologic Disease (NINDS/NCI)
- Molecular Profiling and Drug Discovery for Patients with PTEN Hamartomatous Tumor Syndromes (PHTS) (NCI/NHGRI)
- Immunotherapy for Myelodysplastic Syndrome (NHLBI/VRC/NCI)
- Intermediate Phenotype and Genetic Mechanisms for Psychosis and Cognitive Disturbance in 22q11.2-Hemideletion Syndrome
- Childhood Cancer and Plexiform Neurofibroma Tissue Microarray for Molecular Target Screening and Clinical Drug Development

Education, Public Information, and Public Input

*Genetic and Rare Diseases Information Center*

The ORD supports with the NHGRI the Genetic and Rare Diseases Information Center (GARD). The information center provides information to patients and their families, health professionals, researchers, and to the general public in English and in Spanish. Since its inception in September 2001, the information center has responded to more than 12,000 inquiries for more than 4,000 rare and/or genetic diseases. NHGRI and ORD are in the process of evaluating user satisfaction to shape future activities.

*Database of National Patient Advocacy Groups for Rare Diseases*

ORD supported the Medical Genetics and Rare Disorders Subfile of the Combined Health Information Database (CHID). In October 2005, CHID reported 84,805 searches. Every two years, ORD reviews, updates, and expands one of the six subfiles (medical genetics and rare disorders) through a contract with the National Organization for Rare Disorders. The subfile provides information about and available from voluntary patient support groups and includes national and international organizations and their publications in English and Spanish. At this time, over 7,000 indexed documents are in the subfile as well as 1,400 national and international patient support organizations. CHID has been undergoing an NIH-wide assessment of user satisfaction to assist in future direction.

*Genetics Education for Healthcare Providers*

ORD also supported the National Coalition for Health Professional Education in Genetics (NCHPEG). Established in 1996 by the American Medical Association, the American Nurses Association, and the NHGRI, NCHPEG is a national effort to promote health professional education and access to information about advances in human genetics. NCHPEG members are an interdisciplinary group of leaders from approximately 120 diverse consumer and voluntary groups, medical societies, government agencies, private industry, managed care organizations, and genetics professional societies. By promoting frequent and open communication between stakeholders, NCHPEG seeks to capitalize on the collective expertise and experience of members and to reduce duplication of effort.

As patients ask more questions about genetic tests and disease risk, more responsibility for the use and interpretation of genetic tests and information will fall to primary care physicians, nurses, physician assistants, advanced practice nurses, and other health professionals who may not be formally trained in genetics. This is of importance to ORD since it is estimated that 80 percent of rare diseases have a genetic basis. Core competency educational materials have been produced in English and in Spanish.

In FY 2005, NCHPEG developed Web-based genetics education for family physicians, developed a newsletter of genetic family history for the practice; installed a clearinghouse of genetics education resources; coordinated *Genetic Resources on the Web (GROW)* a source of information about human genetics for health professionals and the public; and organized diversity, cultural competency, and outreach efforts.

*Institute of Medicine (IOM) Forum on Drug Discovery, Development, and Translation*

Together with NIH ICs, ORD is supporting and participating in the Institute of Medicine's Forum on Drug Discovery, Development, and Translation. The forum brings together leaders from private sector sponsors of biomedical and clinical research; federal agencies sponsoring and regulating biomedical and clinical research; foundations; the academic community; consumers; and federal and private health plans. The forum is focusing on four priority areas in drug discovery, development, and translation; scientific challenges that require a coordinated response; public communication and engagement in clinical research; role of the pubic and private sector in drug development; and alternatives to the current business model.

*Public Input*

ORD continued to support the annual meetings of the Genetic Alliance and the National Organization for Rare Disorders (NORD). These two umbrella organizations represent collectively more than 600 rare diseases patient advocacy groups. ORD utilized these meetings to conduct focus groups to determine the needs of member organizations and to identify programs ORD should consider implementing.

In FY 2005, ORD supported a third regional workshop in Philadelphia to discuss with leaders of national patient advocacy groups aspects of research and research opportunities in NIH's extramural and intramural research programs and other research related issues they need to know. These outreach workshops enable patient advocacy groups to become partners in NIH research endeavors.

Identifying Future Research Opportunities for Rare Diseases

*Scientific Conferences*

ORD collaborates with institutes, centers, and offices at NIH and other Federal agencies to stimulate rare diseases research by co-sponsoring scientific conferences where research is lagging or to take advantage of scientific opportunities. The outcomes of these scientific

conferences have included the establishment of research priorities, development of collaborative research protocols, criteria for diagnosing and monitoring rare diseases, specific discoveries, publications, and new research endeavors. These scientific conferences have also contributed to the exchange of ideas and information among basic and clinical investigators, patient advocacy groups, NIH staff, and the pharmaceutical industry.

In FY 2005, ORD co-funded 112 national and international scientific conferences. Examples of the scientific conferences include childhood cancers, bone marrow failure, sickle cell disease, congenital heart disease, dystonias, pediatric stroke, neurofibromatosis, and primary lateral sclerosis. Primary sponsors and titles of ORD cosponsored scientific conferences in FY 2005 as well as FY 2006 are provided in Tables 5 and 6.

**Table 5. ORD Cosponsored Scientific Conferences in FY 2005**

| Primary Cosponsor | Title |
|---|---|
| National Institute on Aging (NIA) | • Mitochondrial DNA Transactions in Health and Disease |
| National Institute on Alcohol Abuse and Alcoholism (NIAAA) | • Mechanisms of Alcohol-Induced Hepatic Fibrosis |
| National Institute of Allergy and Infectious Diseases (NIAID) | • Bacillus-ACT 2005, Bacillus anthracis, B. cereus, and B. thuringiensis International Conference<br>• Changing Host Ranges of Viruses Leading to Emergence of New Pathogens<br>• Electrophysiological Approaches to Plasmodium falciparum-Induced Erythrocyte Permeability Changes<br>• Eosinophil-Associated Disease: Approaches to Treatment<br>• First Meeting of the Nontuberculous Mycobacteria Consortium: Pulmonary Nontuberculous Mycobacterial Infections - Phenotype and Genotype of an Autosomal-Dominant Disorder Primarily Affecting Older Women (NTMC)<br>• Human African Trypanosomiasis Workshop<br>• Hydatidosis—New Approaches to Vaccines and Prevention<br>• Molecular Biology of Spirochetes<br>• NIAID Leprosy Workshop II: Translation of Research Advances into Applications<br>• Primary Immune Deficiency Consortium Conference<br>• Sixth International Conference on Cryptococcus and Cryptococcosis<br>• Symposium on Primary Immunodeficiency Diseases |

| | |
|---|---|
| | • Treatment of Neurocysticercosis |
| National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) | • 12th International Symposium on Basement Membranes<br>• Familial Mediterranean Fever (FMF) and Beyond: The Fourth International Congress on Systemic Autoinflammatory Diseases<br>• Infantile Hemangiomas: Current Knowledge, Future Directions<br>• NIH Burden of Muscle Disease Workshop<br>• Pemphigus 2005: Progress and Future Directions |
| National Cancer Institute (NCI) | • 27th Annual Meeting of the International Association of Cancer Registries: Cancer in Low-Resource Populations<br>• ATM Workshop: Population-based Studies of Breast Cancer Risk in Relatives of Ataxia-Telangiectasia Patients<br>• Birth Defects and Cancer<br>• Bloom Syndrome: Molecular Basis of Genomic Instability<br>• Brain Tumor Diagnostic Quality Control Workshop, Brain Tumor Epidemiology Consortium (BTEC)<br>• Chronic GvHD: The Next Frontier in Transplantation Research<br>• Hereditary Leiomyomatosis and Renal Cell Carcinoma: A New Genodermatosis with an Increased Risk of Internal Malignancy<br>• International Consortium for Familial Chronic Lymphocytic Leukemia<br>• International Consortium for Studies of Cancer and other Diseases in Agricultural Populations<br>• International Lymphoma Epidemiology Consortium: Workshop on Genetics and Immunology<br>• Kidney Cancer—Current Perspectives and Future Directions<br>• National Children's Study International Childhood Cancer Cohort Consortium Workshop<br>• NCI-MMHCC Working Group on Brain Tumors<br>• New Therapeutic Options in Thyroid Cancer<br>• Second Annual North American ABC Genetic Workshop<br>• Second NCI Leadership Workshop on Understudied Rare Cancers |

| | |
|---|---|
| | • Translational Genomics of Neuroblastoma (TGiN)<br>• The ALARA Concept in Pediatric Interventional and Fluoroscopic Imaging—2006<br>• The Eighth International Feline Retrovirus Research Symposium: Cat Genomics, Models for AIDS, Cancer, and other Infectious Diseases in the 21st Centuries<br>• Workshop on Chronic Inflammation and Cancer: Biology, Pathology, Immunology, and Epidemiology<br>• Translational Science Workshop on Epigenetics in Cancer |
| National Institute of Child Health and Human Development (NICHD) | • Artificial Reproductive Technology and Adverse Pregnancy Outcomes<br>• Asphyxia and Hypothermia: Opportunities and Challenges<br>• Clinical Trials in Rett Syndrome: Potential for Early Intervention<br>• Expanded Newborn Screening: Building the Infrastructure<br>• First International Symposium on Pheochromocytoma (ISP 2005)<br>• Mechanisms of Follicular Dysfunction in Ovarian Insufficiency and Premature Ovarian Failure<br>• Ninth International Conference on Osteogenesis imperfecta<br>• Optimizing Care and Long-term Outcome of Near-term Pregnancy and Near-term Newborn Infant<br>• Oxygen and Neonatal Therapies: Controversies and Opportunities for Research<br>• Perinatal-Neonatal Arterial Stroke: Epidemiology, Etiology, Treatment, and Prevention<br>• Translating Civilian and Defense Technologies for Pediatric Critical Care and Rehabilitation Research<br>• Cortisol Secretion Abnormalities and Related Disorders of the Hypothalamic-Pituitary-Adrenal Axis |
| National Institute of General Medical Sciences (NIGMS) | • Gordon Research Conference on Glycobiology 2005<br>• The Third Symposium on the Functional Genomics of Critical Illness and Injury: Identifying Research Priorities |
| National Institute of Biomedical Imaging and Bioengineering (NIBIB) | • Third International Brain Computer Interface (BCI) Meeting |

| | |
|---|---|
| National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) | • Inherited Metabolic Disorders<br>• Primary Sclerosing Cholangitis: Research Workshop<br>• Sixth International Symposium on Familial Amyloidotic Polyneuropathy and other Transthyretin-Related Disorders and the Fifth International Workshop on Liver Transplantation in Familial Amyloidotic Polyneuropathy l |
| National Institute of Dental and Craniofacial Research (NIDCR) | • Sjögren's: Transition from Autoimmunity to Lymphoma<br>• Individual Responder Approaches for Pain and Symptoms Research |
| National Institute of Environmental Health Sciences (NIEHS) | • 2005 FASEB Summer Research Conference: Regulation of Ion Channels<br>• 2005 Mycotoxins and Phycotoxins Gordon Conference<br>• Gene/Environment Interactions in Rare Diseases that Include Common Birth Defects<br>• Ninth International Conference on Environmental Mutagens<br>• Omega-3 Fatty Acid Supplementation during Pregnancy and Infancy: New Opportunities for Prevention Trials |
| National Heart, Lung, and Blood Institute (NHLBI) | • 28th Annual Meeting of the National Sickle Cell Disease Program<br>• Barriers to Late Stage Drug Development for Hemoglobinopathies<br>• Bone Marrow Failure Scientific Symposium<br>• Eighth Cooley's Anemia Symposium<br>• Eighth International Kawasaki Disease Symposium<br>• Eighth International Meeting of the World Association of Sarcoidosis and other Granulomatous Disorders (WASOG)<br>• Host Response to Persistent Airway Bacterial Load in Cystic Fibrosis<br>• NHLBI Working Group: Hemoglobin Gene Transfer in Sickle Cell Disease and Cooley's Anemia<br>• Pulmonary Alveolar Proteinosis (PAP) Research Conference<br>• Renal and Urologic Complications in Sickle Cell Disease<br>• Research Collaborations in Sickle Cell Disease and Thalassemia<br>• Role of Nitrite in Physiology, Pathophysiology, and |

| | |
|---|---|
| | Therapeutics<br>• The Weinstein Cardiovascular Development Conference<br>• Working Group on Cardiomyopathies of Rare Diseases |
| National Human Genome Research Institute (NHGRI) | • 15th International Workshop on the RUNX/CBFB Gene Family<br>• Essential Nursing Competencies for Genetics and Genomics<br>• First ORD-NIH Workshop on Autosomal Recessive Polycystic Kidney Disease and Congenital Hepatic Fibrosis<br>• Fourth International Conference on Smith-Magenis Syndrome (SMS)<br>• ORD-NHGRI Conference on the Pulmonary Fibrosis of Hermansky- Pudlak Syndrome (HPS)<br>• Workshop on Glucocerebrosidase and the Synucleinopathies |
| National Institute of Mental Health (NIMH) | • Assessing Suicidality during Antidepressant Treatment<br>• Indigenous Suicide in the Americas |
| National Institute of Neurological Disorders and Stroke (NINDS) | • 10th International Congress on Neuronal Ceroid Lipofuscinosis<br>• Hydrocephalus: Myths, New Facts, Clear Directions<br>• Lysosomal Disease Network—Second Annual World Symposium<br>• Meeting of the Working Group on Ideomotor Apraxia<br>• Neural Interfaces Workshop<br>• Neural Tube Defects 2005 and Beyond<br>• Neurosarcoidosis Workshop<br>• New Perspectives in Transporter Biology<br>• Research Planning Workshop on Spasmodic Dysphonia<br>• Restoring Mobility: Stem Cells and Sensory/Motor Systems of the Spinal Cord Second Scientific Workshop on Neurodegeneration with Brain Iron Accumulation<br>• Third Gordon Research Conference on CAG Triplet Repeat Disorders 2005<br>• TSC/LAM International Research Symposium |

| | |
|---|---|
| | • Vascular Cognitive Impairment Harmonization Criteria Workshop<br>• Advances in Midbrain/Hindbrain Malformations |
| National Institute of Nursing Research (NINR) | • Cultural Dynamics in HIV/AIDS Biobehavioral Research among Young People |
| Office of Dietary Supplements (ODS) | • Workshop on Dietary Supplements, Coagulation, and Antithrombotic Therapies |
| Centers for Disease Control and Prevention (CDC) DC | • Access to Quality Testing for Rare Diseases: A National Conference |
| **International Conferences** | |
| European Platform for Patient Organizations, Science and Industry (EPOSSI), London, UK | • Sixth Workshop on Partnering for Rare Disease Therapy Development People with Rare Diseases – No Longer Alone in the World. |
| Karolinska Institute, Stockholm, Sweden | • 1st International Conference on Rare Diseases and Orphan Drugs |
| **Other Workshops and Conferences** | |
| ORF/NIH | • 4th Annual Environmental Workshop – Environmental Management Systems (EMS): Tying It All Together. |
| National Institutes of Health (NIH) | • Share the Health: Health and Fitness Expo |
| Genetic Alliance | • Leadership in Alliance: Leveraging Voices, Advancing a Vision |
| National Organization for Rare Disorders (NORD) | • Annual Family Conference |

**Table 6. ORD Cosponsored Scientific Conferences in FY 2006**

| Primary Cosponsor | Title |
|---|---|
| National Institute on Aging (NIA) | • Biology and Therapy for Malignant Salivary Gland Tumors<br>• RecQ Helicases and Other Helicases in Telomere Maintenance and Related Pathways<br>• DNA Repair Deficient Syndromes: Molecular and Clinical Abnormalities |
| National Institute of Allergy and Infectious Diseases (NIAID) | • 20th Meeting of the American Society for Rickettsiology<br>• Gene Therapy for Primary Immune Deficiencies: |

| | |
|---|---|
| | Advances and Safety Issues<br>• Gordon Research Conference on the Biology of Spirochetes<br>• Prevention of Neonatal Herpes<br>• Pulmonary Nontuberculous Mycobacterial Infections: Development of a Research Agenda April 2006 |
| National Eye Institute (EI) | • Autoimmune Retinopathies (AIR) |
| National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) | • New Research Strategies in Osteogenesis imperfecta April 26-27, 2006<br>• Obstacles to Translating Basic Knowledge of Genetic Skin Diseases into Therapies<br>• Paget's Disease of Bone/Fibrous Dysplasia: Advances and Challenges<br>• Multiple Hereditary Exostoses: Insights into Pathogenesis<br>• Reaching Clinical Trials for Pachyonychia Congenita |
| National Cancer Institute (NCI) | • ABC Transporters and Genetic Disease<br>• Biology and Therapy for Malignant Salivary Gland Tumors<br>• FASEB Summer Research Conference on Biological Methylation<br>• Immunotherapy for Cutaneous T-Cell Lymphoma (CTCL)<br>• Interagency Workshop on the Science and Practice of Informal Caregiving<br>• Large Granular Lymphocyte (LGL) Leukemia: Pathogenesis, Pathobiology, and Treatment<br>• Mechanisms and Consequences of c-MY-Deregulating Chromosomal Translocations<br>• Ninth Meeting of the Society for Natural Immunity "NK Cells and Innate Immunity"<br>• Pediatric CT Scanning and Risk for Childhood Cancer: International Collaborative Study<br>• Testicular Cancer and Testicular Dysgenesis Syndrome: Current Perspectives and Future Directions<br>• Translational Genomics of Neuroblastoma (TgiN) Year 2 |

| | |
|---|---|
| National Institute of Child Health and Human Development (NICHD) | • Defining the Metabolic Syndrome in Children and Adolescents<br>• International Conference on Adrenal Cortex and Molecular Steroidogenesis<br>• Mechanisms of Follicular Dysfunction in 46,XX Spontaneous Premature Ovarian Failure<br>• New Horizons in GnRH Research<br>• New Therapies for Necrotizing Enterocolitis (NEC)<br>• Preeclampsia: A Pressing Problem<br>• Prenatal Imaging: Ultrasound and MRI<br>• Critical Pertussis in US Children |
| National Institute on Deafness and Other Communication Disorders (NIDCD) | • Brain-Computer Interfaces for Speech Synthesis |
| National Institute of Dental and Craniofacial Research (NIDCR) | • Seventh Research Workshop on the Biology, Prevention, and Treatment of Head and Neck Cancer |
| National Heart, Lung, and Blood Institute (NHLBI) | • 29th Annual Meeting of the National Sickle Cell Disease Program<br>• Cardiofaciocutaneous Syndrome and Noonan Syndrome Scientific Meeting 2006<br>• Conference on Adult Sickle Cell Disease (SCD) Care: Guidelines for Pain Management<br>• Evolution of Pulmonary Hypertension: Emerging Diseases and Novel Therapeutics<br>• Neuroimaging of Sleep Disorders<br>• The Progeria Research Foundation International Progeria Workshop<br>• Workshop on Recognition and Treatment of Rare Inherited Arrhythmias<br>• Vascular Anomalies: Research and Controversies |
| National Institute of Biomedical Imaging and Bioengineering (NIBIB**)** | • Stem-Cell-Based Tissue Engineering in Regenerative Medicine Conference<br>24th Scientific Conference of the Society for Physical Regulation in Medicine and Biology |
| National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) | • Alpha 1-Antitrypsin Deficiency and other Liver Diseases Caused by Aggregated Protein<br>• Insulin Signaling and Hamartoma Syndromes<br>• Screening for Biliary Atresia |

| | |
|---|---|
| | • Congenital Hyperinsulinism in Infants |
| National Institute of Environmental Health Sciences (NIEHS) | • Environmental Mutagen Society 37th Annual Meeting<br>• Molecular Mechanisms of Chemical Teratogenesis<br>• Seventh International Conference on Lactoferrin: Structure, Function, and Applications |
| National Institute of Mental Health (NIMH) | • Childhood Onset Schizophrenia: Research Challenges and Opportunities on the Neurobiology, Developmental Trajectory, and Treatment |
| National Institute of Neurological Disorders and Stroke (NINDS) | • 11th International Symposium on Neural Regeneration<br>• Advances in Midbrain/Hindbrain Malformations<br>• American Society for Neurochemistry: Cellular and Molecular Mechanisms of Neural Development and Disease<br>• Ataxia-Telangiectasia Clinical Research Workshop<br>• Conference on the Diagnosis of Multiple System Atrophy<br>• Gangliosides in Health and Disease<br>• International Conference on HHV-6 and Workshop on HHV-6 Acute and Sub-acute Encephalitis and Meningoencephalitis<br>• Neurobiology of Disease in Children Conference, a Satellite Symposium of the Child Neurology Society Annual Meeting<br>• New Directions in Biology and Disease of Skeletal Muscle<br>• The NIH Pain Consortium First Annual Symposium - Advances in Pain Research<br>• International Conference on Episodic Ataxia Syndromes |

**New Initiatives**

*Expanding Genetic Testing*

After the Collaboration, Education, Test Translation pilot project for rare diseases (CETT) has advanced, ORD plans to include a network collaboration of biochemical geneticists to improve the genetic testing of rare metabolic disorders, especially in light of the newborn screening initiative which may identify more individuals with these rare conditions.

*Bringing Clinician Scientists Together*

In addition, ORD plans special workshops to bring clinician scientists together internationally to work on specific rare diseases in developing common protocols, standards, terminology, validated assessment measures and biomarkers. These collaborative meetings are building on the Rare Diseases Clinical Research Network experience and are necessary for rare diseases research to take the next step to clinical trials with new therapeutics. Scientists working on basic science, translation, patient advocacy representation and industry will participate in these meetings to develop strong clinical research study designs and new treatments.

*The amyloidoses*

The amyloidoses are a group of devastating diseases in which one or more organ systems in the body accumulate abnormal proteins. In primary amyloidosis, the heart, lung, skin, tongue, thyroid gland, and intestinal tract may be involved as well as the liver, spleen, kidney, and the vascular system, especially the heart. Secondary amyloidosis usually affects the spleen, liver, kidney, adrenal glands, lymph nodes, and the vascular system. Hereditary amyloidosis is characterized by peripheral sensory and motor neuropathy, autonomic neuropathy, and cardiovascular and renal involvement. In FY 2005, the NIH held several scientific conferences on various aspects of the amyloidoses. In FY 2006, ORD is moving forward with a comprehensive conference on amyloidosis that will identify the next steps that need to be taken to increase the understanding, prevention and treatment of this devastating group of diseases.

*Rare Diseases Biospecimen Repositories*

Following recommendations from the Trans-NIH Rare Diseases Research Working Group for a major database that identifies existing biorepositories and assesses rare disease biospecimen collection, storage, and delivery issues that impede research on rare diseases, ORD and the RAND Corporation will develop a database of repositories of DNA, tissue, blood, and other biomaterials. This project will facilitate research leading to treatments and cures for rare diseases by facilitating access to human biomaterials for use in research. The end product will be a publicly accessible, searchable, Web-based database of repositories that collect, store, and distribute human biological materials for research use. The database will be regularly updated and will also identify unmet needs of researchers in obtaining human biomaterials for research on rare diseases. This database will be the first of its kind and will be extremely useful as a comprehensive reference. The database will contain up-to-date information for biomedical researchers, and for other segments of the biomedical research community (e.g., research funding entities, professional societies, patient advocacy groups, research administrators, and the biotechnology and pharmaceutical industries). All interested parties will be able to use the database to identify and obtain resources for basic and translational research as well as drug development in rare diseases, genetics, rare cancers, immunology, physiology, and cell biology, among other disciplines.

# NATIONAL INSTITUTE ON AGING (NIA)

## Overview of Rare Disease Research Activities

The National Institute on Aging (NIA) conducts and supports research on a variety of rare diseases and conditions. Much of the work in this area focuses on the progeroid syndromes -- Werner, Cockayne, Hutchinson-Gilford, and Rothmund-Thomson -- because research findings on these diseases that cause premature aging may have implications for our understanding of normal and pathological aging. Other rare diseases or conditions currently under study at the NIA include Bloom syndrome, Fanconi anemia, ATRX syndrome, and premature ovarian failure.

## Recent Scientific Advances in Rare Diseases Research

*Werner syndrome*

Werner syndrome (WS) is a recessive genetic disease characterized by early onset of many characteristics of normal aging such as wrinkling of the skin, graying of the hair, cataracts, diabetes, and osteoporosis. The symptoms of WS begin to appear around puberty, and most patients die before age 50.

The WS gene (*WRN*) has been identified as having a number of pathogenic mutations. Research is ongoing to elucidate the role of *WRN* protein (WRNp) in pathways of DNA metabolism and to define the protein interactions of *WRN*, which will help to elucidate cellular processes necessary to maintain genomic stability.

*Hutchinson-Gilford progeria syndrome*

Hutchinson–Gilford progeria syndrome (HGPS) is a rare developmental disorder characterized by craniofacial disproportion, short stature, skin and hair abnormalities, and loss of subcutaneous fatty tissue. These features give the patients a characteristic "old-like" appearance. Intellectual development is entirely normal. The individuals afflicted with this syndrome are clinically unaffected at birth, and the diagnosis is often not established until the second year of life. During the advancing years of the disease, the cardiovascular system becomes increasingly affected by atherosclerosis, and the patients die at an average age of 13 years from cardiovascular complications.

Although HGPS has been considered as a prototype of premature aging syndromes, the degree to which it truly recapitulates innate aging phenomena is still being studied. The NIA has continued to study HGPS because the identification of the culprit gene, designated as *LMNA*, has opened new avenues for research to explore the actual relationship between HGPS and normal aging. With the National Heart, Lung, and Blood Institute, the NIA has supported Program Announcement PA-03-069 to encourage research on the biology of HGPS and related disorders. Several new studies of HGPS's pathogenic mechanisms were funded through this PA in 2005.

*Cockayne syndrome*

Cockayne syndrome (CS) is characterized by severe mental and growth retardation, microcephaly, progressive neurological and retinal degeneration, skeletal abnormalities, and a hypersensitivity to sunlight. This condition results in premature aging and death. Two CS genes, *CSA* and *CSB*, have been identified. Findings from the NIA have focused on *CSB*, which appears to be implicated in DNA repair. An associated *CSB* protein has been shown to play a role in repair of oxidative DNA damage, and studies to further clarify its function are ongoing.

*Rothmund-Thomson and RAPALIDINO syndromes*

Rothmund-Thomson syndrome (RTS) is associated with genome instability, predisposition to cancer, skin and skeletal abnormalities, and some features of premature aging. The disease is caused by mutation in the *RECQL4* gene -- the same RecQ family that includes the *WRN* and *BLM* proteins defective in Werner and Bloom syndromes, respectively. An RTS-related disease, termed the RAPADILINO syndrome, with a lower predisposition to cancer, was found to be caused by mutations in the same *RECQL4* gene. However, the molecular mechanism of RTS and RAPIDILINO syndromes is poorly understood.

The data suggest that *RECQL4* may play a role in maintaining genomic stability and to understand the mechanism of this disease. NIA investigators have completed the first biochemical characterization of *RECQL4* and its associated complex and are working to elucidate the role of *RECQL4* in maintaining genomic stability.

*Fanconi anemia*

Fanconi anemia (FA) is a genetic disease characterized by congenital defects, bone marrow failure, and cancer susceptibility. A group of FA proteins function as a complex machine that is thought to participate in DNA repair. However, the interactions between FA proteins and DNA are poorly understood. NIH researchers have identified a new gene, *FANCM*, which is mutated in one subgroup of FA patients. *FANCM* appears to act as an engine that moves the FA DNA repair complex along damaged DNA and may provide a target for therapies that enhance the FA DNA damage response in patients.

*Bloom syndrome*

Patients with Bloom syndrome exhibit growth retardation, immunodeficiency, infertility, photosensitivity, and predisposition to cancer. Investigators have recently identified the Bloom syndrome gene, *BLM*. Interestingly, the *BLM* gene belongs to the helicase family, like the genes mutated in Werner and Rothmund-Thomson syndromes. All three diseases have some common features, such as genetic instability and predisposition to cancer. NIA investigators have also identified a possible connection between the *BLM* and Fanconi anemia pathways of genomic maintenance. NIA researchers are currently working to understand the molecular mechanisms of each of these diseases.

*ATRX syndrome*

ATRX syndrome represents a combination of alpha-thalassemia, mental retardation, and multiple associated developmental abnormalities. The gene defective in ATRX has been localized to the X chromosome and cloned. Mutations in the same gene also cause several other forms of syndromal X-linked mental retardation, and it has been hypothesized that the ATRX gene could function as part of a complex involved in regulation of gene expression. Further study of the molecular underpinnings of ATRX syndrome is ongoing.

*Premature ovarian failure*

Premature ovarian failure (POF) affects 1-3 percent of all women and is characterized by early menopause resulting from inadequate formation or maintenance of the pool of ovarian follicles. Researchers recently determined that in mice, POF can arise from disrupted follicle development, with the *Foxl2* gene as a selective determinant of perinatal ovarian development. *Foxl2* disruption in mice provides the first model directly relevant to POF in humans, along with a route to genes selectively involved in the determination of the critical follicle pool. Such genes should include candidates for mutation in other instances of POF, where affected genes have been difficult to identify. In the long run, they may provide targets for therapeutic intervention.

## NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM (NIAAA)

### Overview of Rare Diseases Research Activities

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) conducts and supports research on the causes, consequences, prevention, and treatment of problems and diseases that arise from alcohol use. In addition to alcohol abuse and alcoholism, these adverse consequences include alcoholic liver diseases such as alcoholic hepatitis and cirrhosis, alcoholic pancreatitis, alcoholic cardiomyopathy, alcoholic dementia, fetal alcohol spectrum disorders, as well as diseases of the lung, endocrine system, and brain among other organs and systems. Given the breadth of organs affected by alcohol, knowledge gained in alcohol-related research will have broad application to other areas of human health and disease.

While many of the disorders associated with alcohol use would not be considered rare, a number of the adverse consequences are rare diseases.

### Recent Scientific Advances in Rare Diseases Research

*Alcoholic pancreatitis*

Long-term heavy alcohol consumption is associated with both acute and chronic pancreatitis. Pancreatitis is an inflammation of the pancreas. Acute pancreatitis occurs suddenly and lasts for a short period of time and usually resolves. Chronic pancreatitis does not resolve itself and results in a slow destruction of the pancreas. Either form can cause serious complications and may lead to multiple co-morbidities including maldigestion, diabetes, and pancreatic cancer. NIAAA-funded researchers have made significant progress in understanding the underlying mechanisms by which alcohol intake leads to the development of pancreatitis.

*Role of connective tissue growth factor (CCN2) in chronic pancreatitis*

This study investigated the role of connective tissue growth factor (CCN2) in the activation of rat pancreatic stellate cells (PSCs), which is a first step in the development of fibrosis that may result in chronic pancreatitis. The results showed that CCN2 was produced by activated PSCs, and its gene activity was stimulated by alcohol, acetaldehyde, and a fibrogenic cytokine transforming growth factor beta1. CCN2 stimulated adhesion, migration, and collagen I synthesis in PSCs, which are features of activated PSCs. These results suggest that alcohol may contribute to the development of chronic pancreatitis by increasing the synthesis of CCN2 in the pancreas.

*A mouse model of ethanol dependent pancreatic fibrosis (chronic pancreatitis)*

Researchers have developed a mouse model of alcohol dependent chronic pancreatitis by administering ethanol to mice for eight weeks and simultaneously injecting caerulein, a more active form of cholecystokinin hormone. These mice exhibited necrosis, and increased expression of collagen I, alpha-smooth muscle actin, transforming growth factor beta1, and tissue inhibitor of metalloproteinase, which are important features of fibrosis and chronic

pancreatitis. The mice administered with ethanol or caerulin alone did not show significant changes compared to those administered with both agents together. This mouse model may be useful in understanding the underlying mechanism by which ethanol ingestion leads to the development of chronic pancreatitis.

*Pancreatic response to endotoxin after chronic alcohol exposure*

This study examined the combined effect of endotoxin and alcohol on rat pancreas. Endotoxin is an outer covering of gram negative bacteria residing in the intestine. Chronic alcohol exposure alone inhibited cell death in the pancreas of rats. On the other hand, pancreatic cell death and inflammation increased after endotoxin injection in control and alcohol-fed rats in a dose-dependent fashion but with a significantly greater response in the alcohol-fed animals. These findings suggest that the pancreas exposed to alcohol is more sensitive to endotoxin-induced cell injury.

*Alcohol-induced hepatic fibrosis*

Chronic heavy alcohol consumption is a major cause of liver cirrhosis, which ultimately results in death. Liver cirrhosis is a progression of fibrosis that results from excessive deposition of extracellular matrix components, especially collagen, in the liver. Although various hepatic cells are involved in the development of fibrosis, hepatic stellate cells (HSCs) are the primary source of excessive extracellular matrix components. A major feature of fibrosis is the activation of HSCs, consisting of an early initiation phase followed by a perpetual phase. NIAAA-funded researchers have made progress in terms of understanding the mechanisms of HSC activation.

*Role of acetaldehyde in human hepatic stellate cell activation*

Acetaldehyde, an immediate metabolite of alcohol, is fibrogenic and induces the expression of type I collagen genes in hepatic stellate cells (HSCs). This study investigated the mechanisms whereby acetaldehyde stimulates and modulates collagen production. The results showed that acetaldehyde can increase collagen production by two mechanisms. In the early stage, it can increase collagen production directly, which is independent of a fibrogenic cytokine transforming growth factor-beta1 (TGF-beta1). In the late stage, however, the effect of acetaldehyde is mediated through TGF-beta1.

*Sinusoidal liver endothelial cells (SECs) produce pro-fibrotic factors in response to adducts formed from the metabolites of ethanol*

Acetaldehyde can react with many proteins covalently, which results in the formation of acetaldehyde-protein adducts. Similarly, a lipid peroxidation product, malondialdehyde (MDA), can also react with proteins to form adducts. Acetaldehyde and MDA both can react with the same protein that results in the formation of acetaldehyde-MDA hybrids adducts (MAA). The plasma levels of MAA are elevated in alcoholics as well as in rats exposed to alcohol. The MAA has been shown to increase the secretion of pro-inflammatory mediators (cytokines/chemokines) by rat sinusoidal liver endothelial cells (SECs), which line blood vessels of the liver. This study

demonstrated that MAA-modified protein increased the expression of cellular fibronectin in the SECs. Since fibronectin is fibrogenic via activation of hepatic stellate cells, it is suggested that MAA may contribute to fibrogenesis by increasing the production of fibronectin in SECs.

*Mechanistic basis of alcohol related carcinogenesis*

Alcoholic beverage consumption is known to be causally related to an increased risk of cancer of the upper gastrointestinal tract. The formation of acetaldehyde from ethanol metabolism seems to be the major mechanism underlying this effect. Acetaldehyde is carcinogenic in rodents and causes sister chromatid exchanges and chromosomal aberrations in human cells. The best-studied DNA adduct from acetaldehyde is N(2)-ethyl-2'-deoxyguanosine, which is increased in liver DNA obtained from ethanol-treated rodents and in white blood cells obtained from human alcohol abusers. However, the carcinogenic relevance of this adduct is unclear in view of the lack of evidence that it is mutagenic in mammalian cells. A different DNA adduct, 1,N(2)-propano-2'-deoxyguanosine (PdG), can also be formed from acetaldehyde in the presence of histones and other basic molecules. PdG has been shown to be responsible for the genotoxic and mutagenic effects of crotonaldehyde. The PdG adduct can exist in either of two forms: a ring-closed form or a ring-opened aldehyde form. Whereas the ring-closed form is mutagenic, the aldehyde form can participate in the formation of secondary lesions, including DNA-protein cross-links and DNA interstrand cross-links. The formation of these types of complex secondary DNA lesions resulting from PdG may explain many of the observed genotoxic effects of acetaldehyde described above. Repair of PdG and its associated adducts is complex, involving multiple pathways. Inherited variation in the genes encoding the proteins involved in the repair of PdG and its secondary adducts may contribute to susceptibility to alcoholic beverage-related carcinogenesis.

*Polyamines stimulate the formation of mutagenic 1,N2-propanodeoxyguanosine adducts from acetaldehyde and DNA*

Acetaldehyde (AA), the first metabolite of ethanol, is a suspected human carcinogen, but the molecular mechanisms underlying AA carcinogenicity are unclear. These studies produced a working model to explain the mechanistic basis of alcohol related carcinogenesis after testing the hypothesis that polyamines could facilitate the formation of mutagenic alpha-methyl-gamma-hydroxy-1,N2-propano-2'-deoxyguanosine (Cr-PdG) adducts from biologically relevant AA concentrations. Researchers found that Cr-PdG adducts could be formed by reacting deoxyguanosine with muM concentrations of AA in the presence of spermidine, but not with either AA or spermidine alone. The identities of the Cr-PdG adducts were confirmed by both liquid and gas chromatography-mass spectrometry. Using a novel isotope-dilution liquid chromatography-mass spectrometry assay, researchers found that in the presence of 5 mM spermidine, AA concentrations of 100 microM and above resulted in the formation of Cr-PdG in genomic DNA. These AA levels are within the range that occurs in human saliva after alcoholic beverage consumption. They also showed that spermidine directly reacts with AA to generate crotonaldehyde (CrA), most likely via an enamine aldol condensation mechanism. They propose that AA derived from ethanol metabolism is converted to CrA by polyamines in dividing cells,

forming Cr-PdG adducts, which may be responsible for the carcinogenicity of alcoholic beverage consumption.

*Fetal alcohol syndrome and other alcohol-related birth defects*

Fetal alcohol spectrum disorders (FASD) describes a spectrum of prenatal alcohol effects resulting from drinking by the mother during pregnancy. The most serious disorder arising from prenatal alcohol exposure is fetal alcohol syndrome (FAS), a cluster of defects that includes mild craniofacial abnormalities, growth retardation, and central nervous system impairments manifested by deficits in executive function, memory and learning, and motor activity. FAS is considered the most common non-hereditary form of mental retardation. Alcohol-related neurodevelopmental disorder (ARND) is more variable in phenotype but can be equally debilitating. Various alcohol-related organ system birth defects have also been reported among children with FAS or ARND, including congenital heart defects, ocular abnormalities, and increased susceptibility to infections.

*Possible molecular targets to prevent alcohol induced neuronal death*

It has been shown previously that developing serotonin (5-HT) neurons and other fetal neurons are reduced *in vivo* and *in vitro* by exposure to ethanol, effects related to cell death. It was also shown that serotonin receptor agonists diminished the ethanol-associated reduction of these cells by decreasing neuron death. The study demonstrated, by means of selective inhibitors, that the neuroprotective effect of serotonin receptor agonists against ethanol-induced cell death of developing serotonin neurons is mediated by activation of at least two signaling pathways, PI-3K and MAPKK. The findings identify new molecular targets for drug discovery to prevent ethanol-induced neuronal death.

In another study, a systematic investigation was conducted of the expression profile of the serotonin 5HT1A receptor throughout the time period for development of the hippocampus. This receptor is sensitive to prenatal ethanol insult. Results indicate robust and early expression of the receptor by all populations of hippocampal neurons, as well as in immature astrocytes. This suggests a role for 5HT1A in the regulation of hippocampal neuronal development, which can be affected by prenatal alcohol exposure. Understanding the expression profile may lead to the development of agents that prevent ethanol's action in the brain.

*Vitamin E and mitochondrial defense systems*

Ethanol (EtOH) has been shown to disrupt the structure and function of the developing nervous system, sometimes leading to birth defects associated with fetal alcohol syndrome (FAS). Cellular membrane peroxidation, intracellular oxidant accumulation, and suppression of endogenous antioxidant enzymes contribute to the toxic effects of EtOH. A mitochondrially targeted vitamin E (MitoVit E) was chemically engineered to accumulate in the mitochondria of cells. This designer MitoVit E has been shown to inhibit intracellular oxidant accumulation and cell death more effectively than unaltered vitamin E, though both are effective. Further testing will determine the therapeutic potential of this compound.

**Significant Ongoing Rare Diseases Research Initiatives**

Work is continuing on the mechanisms of neurological disease in Xeroderma Pigmentosa (XP), Ataxia Telangiectasia (AT), and Cockayne syndrome (CS). The focus of the work on XP is on the role of a specific type of DNA damage called cyclopurine in causing brain cell death. In CS, the intracellular localization of the truncated CSB protein in affected brain cells is being studied. The work on AT is designed to investigate the role of the ATM protein in cerebellar Purkinje neurons, which are severely affected in AT patients.

Work is also ongoing in the area of alcohol related carcinogenesis, using cell culture systems.

*Perinatal alcohol, sudden infant death* syndrome *(SIDS), and stillbirth initiative*

In collaboration with the NICHD, the NIAAA has launched an initiative to develop multi-disciplinary research projects in communities, both nationally and internationally, where prenatal maternal alcohol consumption is high to determine the relationship between prenatal alcohol exposure and other variables in the risk for SIDS and adverse pregnancy outcomes such as stillbirth and FAS. Pilot studies are ongoing.

*Collaborative initiative on fetal alcohol spectrum disorders (FASD)*

The NIAAA continues to support the CIFASD, a collaborative, multi-disciplinary and cross-cultural research program aimed at developing effective interventions and treatment for FASD. The consortium coordinates basic, behavioral, and clinical investigations that utilize novel and cutting edge techniques. One of the first steps will be to definitively outline a diagnostic schema so that the full range of effects from exposure to moderate or large amounts of alcohol can be determined. The goal of the CIFASD is to bring together researchers from around the world who are conducting research on FASD or are interested in the global problem of FASD, and who have the capabilities and resources to utilize international subject populations to further knowledge in this area. Advances in science often require the appropriate technological, social, and cultural climates to foster those advances. Studies that could not be conducted in any one site due to lack of study participants or given expertise will become possible through this collaborative initiative.

**Rare Disease-Specific Conferences, Symposia, and Meetings**

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) and Office of Rare Diseases (ORD) organized a symposium on the Mechanisms of Alcohol-Induced Hepatic Fibrosis in Santa Barbara, California, June 25, 2005. The following topics were covered: 1) Alcoholic Liver Fibrosis: Evolving Concepts and Future Directions; 2) Role of Acetaldehyde in Hepatic Stellate Cell Activation; 3) Cross Talk Between Liver Cells and Fibrogenic Response; 4) Signaling, HCV, and Death in Activated Hepatic Stellate Cells ; 5) Fat Paradox in Hepatocytes and Stellate Cells; 6) Alcohol, Innate Immunity and Liver Fibrosis; 7) Genetics of Alcoholic Liver Fibrosis; 8) Mechanisms of Reversion of Liver Fibrosis; and 9) Systems Biology Approach in Liver

Research. The proceedings of the symposium have been submitted to the journal, Hepatology, for publication.

The Interagency Coordinating Committee on Fetal Alcohol syndrome was convened by the National Institute on Alcohol Abuse and Alcoholism in Rockville, Maryland on November 18, 2005.

*Publications Related to ORD-sponsored Symposia*

Purohit V, Khalsa J, and Serrano J (2005). Mechanisms of alcohol-associated cancers: introduction and summary of the symposium. Alcohol 35:155-160.

Purohit V, Brenner DA. Mechanisms of Alcohol-Induced Hepatic Fibrosis: A Summary of the Ron Thurman Symposium (Submitted to Hepatology journal).

Brooks, P.J. and Theruvathu, J.A. (2005) DNA adducts from acetaldehyde: Implications for alcohol-related carcinogenesis. Alcohol 35(3) : 187-193.

**Activities with Voluntary Rare Diseases Organizations to Stimulate Research**

Cockayne Syndrome Care and Share Network. (http://www.cockayne- syndrome.org/.)

NIAAA researcher, P. J. Brooks consults with some Rare Disease patient organizations. Specifically, one ongoing project concerns the mechanisms underlying the neurological abnormalities suffered by patients with Cockayne syndrome. For this work, he is in contact with the parents of CS children via the network.

Dr. Brooks also consults periodically with the network Ataxia Telangiectasia Children's Project (www.atcp.org.) regarding his ongoing research on AT. He plans to attend their annual sponsored meeting in 2006.

## NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (NIAID)

### Overview of Rare Diseases Research Activities

The National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports basic and applied research to better understand, treat, and ultimately prevent infectious, immunologic, and allergic diseases. Many of the disorders encompassed by the NIAID mission are rare. Rare immune disorders affect, in aggregate, a large number of Americans, and understanding these disorders will provide important insights into how the healthy immune system functions. Some infectious agents that cause rare diseases are common worldwide but are not prevalent in the United States. Others affect very few people at present, but have the potential to emerge among a much larger population—either naturally, which public health authorities fear may occur with H5N1 avian influenza infection in humans, or deliberately, such as might occur with anthrax or Ebola should these agents be used in a bioterror attack. For these reasons, the NIAID research portfolio has recently been expanded considerably to meet the challenges posed by newly emerging and re-emerging infectious diseases and bioterrorism. In 2002, for example, NIAID published a strategic plan for biodefense research[2], as well as research agendas for high-priority "Category A" biodefense pathogens and less immediately threatening but still dangerous Category B and C pathogens. [Category A diseases/agents are the highest priority agents, which include organisms that pose a risk to national security because they can be easily disseminated or transmitted from person to person, result in high mortality rates and have the potential for major public health impact, might cause public panic and social disruption, and require special action for public health preparedness. Category B diseases/agents are the second highest priority agents, which include those that are moderately easy to disseminate, result in moderate morbidity rates and low mortality rates, and require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance. Category C diseases/agents are the third highest priority agents, which include emerging pathogens that could be engineered for mass dissemination in the future because of availability, ease of production and dissemination, and potential for high morbidity and mortality rates and major health impact (from http://www.bt.cdc.gov/agent/agentlist-category.asp#catdef).] The NIAID documents describe three distinct priority areas for the biodefense research: *basic research* on microbes and host immune defenses; targeted, milestone-driven *medical countermeasure development* to create the vaccines, therapeutics, and diagnostics; and development of *infrastructure* needed to safely conduct research on dangerous pathogens.

For the purpose of reporting on NIAID research activities in rare diseases, this report is divided into four areas: infectious diseases, primary immunodeficiency diseases, autoimmune diseases, and other immune system-mediated conditions. *Infectious diseases* are caused by bacteria, viruses, fungi, protozoans, and parasites. *Primary immunodeficiency diseases* are hereditary disorders caused by intrinsic defects in the cells of the immune system and are characterized by unusual susceptibility to infection. *Autoimmune diseases* result when the immune system in the body attacks its own organs, tissues, and cells. *Other immune system-mediated diseases*, such as asthma and allergic diseases, are caused by inappropriate or destructive immune responses.

---

[2] *NIAID Strategic Plan for Biodefense Research, the NIAID Research Agenda for Category A Agents* and the *NIAID Research Agenda for Category B and C Agents;* http://www.niaid.nih.gov/biodefense

NIAID research seeks to understand the mechanisms by which these diseases harm people and how the immune system responds to them. The ultimate goal is to develop new and more effective strategies of disease diagnosis, treatment, and prevention.

## Scientific Advances in Rare Diseases Research

### Rare Infectious Diseases

*Anthrax*

Anthrax is an acute infectious disease caused by the rod-shaped bacterium *Bacillus anthracis*. The disease is transmitted through contact with *B. anthracis* spores—hard, desiccated structures that contain the organism's DNA. Spores that lodge in the lungs cause inhalational anthrax, which is almost always fatal if not treated. Cutaneous anthrax results when spores pass through a break in the skin, and gastrointestinal anthrax results from ingesting spore. Anthrax primarily occurs among ruminants such as cattle and sheep; human anthrax is rare, with the cutaneous form being most common. Because the spores are very stable and thus easy to disseminate in an active form, *B. anthracis* is categorized as a Category A priority pathogen.

By uncovering the molecular pathways that enable the bacterium to form spores, infect people, and cause illness, NIAID hopes to identify new ways to diagnose, prevent, and treat anthrax. NIAID scientists and their colleagues from the Protein Biophysics Resource of NIH developed and characterized a monoclonal antibody that binds to and neutralizes the anthrax toxin more efficiently than any other such antibody characterized to date. These results indicate that passive immunization—immunity acquired by injection of antibodies against anthrax toxin—may provide immediate protection to people exposed to anthrax; future studies will test whether the protection lasts long enough to prevent illness weeks after exposure, when inhaled *B. anthracis* spores lodged in a person's lungs begin to grow.

Little is known about the clinical significance of spores that are inhaled but do not go on to germinate into bacteria or about the clinical course of the disease after recovery. NIAID intramural clinical researchers, in collaboration with the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), completed a preclinical animal study demonstrating that the duration of post-exposure antibiotics could be shortened from 60 days to 14 days if the antibiotic were given in combination with the currently licensed anthrax vaccine. NIAID intramural researchers have also been investigating potential long-term effects on some survivors of the 2001 anthrax attacks; this work is part of a larger clinical study to examine anthrax from initial infection through post-recovery.

NIAID researchers explored mechanisms to explain differences in sensitivity among mice to anthrax toxin. Mice from which the adrenal glands had been removed showed an increased susceptibility to the toxin. Attempts to restore adrenal function with a synthetic hormone called dexamethasone had the opposite, unexpected effect of causing the mice to become highly toxin sensitive. These results indicate that susceptibility of mice to anthrax toxin depends on a fine but

easily perturbed balance of endocrine functions and suggests that steroid therapy for anthrax could have detrimental consequences.

*Avian Influenza*

Thousands of strains of influenza viruses circulate among both mammals and birds. Scientists group these viruses on the basis of two surface proteins, hemagglutinin (H) and neuraminidase (N). Of known influenza viruses, the H5N1 avian influenza strains spreading in domestic and migratory fowl in Asia, Eastern Europe, and Africa are of greatest concern. Although H5N1 influenza is primarily an animal disease, it has infected over 200 people, approximately half of whom have died. If the H5N1 virus mutates further, remains highly virulent, and acquires the capability to spread efficiently from person to person, it could spark a global pandemic with potentially catastrophic consequences. Development of effective vaccines against H5N1 influenza is therefore an urgent public health priority.

NIAID-supported scientists have recently completed a multicenter, double-blind phase I/II clinical trial of an experimental inactivated H5N1 influenza virus vaccine. Researchers found that the H5N1 influenza vaccine is safe and generates immune responses likely to provide protection against H5N1 influenza virus. The H5N1 influenza vaccine used in the trial was manufactured using a very similar process to the one used to make the seasonal influenza vaccine each year, which may allow the United States Food and Drug Administration (FDA) to expedite approval of the new vaccine if needed.

Under a Cooperative Research and Development Agreement, NIAID intramural researchers are working with colleagues from MedImmune, Inc., to create live, attenuated vaccine candidates for each of the 16 known forms of hemagglutinin. So far, three similar H5N1 vaccine candidates—based on the same technology used in MedImmune's FluMist vaccine—have been developed and were found to be protective in mice. Clinical testing of one of these is planned for spring 2006. In addition, an H9N2 live, attenuated, nasal spray vaccine has already undergone Phase I clinical testing and was found to be safe in study volunteers. Laboratory testing is under way to determine whether this vaccine induced a level of immunity that is likely to be protective.

*Cholera*

The bacterium *Vibrio cholerae* causes cholera, a severe diarrheal disease. A recent NIAID-supported study surveyed water samples from Dhaka, Bangladesh over a three-year period spanning several cholera outbreaks. The study showed that water samples contained either susceptible cholera bacteria or specific viruses, called bacteriophages, that attack the bacteria. Over the same period, the incidence of cholera varied; between epidemics, water samples contained cholera phages but no viable bacteria. The study demonstrated that cholera phages play an important role in reducing cholera bacteria in the environment and reducing human illness.

In evaluating vaccines for public health use, two factors are important: how well a vaccine protects a single vaccine recipient, and how well it induces "herd immunity," which is where

enough people in a population are resistant to infection that even unvaccinated people are protected. NIAID-supported scientists recently re-analyzed data from a 1985 vaccine clinical trial in Bangladesh. Their analysis indicates that killed oral cholera vaccines conferred significant herd immunity to nonvaccinated individuals living in community with those who had been vaccinated. These results suggest that use of cholera vaccines could have a major effect on controlling the burden of cholera in endemic areas.

*Chicken pox and shingles*

Varicella zoster virus (VZV) causes chicken pox as a result of primary infection, and shingles, also called herpes zoster (HZ), when it re-emerges from latency years to decades later. Although HZ is not typically life-threatening, it reduces quality of life and is often followed by post-herpetic neuralgia, a painful and often debilitating complication. Antiviral drugs have only a modest impact on the acute phase of HZ, and do not prevent post-herpetic neuralgia. A collaborative Phase III clinical trial was conducted by NIAID, the Veteran's Administration, and Merck, Inc., to assess whether vaccinating older adults with live-attenuated VZV vaccine reduces the incidence and/or severity of HZ and its complications. Involving more than 38,500 volunteers, the trial was one of the largest adult vaccine clinical trials ever performed. VZV vaccine reduced the number of shingles cases by about half, and dramatically reduced illness severity and complication rate among those who got the disease. These striking results are the first demonstration that a vaccine against VZV can prevent shingles. The Food and Drug Administration (FDA) is currently considering Merck's application to market the vaccine for this use.

VZV enters the body through the respiratory tract, but it takes a few weeks for the hallmark chicken pox rash to develop. Scientists previously thought the delay was caused by VZV spreading from its initial entry site to mononuclear cells in regional lymph nodes, then to organs such as the liver, and finally to the skin. However, using an immunodeficient mouse model with grafts of human thymus, liver and skin, NIAID-supported researchers showed that VZV immediately infects T cells which then mediate VZV transfer to the skin. The delay between infection and rash development is probably due to the virus having to overcome a strong host innate immune response, particularly alpha interferon production. This new information about how VZV spreads and VZV pathogenesis should be helpful in designing "second-generation" live-attenuated varicella vaccines.

*Congenital cytomegalovirus*

Cytomegalovirus (CMV) is a common infection that affects fetuses and neonates. It is also the leading infectious cause of hearing loss, although this complication affects only 10-15 percent of CMV-infected children; hearing loss is usually delayed and gets progressively worse. It has been unknown why only some children are affected, and why the symptoms progress as they do. NIAID-supported researchers examined a large number of congenitally CMV infected children, and measured the amount of infectious virus in their blood and urine. They found that the risk of developing hearing loss increased with larger amounts of infectious CMV. This suggests that

such measurements may help clinicians decide when to treat infected children with antiviral drugs.

*Cryptococcosis*

Cryptococcosis, which is caused by the fungus *Cryptococcus neoformans*, is a life-threatening infection of the central nervous system (CNS) that commonly affects people, such as AIDS patients, with impaired immune systems. Cryptococcosis is a predictor of HIV infection in countries without ready access to HAART (highly active antiretroviral therapy). Untreated persons with HIV infection become infected more rapidly and cryptococcosis is one of the first infections to occur. NIAID-supported researchers recently sequenced the full genome of *C. neoformans*, and identified several unique genes that may contribute to its unusual virulence properties. Access to the genomic sequence has allowed researchers to develop oligonucleotide-based microarrays that are being used to identify potential targets for new antifungal drugs and diagnostics. The availability of the sequence also allows other investigators to compare these gene sequences with other pathogens to understand which genes influence pathogenicity, and provides investigators with access to the total protein complement of the *Cryptococcus* genome.

Preclinical assessment of promising new drug candidates and formulations for treatment of cryptococcosis is ongoing in NIAID-supported research laboratories. One candidate is amphotericin B (AmB) formulated for oral drug delivery by scientists at BioDelivery Sciences International. AmB is an intravenous antifungal agent used in the first line treatment of severe fungal infections but is frequently accompanied by toxicities such as nephrotoxicity. This new formulation of AmB is less toxic, which could advance clinical therapy not only for cryptococcosis but for many other fungal infections.

Current antifungal therapies for cryptococcal meningitis in AIDS patients fail more than 25 percent of the time. Recently, investigators from the NIAID Mycoses Study Group completed the initial phase using monoclonal antibodies (MAb) therapy for the treatment of fungal infections in humans. In a safety and tolerability study, the researchers administered a MAb called 18B7 to HIV-positive patients who had previously been treated for cryptococcal meningitis. The investigators found the MAb regimen to be well tolerated and observed evidence of pharmacologic benefit at the higher doses tested. The results are promising and support the continuation of the study to assess the antibody's effectiveness in treating fungal diseases.

*Ebola*

Ebola virus is a rare virus that causes fatal high fever and massive internal bleeding. Because it is up to 80 percent fatal, Ebola virus is a Category A priority pathogen. The hemorrhagic fever syndrome seen with Ebola is accompanied by a profoundly dysfunctional immune response. Although the molecular events that cause these problems are not well understood, scientists at the NIAID Vaccine Research Center (VRC) have identified a cellular mechanism that may explain them. Ebola virus disrupts several processes essential for immune activation and recognition, such as cell trafficking and antigen presentation. By altering the trafficking of select cellular proteins, Ebola GP inflicts cell damage and may facilitate immune escape by the virus.

This mechanism is likely responsible for inflammatory dysregulation, immune suppression, and vascular dysfunction that are the hallmarks of lethal Ebola virus infection, and understanding it better will aid the development of countermeasures against the pathogenic effects of the virus.

*Escherichia coli (Enterotoxigenic)*

Enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of infant mortality in less developed countries and the cause of most cases of "travelers' diarrhea." Like many pathogenic bacteria, ETEC produces toxins that contribute to the disease process—in this case, a toxin called LT that induces copious watery diarrhea. NIAID-supported researchers found that membrane vesicles with LT embedded in their surfaces have a mechanism to specifically target host cells. LT allows the vesicle to adhere to the cell, which in turn allows the toxin-containing vesicle to gain entry into the cell. The discovery that toxin-containing vesicles play a role in pathogenesis may be extended to other toxin-producing bacteria and may represent a therapeutic opportunity.

Another NIAID-supported study showed that intestinal cells of newborn mammals produce an innate anti-bacterial defense that can reduce the number of bacteria present. Paneth cells are specialized surface cells in the small intestine of mammals that release antimicrobial proteins for disease protection. For this reason, Paneth cells are crucial for the defense of the small intestine, but their function during infections in newborns has not been well characterized. The NIAID study showed that in newborn rats, chemical treatment that selectively damages Paneth cells increases the number of E. coli bacterium in their small intestine, compared to controls. This research supports the hypothesis that Paneth cells are needed for newborn antibacterial defense.

*Hepatitis E*

Hepatitis E disease, caused by hepatitis E virus (HEV), generally affects young adults. It usually is not life-threatening, except in pregnant women infected with the virus where fatality rates of 15 to 20 percent have been reported. NIAID researchers recently developed a cell culture system that permitted limited infection by wild-type HEV. Using this cell culture system they identified specific parts of viral proteins that can be neutralized by antibodies. This research provides a powerful new way to evaluate the results of vaccine trials, including the current phase II/III trial of HEV recombinant vaccine. Identification of neutralizing monoclonal antibodies will be useful for formulating antibodies for prophylaxis for HEV infection.

*Lassa fever*

The *Arenaviridae* family of viruses cause a fatal hemorrhagic fever, and are considered Category A pathogens. The only treatment for this deadly family of viruses is ribavirin for Lassa fever. NIAID-supported researchers have found that a bioengineered alpha interferon called interferon alfacon-1 is active in cell culture against another arenavirus called Pichinde, which in hamsters is a model for Lassa fever. In Pichinde-infected hamsters, interferon alfacon-1 treatment significantly protected the animals from death, and the treated animals that ultimately died survived longer and had a reduced virus load and less liver damage than untreated animals. This is the first reported effective treatment of acute arenavirus with alpha interferon therapy.

*Leishmaniasis*

*Leishmania* parasites are responsible for an array of disfiguring, fatal diseases worldwide, including sand fly disease, kala-azar, Dum-Dum fever, and "Baghdad boil." It has been generally held that the protective immune response and long-term resistance that develops after *Leishmania major* infection depends on the parasite persisting in the body. NIAID supported researchers used a mouse model to show that although effector T cells—immune cells directly involved in fighting the infection—disappear after the parasite is removed, memory T cells remain. Upon secondary infection, these memory T cells become tissue-homing effector cells that mediate immune protection. The findings suggest that expansion of the memory T cell population, rather than short-lived effector T cells, should be the goal for vaccines against leishmaniasis and possibly other infectious diseases that are most effectively fought by T-cell-mediated responses.

Transmission of *Leishmania* parasites by sand flies requires that the parasites attach to midguts of the insects. Only then can the parasites develop into forms that are transmitted to humans or other mammalian hosts by insect bites. Using sequence information from the Leishmania genome, NIAID scientists have identified and characterized the receptor on cells in the sand fly midgut that binds *Leishmania* parasites and controls their ability to transmit the most common form of cutaneous leishmaniasis found in North Africa, the Middle East, and South Asia. Feeding flies antibodies against this receptor, PpGalec, prevents infections by *Leishmania* parasites.

A multi-center project funded in part by NIAID sequenced the genomes of three Trypanosomatids, *Leishmania major*, *Trypanosoma cruzi,* and *Trypanosoma brucei*. The species have a share core of approximately 6,200 genes which may be targets for intervention across the Trypanosomatid protozoa. However, each species had several unique features, such as large families of genes that encode surface proteins that help to explain differences in modes of transmission and pathogenesis.

*Lyme disease and other tick-borne pathogens*

Lyme disease, caused by the bacterium *Borrelia burgdorfer,* is typically transmitted by ticks and can usually be treated successfully with antibiotic therapy, especially when treated early in the disease. Unfortunately, some patients do not respond completely to therapy. NIAID researchers are conducting three clinical trials of patients with Lyme disease. One study addresses patients with post-treatment Lyme disease syndrome, which occurs when therapy is not completely successful. A second study focuses on patients with classical Lyme disease, and the third protocol examines the host response in skin biopsies from patients with the typical rash called erythema migrans that accompanies Lyme disease. These studies will help to improve laboratory diagnosis of Lyme disease and increase understanding of clinical manifestations and immunological responses to *B. burgdorferi* infection.

NIAID funded scientists have gained several insights into the molecular mechanisms of *B. burgdorferi* infection. To successfully colonize ticks, a protein on the outer surface of *B.*

*burgdorferi* called OspA must bind to a newly identified receptor protein in the tick midgut called TROPSA. When the binding of OspA to TROSPA is blocked, either with antibodies that block the binding site or by inhibition of TROSPA synthesis, *B. burgdorferi* does not adhere as well to the midgut of ticks and can therefore no longer colonize them. This greatly reduces the bacteria's ability to transmit disease to humans and other mammals and suggests new possibilities for controlling the spread of Lyme disease in endemic areas.

No human vaccine is currently available for Lyme disease. NIAID supported researchers have therefore experimented with vaccinating wild mice, in the hope that such a strategy might prove useful for reducing disease transmission to people. White-footed mice, a major Lyme disease reservoir, were immunized with either recombinant OspA or a control antigen and then released to a field site monitored for *B. burgdorferi* in captured ticks. OspA vaccination substantially reduced the prevalence of infected ticks, indicating that this type of approach may limit the spread of Lyme disease in endemic areas.

*Relapsing fever virus (Borrelia)*

Tick-borne relapsing fever, caused by *Borrelia hermsii*, is endemic in scattered areas throughout many regions of higher elevation in the western United States. Genome comparisons of *B. hermsii*, *Borrelia turicatae*, and the Lyme disease bacterium, *B. burgdorferi* may shed light on the mechanisms and pathways for infection in vertebrate and tick hosts. Researchers at NIAID's Rocky Mountain Laboratories are investigating several unique genes that may help clarify different mechanisms in the pathogenicity of Lyme disease and relapsing fever spirochetes. Using a genetic analysis of bacteria from various host species at several locations in the United States, researchers identified a new focus of tick-borne relapsing fever in Florida.

*Rocky Mountain spotted fever*

Unlike most bacteria, the organism that causes Rocky Mountain spotted fever (RMSF) and related diseases must live inside the cells of its hosts, and typically lacks the transferable genetic elements that allow most bacteria to exchange genes with one another. These factors all make the RMSF pathogen, *Rickettsia rickettsii* and similar agents difficult to study in the laboratory. However, NIAID-funded investigators recently identified a novel transferable genetic element in *Rickettsia peacockii*, a close relative of *R. rickettsii*. The investigators showed that the insertion sequence appeared to be active and present in ten locations within the *R. peacockii* genome. One such insertion disrupts a key gene involved in the cell-to-cell spread in *R. rickettsii*, and thus partially explains why *R. peacockii* does not cause disease. The discovery of this transposable genetic element also offers new opportunities for studying other elements of rickettsial pathogenesis and provides a potential new tool for genetic analysis of these difficult-to-study bacteria.

*Tick-borne encephalitis virus*

Tick-borne encephalitis (TBE) is caused by a variety of flaviviruses, including Tick borne encephalitis virus (TBEV), Omsk hemorrhagic fever virus, Kyasanur forest disease virus, Langat

virus, and Powassan virus. These viruses all cause encephalitis, meningitis or hemorrhagic fevers with mortality rates as high as 40 percent. NIAID researchers found that a protein called NS5 from the tick-borne Langat flavivirus binds to a receptor for interferon, a human defense protein, and thus prevents interferon from inhibiting viral replication. That NS5 inhibits interferon is unique among this genus of viruses, and provides a potential candidate for novel therapeutic intervention.

*Plague*

Plague, a Category A priority pathogen, is caused by the bacterium *Yersinia pestis.* It is usually transmitted to people through the bite of an infected flea, which usually causes the bubonic form of the disease characterized by severe swelling of the lymph nodes. Inhalation of plague bacteria can cause the pneumonic form of the disease, which is virtually always fatal without treatment.

In the early stages of the disease, the symptoms of plague are hard to distinguish from other diseases such as influenza—which might delay detection of a bioterrorism attack using plague. Recently, NIAID-funded scientists developed a six-hour test to assist in the diagnosis of plague in patients presenting with pneumonia symptoms at a hospital; current tests rely on culturing organisms present in patient samples and typically take from 24 to 72 hours to generate a result. A rapid diagnostic would enable health care providers to identify and isolate the pneumonic plague patient away from other patients, begin a best course of treatment quickly, and take appropriate precautions.

Researchers at NIAID's Rocky Mountain Laboratories have developed a model of bubonic plague using the brown Norway rat. The researchers showed that the gross pathology and histopathology in infected lymph nodes closely resembles what is observed in human bubonic plague. Rodent models for plague provide a system for further applied research, such as vaccine efficacy trials, and for basic research into the host response to *Y. pestis*. In other studies, the researchers showed that a large quantity of *Y. pestis* is needed to infect its most proficient vector, the rat flea *Xenopsylla cheopis*, and that subsequent transmission efficiency was low. These results suggest that the evolution of flea-borne transmission led to the emergence and continued maintenance of a hypervirulent *Y. pestis* clone to overcome the high barrier to infecting fleas and the low transmission efficiency of transmission from fleas to mammals. These results may help in devising disease-control strategies.

*Q fever*

Q fever, a Category B biodefense pathogen, is caused by the bacterium *Coxiella burnetii*. Acute Q fever resembles influenza in its symptoms, usually resolves on its own, and responds well to antibiotics. Chronic Q fever, however, is a more severe and sometimes fatal disease caused by long-term infections of the heart or other tissues. Past research suggested that genetic variations among *C. burnetii* bacteria determine whether Q fever will be acute or chronic, but the identity of those variations was unknown. Recently, NIAID-funded researchers identified the gene and encoded protein associated with the acute form of Q fever. Researchers hope that being able to

detect important sequence changes in this gene or the presence of the protein in clinical samples will lead to an effective diagnostic test to distinguish between acute and chronic Q fever.

*Schistosomiasis*

*Schistosoma mansoni* infection can cause chronic and potentially fatal shistosomiasis. Researchers now have discovered a new biochemical pathway that results in the damaging formation of fibrous tissue that accompanies these infections. The results may be applicable to other disorders as well, because inflammation, fibrosis, and tissue scaring are common manifestations of a number of autoimmune, allergic, and chronic infectious diseases; further research on this topic may yield new approaches to treat a variety of chronic inflammatory disorders.

*Severe acute respiratory syndrome (SARS)*

Severe acute respiratory syndrome (SARS) emerged in 2003, but has since disappeared from the human population. But the concern that the SARS coronavirus (SARS-CoV) could reemerge makes it imperative that effective means to prevent and treat the disease be developed. In August 2004, SARS-CoV was added to NIAID's list of Category C priority pathogens for biodefense.

NIAID scientists and their collaborators have learned a great deal about SARS-CoV. Several animal models for SARS have been developed, including mouse, hamster, and non-human primate models. Several candidate vaccines against SARS have been developed. For example, the VRC contracted with Vical, Inc. to manufacture a DNA-based vaccine encoding the S protein of SARS-CoV. VRC mouse studies demonstrated that this vaccine induces T cell and neutralizing antibody responses, as well as protective immunity. A Phase I clinical study to evaluate safety, tolerability, and immune response was initiated in FY 2005. Interim study results indicate that the vaccine is well tolerated with no systemic or local reactogenicity greater than mild in severity, with no serious adverse events. The study is expected to be complete in FY 2006.

*Smallpox*

Smallpox is a highly lethal infectious disease caused by the *Variola major* virus it is a Category A priority pathogen. After smallpox was eliminated from the human population in the late 1970s, immunization programs worldwide were discontinued. Very few people born since then have ever been vaccinated, and the protection for people vaccinated decades ago has declined substantially. Development of smallpox treatments that could help people with little or no smallpox immunity is therefore a high priority.

Antiviral drugs are generally directed against the proteins and functional pathways of the virus itself. However, NIAID-funded scientists recently took the novel approach of targeting normal cellular signaling pathways that the pox virus uses to reproduce itself. Over the past several decades, thousands of promising anticancer drugs have been developed by pharmaceutical companies to interfere with cell signaling pathways. Two different studies conducted by two

groups of NIAID-supported researchers have shown that the anticancer drugs CI-1033 and Gleevec, which inhibit separate cell signaling pathways, each inhibit a late step in the orthopoxvirus life cycle. The consequence of this inhibition is a reduction in the spread of the virus and prevention of poxvirus-induced disease. This strategy may lead to advances for infectious diseases that rely on similar host-cell enzymes. Because the drugs target host rather than viral targets, they are much less likely to cause drug-resistance than conventional antiviral therapies. These new studies suggest that anticancer drugs represent a largely untapped source of potential antiviral drugs and merit further exploration.

*Spongiform encephalopathies*

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases. They include scrapie of sheep, Creutzfeldt-Jakob disease of humans, bovine spongiform encephalopathy (BSE) or "mad cow" disease, and chronic wasting disease (CWD) of deer and elk. TSEs are caused by accumulation of an abnormal form of a protein found in humans and animals called prion protein (PrP). The biological agent that transmits prion diseases is highly resistant to inactivation by heat and chemicals and has been extremely difficult to identify with precision. Previous evidence has suggested that a misfolded version of prion protein (PrP) might be the causative agent though there is disagreement over whether single, misfolded PrP molecules or larger aggregates of misfolded PrP might be required for transmission. Using a new method to separate proteins on the basis of molecular size, NIAID scientists have recently shown that the minimal size of the infectious agent is similar to six PrP molecules, and that the agent with peak infectivity has a size in the range of 14 to 28 PrP molecules. Although this work does not prove that PrP alone is the infectious agent, these studies appear to exclude the hypothesis that single misfolded PrP molecules can transmit disease, and indicate that drugs that prevent PrP aggregation into polymeric forms might be effective in treating or preventing disease.

*Streptococcal group A invasive disease*

Group A streptococci (GAS) cause a wide spectrum of bacterial diseases, ranging from minor to life-threatening infections. Research to create vaccines and therapeutics has been hampered by insufficient animal models to study GAS, but recently a monkey model was developed. NIAID funded researchers further classified this GAS monkey model using microarrays, a technology that simultaneously measures the expression of many bacterial genes, during a study of people with sore throats. Different phases of disease–such as the asymptomatic phase and the acute phase –were associated with different patterns of bacterial gene expression. The understanding of which genes are active at different times during the course of disease will be useful in the development of GAS vaccine candidates and more effective treatments for severe invasive GAS disease.

*Streptococcus group B*

Group B streptococci (GBS) are the leading cause of life-threatening bacterial infections in newborns such as sepsis and meningitis. Most infected infants acquire GBS infection from their mothers during birth. Current neonatal disease prevention strategies in the United States have

focused on giving antibiotics during labor and delivery; however, this has not eliminated GBS disease and encourages the widespread use of antibiotics. A better tool would be a vaccine for the mother that would provide cross protection against all strains of GBS and would protect the infant by transfer of protective antibodies to the baby through the placenta. To this end, NIAID-supported researchers compared genome sequences of eight GBS strains and found four surface proteins that induced protective antibodies in mice. In combination, the four proteins were highly protective against a large group of GBS strains which include all capsule types that are currently causing GBS disease. These data provide the basis for development of a universal GBS vaccine.

S*treptococcus pneumonia, drug-resistant invasive diseases*

Infection with *Streptococcus pneumoniae* can result in pneumonia, meningitis, or sepsis and is a common cause of middle ear infections (otitis media) in infants and young children. Acquired immunity to *S. pneumoniae* (pneumococcus) has long been assumed to depend on the presence of antibodies that bind to an outer surface capsule that surrounds the organism. NIAID supported researchers found a type of immune cell called CD4(+)T cells also participate in the development of acquired immunity against *S. pneumoniae.* These findings suggest that immunity to *S. pneumoniae* can occur through the presence of either specific antibodies to the outer surface capsule or through a cellular component that provides very broad protection against all strains of the organism. Further investigation will explore ways to stimulate this broad protection.

*Syphilis*

Syphilis is a genital ulcerative disease caused by the bacterium *Treponema pallidum.* If untreated during pregnancy, syphilis infection has a 40 percent perinatal mortality rate. The human immune system mounts a significantly less robust response to *T. pallidum* than it does to other bacteria, which helps to explain why *T. pallidum* frequently establishes chronic, lifelong infection. Although scientists have indirect evidence that the immune system can mount a response against at least some of the bacterium's surface proteins, efforts to identify specific immune targets have been hampered by a lack of established methods to demonstrate their presence on the bacterial surface. NIAID-supported investigators, however, have recently provided the first direct physical evidence of an antigen on the surface of *T. pallidum*. Researchers immunized mice with outer membrane vesicles isolated from *T. pallidum* and found that the mice created an antibody that binds to a molecule on the *T. pallidum* surface. In an experimental rabbit model of syphilis, the antibody conveyed partial protection, suggesting that the *T. pallidum* molecule can serve as a protective immunogen.

*West Nile virus*

West Nile virus (WNV) belongs to a group of disease-causing viruses known as flaviviruses, which are spread by mosquitoes and other insects; other flaviviruses include yellow fever virus, Japanese encephalitis virus, dengue virus, and Saint Louis encephalitis virus. WNV was first isolated in Uganda in 1937, but emerged in 1999 for the first time in the Western Hemisphere in

the New York City area. It has subsequently become endemic across North America. There are no drugs to treat the virus and no vaccines available to prevent infection in humans. Because WNV is now well-established in the United States, research on WNV and other arthropod-borne viruses is part of NIAID's comprehensive emerging infectious diseases program.

Using a three-year fast-track grant, NIAID provided initial support to Acambis, Inc., to develop a live, attenuated recombinant human vaccine for WNV. The vaccine developed is a chimera, in that it is composed of the main backbone from the well-established Yellow Fever 17D vaccine, but in which the envelope genes of the Yellow Fever vaccine virus have been replaced with the analogous genes from West Nile virus. The WNV vaccine candidate demonstrated good safety, efficacy, and protection against disease in animal models. Acambis is conducing a Phase I clinical trial of the vaccine in humans (started in November 2003) with excellent results so far with regard to safety and immunogenicity. A different chimeric WNV vaccine based on a dengue virus backbone has been developed by NIAID intramural researchers and is also in Phase 1 clinical trials. In addition, the NIAID Vaccine Research Center is partnering with industry to develop a DNA vaccine for WNV. A clinical trial of this vaccine began in April 2005.

In humans, the natural antibody response to WNV is often successful in neutralizing the virus. In an attempt to create a WNV treatment, therefore, NIAID-supported researchers have developed monoclonal antibodies against WNV. One particular antibody neutralized ten different WNV strains in animal studies, and a single dose was able to cure mice of WNV, even after the virus had entered the brain. The researchers then developed a "humanized" version, in which the genetic material that controls the mouse antibody's targeting was cloned into a human antibody backbone. When tested in mice, the humanized antibodies retained their ability to stop West Nile virus. This successful animal study suggests that humanized antibodies may be a viable treatment for WNV and that antibody-based therapeutics may be useful in treating other infections caused by viruses that invade the brain.

*Clinical Trials Initiated in FY 2005*

- West Nile Virus vaccines: Two Phase I clinical trials testing WNV vaccines were initiated in FY 2005. One is a chimeric WNV using a dengue virus as a backbone to carry West Nile virus genes and another is a DNA-based vaccine, developed in collaboration with Vical, Inc.

*Ongoing Clinical Trials*

- Cytomegalovirus vaccines: Phase I/II trials are ongoing for three different candidate vaccines for human CMV. One example is testing a vaccine's effectiveness to prevent infection for post-partum CMV-seronegative women.
- Cytomegalovirus antiviral: The Adult AIDS Clinical Trials Group is analyzing data collected during a recently closed phase III clinical trial that examined valganciclovir's safety and effectiveness in preventing cytomegalovirus organ damage in HIV-infected subjects.
- Ebola vaccine: The VRC has recently completed the first Phase I clinical study of a preventive DNA vaccine against Ebola virus infection.

- GBS vaccine: The Medical College of Georgia and Planned Parenthood of Houston and SE Texas are conducting a Phase II randomized, double-blinded, comparative clinical trial for a group B streptococcal (GBS) type III polysaccharide-tetanus toxoid vaccine in 18-40 year old women to evaluate prevention of vaginal acquisition of GBS type III. Recruitment is anticipated to be completed by 2007.
- SARS vaccine**:** The VRC is conducting a Phase I clinical study to evaluate safety, tolerability, and immune response of a single closed, circular DNA plasmid based vaccine encoding the S protein of SARS-CoV. Interim study results indicate that the vaccine is well tolerated with no systemic or local reactogenicity greater than mild in severity, with no serious adverse events. The study is expected to be complete in FY 2006.
- Shiga toxin-producing *Escherichia coli* antivirals: The VTEU will continue Phase I testing of monoclonal antibodies against Shiga toxin of *E. coli* O157:H7 and other Shiga toxin-producing *Escherichia coli* strains as a way to prevent the development of hemolytic-uremic syndrome in infected children.
- Small pox antiviral: NIAID has supported development of an IND to support the use of cidofovir as primary treatment of smallpox. The VTEU has developed clinical protocols to assess activity of cidofovir as back-up therapy after vaccine immune globulin (VIG) for complications related to smallpox vaccine. Thus far, no one has needed to be enrolled in this protocol.
- Small pox vaccine: The modified vaccinia ankara (MVA) vaccine as an attenuated poxvirus has several clinical trials underway to demonstrate the safety and immunogenicity of MVA in healthy adults, and in adults with atopic dermatitis.
- *Streptococcus pneumoniae* vaccine: The Comprehensive International Program for Research on AIDS (CIPRA) project at the University of Witwatersrand is enrolling pediatric subjects into a Pediatric Vaccine Protocol to study antibody responses to *Streptococcus pneumoniae* and *Haemophilus influenzae* type b conjugate vaccines.
- Syphilis therapeutic: The STD Clinical Trials Unit is conducting a randomized Phase III trial to evaluate the equivalency of oral azithromycin versus injectable benzathine penicillin for treatment of primary syphilis.
- Eosinophilic gastroenteritis therapeutics: Omalizumab (therapeutic monoclonal anti-IgE) for eosinophilic gastroenteritis study is currently enrolling subjects. Also intravenous immunoglobulin G (Omr-IgG-am$^{TM}$) is being tested in an FDA-cleared Phase I/II randomized, placebo-controlled clinical trial under the NIAID Collaborative Antiviral Study Group (CASG).
- West Nile Virus natural history: The CASG is conducting a Phase I/II clinical trial to assess the natural history of WNV encephalitis to characterize the serious disease patterns and evaluate potential prognostic indicators of disease progression. The CASG is also testing intravenous immunoglobulin G (Omr-IgG-am$^{TM}$) in an FDA-cleared Phase I/II randomized, placebo-controlled clinical trial.

*Planned Clinical Trials and IND applications*

- Ebola vaccine: The VRC will continue to develop an accelerated adenoviral Ebola vaccination strategy to be used as a prophylactic vaccine or in an acute outbreak or occupational exposure. A Phase I trial is scheduled for late 2006. The VRC will also

investigate the immune mechanism of protection of a vaccine against Ebola in hopes of developing immune therapy for acute intervention and will continue to develop improved or second generation vaccine candidates for Ebola and other viral hemorrhagic fevers (VHFs), including Marburg virus.

- Typhi vaccine: The Vaccine Treatment and Evaluation Unit (VTEU) will begin a clinical trial early in 2006 on the Safety and Immunogenicity of a Live, Attenuated *Salmonella* Typhi vaccine (Ty800).
- Typhoid vaccine: The VTEU plans a clinical study for FY 2006 to evaluate the ability of licensed VI typhoid vaccine to boost the immune response to live attenuated vaccine strain CVD909.
- Enteroaggregative *E. coli*: The VTEU will begin a clinical trial in Fall 2005/Winter 2006 on the Pathology of Enteroaggregative *E. coli* in adult volunteers.
- Cytomegalovirus therapeutic: The CASG plans to assess a new oral derivative of cidofovir in patients with CMV to develop the human safety database for the product as it moves through development.
- SARS vaccines: Two Phase I clinical trials for inactivated whole virus SARS vaccines are planned for FY 2006. NIAID has had Pre-IND meetings with the FDA for both of these vaccines.
- SARS therapeutic: CASG, in consultation with the Center for Disease Control and Prevention (CDC), is developing a clinical protocol to evaluate Interferon alfacon-1 (INFERGEN), an engineered recombinant interferon molecule in preparation for the reemergence of SARS.
- West Nile Virus vaccine: The VRC in collaboration with Vical, Inc., is developing a second generation DNA vaccine using an improved expression vector. A Phase I clinical trial is planned for spring 2006.

**Products Approved in FY 2005**

- Acellular pertussis vaccine combined with tetanus and diphtheria: Boostrix, manufactured by GlaxoSmithKline Biologicals, and ADACEL, manufactured by Sanofi Pasteur, were both licensed in 2005 for use as a single-dose booster vaccine in individuals aged 10 to 18 and 11 to 64 years respectively.
- Meningococcal conjugate vaccine: Menactra (manufactured by Sanofi Pasteur) was licensed in 2005 for use in individuals aged 11-55.

**New Activities**

- Cooperative Research Partnerships for Biodefense is a new RFA that will support discovery/design and development of vaccines, therapeutics, adjuvants, and diagnostics for NIAID Category A, B and C priority pathogens and toxins.
- Regional Biocontainment Laboratories Construction Program funded construction of four more Regional Biocontainment Laboratories bringing the total to 13.
- Intramural Biocontainment Construction NIAID has begun construction of three BSL–3/4 laboratories, one on the NIH campus, one in collaboration with US Army Medical Research

Institute for Infectious Diseases (USAMRIID) in Frederick, MD, and one at the Rocky Mountain Laboratory in Hamilton, MT.

**Ongoing Activities**

- Bacteriology and Mycology Biostatistical and Operations Unit and the Bacteriology and Mycology Study Group initiatives support clinical trials against fungal and resistant bacterial infections. A reserve fund to support orphan drug studies that cannot be funded through industrial sponsors is available through the contract.
- Biodefense and Emerging Infectious Diseases Research Opportunities initiative supports research leading to the diagnosis, prevention, and treatment of diseases caused by emerging infectious diseases, including potential agents of bioterror.
- Biodefense and Emerging Infections Research Resources Program provides high quality resources for the research community, such as sets of West Nile peptides.
- Biodefense Proteomics Research Centers in FY 2005 has generated more than 700 potential targets for vaccines, therapeutics and diagnostics.
- Collaborative Antiviral Testing Group has projects on Yellow Fever, Dengue, West Nile virus, VEE, Pichinde virus (a surrogate for arenaviruses), Punta Toro virus (a surrogate for Rift Valley Fever, Sandfly Fever, and hantavirus), prion disease, SARS-CoV, and smallpox. Approximately 1,600 compounds were screened in FY 2005. Several compounds with efficacy against pathogens of rare diseases identified include; smallpox, Punta Toro Virus, Pichinde Virus, prion disease, WNV, and SARS-CoV. One compound for smallpox, ST-246, was supported by a NIAID SBIR award.
- Challenge Grants Program: Biodefense Product Development facilitates collaborative partnerships between government and the private sector for further development of already-identified products against NIAID Category A, B, and C priority pathogens.
- Collaborative Antiviral Study Group (CASG) is a multi-institute, collaborative network composed of 63 institutions under which clinical studies of therapies for viral infections are conducted. The CASG has four pediatric clinical trials aimed at treating neonatal herpes simplex virus infections, sepsis caused by a group of viruses called enteroviruses, and cytomegalovirus (CMV) infections involving the central nervous system.
- Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense and SARS in FY 2005 had 27 awards to support research on biodefense, including rare diseases such as Ebola virus, SARS and tularemia.
- Cooperative Research Partnerships for Biodefense
- DAIT/DMID Population Genetics Analysis Program: Immunity to Vaccines/Infections has awarded six Centers and studies include examining host response to immunization against smallpox, anthrax, typhoid fever, and cholera.
- Immune Function and Biodefense in Children, Elderly, and Immunocompromised Populations Neutralizing Monoclonal Antibodies for Type A Botulinum Neurotoxins has the objective to develop novel methods for protecting or treating immunocompromised individuals at risk from bioterror threats. The research program covers the identification of biological mechanisms responsible for increased susceptibility to infection or decreased

effectiveness of vaccines in these populations, as well as testing of treatments designed to increase safety or efficacy. Ten awards were made under this program in FY 2005.

- *In vitro* and Animal Models for Emerging Infectious Diseases and Biodefense Program provides a range of resources for pre-clinical testing of new therapies and vaccines including nonhuman primate models such as anthrax, plague, tularemia, smallpox, SARS, ricin, and Burkholderia. This project supported development of four animal models in FY 2005: monkeypox in African Dormice, Venezuelan Equine Encephalitis in mice, SARS Co-V in hamsters, and human metapneumovirus in cotton rats.
- Neutralizing Monoclonal Antibodies for Type A Botulinum Neurotoxins promotes development and manufacture of monoclonal antibodies for botulinum neurotoxins A1 and A2, using authorities provided by Project Bioshield. One award made in FY 2005.
- NIAID International Research in Infectious Diseases (IRID) R03 Program supports the development of local scientific expertise and increases collaborative research partnerships at NIAID international sites.
- Microbial Sequencing Centers which provide rapid and cost efficient resources for producing high quality genome sequences of pathgens. In FY 2005, 30 bacterial pathogen sequencing projects were completed, including *Burkholderia mallei* (5 strains), *Burkholderia pseudomallei*, (6 strains), *Burkholderia thailandensis*, *Ehrlichia spp.*, *Escherichia coli* (8 strains), *Shigella boydii*, *Vibrio cholerae* (6 strains) and *Yersinia pestis* (2 strains). DNA sequencing projects for one protozoan parasite (*Giardia lamblia*), one insect vector (*Aedes aegypti*) and three fungi (*Aspergillus terreus* and *Histoplasma capsulatum* (2 strains)) also were completed.
- Modeling Immunity for Biodefense supports development of user-friendly mathematical modeling tools to understand host immune responses to infection by, or vaccination against Category A, B or C priority pathogens, and to guide laboratory experiments of host immune responses against infectious agents. Four awards were made in FY 2005.
- Mycology Research Units (MRUs) Program project grants under this program support interdisciplinary research to increase understanding of the biology and host-pathogen interactions of the medically important fungi.
- Partnerships for Vaccines and Diagnostic Development Program cooperative agreement program that focuses on the development of vaccines against GAS, GBS, and *Helicobacter pylori*.
- Pathogen Functional Genomics Resource Center at the Institute for Genomic Research (TIGR) provides a wide range of genomic and related resources and technologies for the functional analysis of microbial pathogens and invertebrate vectors of infectious diseases.
- Recombinant Type E Botulinum Neurotoxins Vaccine supports manufacture of a recombinant type E botulinum neurotoxin vaccine, using authorities provided by Project Bioshield. One award was made in FY 2005.
- Regional Biocontainment Laboratories Construction Program funded construction of four more Regional Biocontainment Laboratories (RBLs), for a total of two National Biocontainment Laboratories (NBLs) and 13 RBLs.
- Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs) has centers funded under this initiative that conduct research relevant to rare diseases including new approaches to blocking the action of anthrax, botulinum, and cholera toxins,

and developing new vaccines against anthrax, plague, tularemia, smallpox, and hemorrhagic fevers.

- Respiratory Pathogens Reference Laboratory, a contract with the University of Alabama, is a resource facility to provide reagents and assay development for measurement of the human immune response to targeted bacterial respiratory pathogens.
- The Schistosome Resource Center of the Biomedical Research Institute and the Filaria Resource Center of the University of Georgia are resources for helminth (parasitic worm) research.
- Therapeutics for CDC Category A Agents: Bioshield Accelerated Product Development
- Training and Career Development for Biodefense and Emerging Diseases
- Tularemia Vaccine Development Teams support research to identify and evaluate new tularemia vaccine candidates. Two awards were made in FY 2005.
- Contract with Brigham and Women's Hospital a collaborative multidisciplinary clinical studies in selected populations to further understand GBS infection and on studies of the host immune response.
- Anti-infective drug development contracts test new candidate compounds for efficacy against infectious complications of AIDS in culture and in animals. Microorganisms relevant to rare diseases are: *Mycobacterium avium*, *Pneumocystis*, *Cryptosporidium*, *Cryptococcus*, and *Microsporidium*.
- Cooperative program with the USAMRIID conducts vaccines research for viral hemorrhagic fevers using Ebola virus and (in conjunction with FDA) evaluating the efficacy of antibiotic treatment in a model of pneumonic plague in African green monkeys. In conjunction with the National Cancer Institute (NCI), the F1–V Plague vaccine manufacturing process is being developed.
- In collaboration with USAMRIID and CDC, NIAID supports the *in vitro* screening of candidate drugs against the SARS coronavirus.
- NIAID and FDA, through an interagency agreement, continued to support the screening of compounds that may be effective against biodefense-related and emerging viruses, including vaccinia, cowpox, West Nile, yellow fever, and SARS, among others.
- NIAID and the Department of Defense (DoD) are collaborating to provide for the coordinated development of a recombinant vaccine to protect against serotypes A and B of botulinum toxin. Through a Memorandum of Understanding (MOU) with DoD's Chemical/Biological Defense Program, NIAID is advancing the development of the Army's tularemia LVS vaccine by conducting toxicology testing and phase I clinical trials. The MOU is being extended beyond its original 2004 agreement to continue this collaboration.

**Scientific Conferences, Symposia, and Meetings**

- Pulmonary Nontuberculous Mycobacterial Infections: Phenotype and Genotype of an Autosomal-Dominant Disorder Primarily Affecting Older Women was held March 2005 in Bethesda, Maryland.
- NIAID/DMID/OBRA Ricin Toxin Expert Panel Workshop was held April 1-2, 2005 in Bethesda, Maryland.
- The Bacteriology and Mycology Study Group held its fifth annual meeting on April 7-8, 2005 in Bethesda, Maryland.

- Human African Trypanosomiasis Workshop was held May 16, 2005 in Rockville, Maryland.
- Hydatidosis—New Approaches to Vaccines and Prevention was held May 28-29, 2005, in Lima, Peru.
- Electrophysiological Approaches to *Plasmodium falciparum*-induced Erythrocyte Permeability Changes was held June 2006 in Rockville, Maryland.
- Treatment of Neurocysticercosis was held June 22-23, 2005, in Lima, Peru.
- Sixth International Conference on Cryptococcus and Cryptococcosis was held June 24-28, 2005, in Boston, Massachusetts.
- U.S.-Japan Cooperative Medical Sciences Program's TB and Leprosy Panels and Immunology Board held July 28-30, 2005, in Seattle, Washington.
- NIAID Leprosy Workshop II: Translation of Research Advances into Applications was held July 30, 2005, in Seattle, Washington.
- Changing Host Ranges of Viruses Leading to Emergence of New Pathogens was held September 6-8, 2005, in Washington, D.C.
- Lipid-based Elongated Microstructures: Delivery of Agents Mediated by Cochleates was held September 14, 2005, at Bethesda, Maryland, as a forum to present enabling technology to NIH staff and discuss its potential application to preclinical/clinical drug development.
- Bacillus-ACT 2005, *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis* International Conference was held September 25-29, 2005, in Santa Fe, New Mexico.
- Molecular Biology of Spirochetes was held December 5-8, 2005, in Prague, Czech Republic.
- Interagency Task Force to Assess the Vulnerability of Specific Foods holds meetings to evaluate the contamination with botulinum neurotoxins (March 2003 to present).
- National Inter-Agency Genomics Sciences Coordinating Committee (NIGSCC) is a coordinated Federal effort in biodefense genomics.
- Microbe Project, a Federal Interagency Working Group coordinates genomic and postgenomic initiatives, including those related to biodefense, with other Federal agencies such as the Central Intelligence Agency, the Federal Bureau of Investigation, the FDA, the CDC, the DoD, the Department of Energy, the National Science Foundation, and the U.S. Department of Agriculture.
- Quarterly NIAID/DVC/JVAP Botulinum Vaccine Meetings are used to coordinate Botulinum Vaccine efforts between the Joint Vaccine Acquisition Program of DoD and NIAID.
- The NIAID was invited by the Attorney General of the State of Connecticut to present testimony on the Lyme disease research program as well as current efforts to improve procedures for the diagnosis of Lyme disease.
- Equine Immunization Sub-Group to Evaluate Immunization Protocols for the CDC Equine Anti-Toxin Program has ongoing meetings.

## Primary Immunodeficiency Diseases

### Scientific Advances in Rare Diseases Research

*Autoimmune lymphoproliferative syndrome*

Autoimmune Lymphoproliferative syndrome (ALPS) is a rare disorder that affects both children and adults. It is characterized by an overabundance of immune system cells, and causes a wide spectrum of symptoms including enlarged lymph nodes and anemia. The populations of different types of immune system cells are tightly regulated. A process called apoptosis kills and removes cells at the end of their normal lifespan, and can be triggered early to remove cells that are damaged, virally infected, or potentially cancerous. Failure of apoptosis can lead to ALPS and, in some cases, the development of lymphoid cancers.

NIAID researchers have identified a new disease associated with faulty apoptosis, called "caspase-8 deficiency state" (CEDS), which is caused by a genetic deficiency that prevents cells from making caspase-8, a protein involved in regulating many aspects of the normal cell cycle, including apoptosis. The NIAID scientists demonstrated that caspase-8 deficiency in humans and in mice specifically abolishes normal immune cell activation—to expand the population of immune cells needed to fight a particular infection—as well as apoptosis—to remove the expanded cell population when the infection is over. These studies provide new insights into the molecular mechanisms that underlie autoimmune and immunodeficiency disease, and they reveal crucial steps in the pathway of programmed cell death in lymphocytes.

*Common variable immunodeficiency*

Common variable immunodeficiency (CVID) and Immunoglobulin A (IgA) deficiency, two immunodeficiency diseases, were known to have similar symptoms in a subset of cases and researchers suspected there may be a common genetic cause. IgA deficiency affects 1 in 600 people in the western world but is asymptomatic in many cases; CVID is less common but more severe. Both conditions can result in increased susceptibility to pneumonia and to recurring infections of the ear, sinus, and gastrointestinal tract. Individuals with CVID also have an increased risk of developing B cell tumors. NIAID-funded researchers showed that one mutation in the protein TACI, a protein that controls B cells to switch from the early immune antibody type, immunoglobulin M (IgM), to later immune response antibodies such as IgA and IgG, is one cause of both IgA deficiency and CVID. This research identifies a genetic cause for these primary immunodeficiency diseases and will allow genetic testing and early treatment.

*Chronic granulomatous disease*

Chronic Granulomatous Disease (CGD) is a life-threatening, inherited disorder in which immune cells called phagocytes are unable to kill bacteria and fungi. The specific defect is in an enzyme called phagocyte NADPH oxidase. NIAID scientists recently completed a long-term study of the safety and effectiveness of interferon gamma for the prevention of infections in CGD of childhood. Study patients, all of whom carried the genetic defect, were followed for up to nine

years. Serious infections were 0.3 per patient year. Mild adverse events were common but tolerable in most patients. There were no life-threatening interferon-gamma adverse events and no discernable effect on growth and development. The overall mortality was 6.6 percent over nine years. Thus, interferon-gamma prophylaxis for CGD appears effective and well-tolerated over a prolonged period.

In addition to increased susceptibility to infections, CGD patients frequently have other autoimmune disorders such as juvenile rheumatoid arthritis, rheumatoid arthritis, and Crohn's disease. NIAID researchers recently described another autoimmune disorder, sarcoidosis, in a few CGD patients. Studies of these patients who have both CGD and a defined autoimmune disease may provide new insights into the pathogenesis of autoimmune disorders and may lead to new treatments for these disorders.

*Ongoing Clinical Trials*

- Severe congenital T-cell immunodeficiency therapeutic: A pilot trial is ongoing testing the use of fludarabine and antithymocyte globulin as preparative therapy for hematopoietic stem cell transplant for the treatment of severe congenital T-cell immunodeficiency.
- Severe congenital T-cell immunodeficiency therapeutic: A phase I/II trial is ongoing testing the use of de-escalation of busulfan with fludarabine and antithymocyte globulin as preparative therapy for hematopoietic stem cell transplant for the treatment of severe congenital T-cell immunodeficiency.

**Ongoing Activities**

- Primary Immunodeficiency Diseases Consortium is co-sponsored with the National Institute of Child Health and Human Development (NICHD). The Consortium: (l) provides leadership and mentoring; facilitates collaborations; enhances coordination of research efforts; solicits, reviews, recommends, and makes awards for pilot or small research projects; (2) maintains and expands a primary immunodeficiency diseases registry, which provides data to the research community about the clinical characteristics and prevalence of these diseases; and (3) develops a repository of specimens from subjects with primary immunodeficiency diseases.

Scientific Conferences, Symposia, and Meetings

- Primary Immune Deficiency Consortium Conference was held May 12, 2005 in Boston, MA.
- Symposium on Primary Immunodeficiency Diseases was held June 16-18, 2005 in Budapest, Hungary.

## Autoimmune Diseases

### Scientific Advances in Rare Diseases Research

*Eosinophilic esophagitis and gastroenteritis*

Eosinophilic gastroenteritis (EG) is an inflammatory disease characterized by movement of immune cells called eosinophils into the gastrointestinal tract, where they damage tissue. Other than systemic corticosteroids, few therapeutic options exist. In an NIAID clinical study, patients treated with a monoclonal anti-IL-5 antibody (called SCH55700) showed transient improvement, but by 12 weeks the number of eosinophils had rebounded and symptoms were worse than before. Using purified eosinophils from patients and controls, the NIAID researchers showed the rebound after SCH55700 treatment was due to a blood serum factor that enhances eosinophil survival. Reversing this effect using an antibody against IL-5 suggests that this factor may be IL-5 itself. These findings suggest that blocking more than the single mediator IL-5 may be required to completely suppress the clinical manifestations of this disorder.

*Systemic lupus erythematosus*

Systemic lupus erythematosus (SLE) is a potentially life-threatening autoimmune disease in which the body attacks its own tissues, harming the kidneys, lungs, central nervous system, and heart. Immune responses to infectious organisms can contribute to the development of certain autoimmune diseases in some people. NIAID-funded researchers recently tested the hypothesis that Epstein Barr virus (EBV), a common human virus, triggers SLE. Previously it had been shown that the antigen that initiates the autoimmune response in SLE is an RNA-binding protein called Ro. The researchers hypothesized that an antibody against an EBV protein might cross-react with Ro and thus be an early event in the disease. To test this hypothesis, the scientists immunized rabbits with small proteins matching the cross-reacting portions of Ro or a small fragment of the EBV protein EBNA-1. Strikingly, both groups of rabbits developed lupus-like symptoms. These findings suggest that the antibody response to EBV triggers the onset of SLE through molecular mimicry. Another NIAID-funded study suggests that bacterial infection may contribute to the pathogenesis of SLE. Individuals with a unique mutation in the gene for Toll-like receptor 5 (TLR5) have a reduced risk of developing SLE. TLR5 encodes an immune cell protein that recognizes the bacterial protein flagellin and initiates inflammatory and innate immune responses. People carrying the mutant TLR5 protein are more susceptible to infection, but protected from developing SLE perhaps due to the lower levels of proinflammatory cytokines during infection, which may influence production of autoantibodies. Both of these studies suggest early targets for specific intervention to potentially block the disease process before symptoms appear.

*Ongoing Clinical Trials*

- Systemic lupus erythematosus therapeutic: A Phase II study is testing CTLA-41g plus cyclophosphamide for efficacy in treating SLE.

- Systemic lupus erythematosus therapeutic: A Phase I open-label safety and efficacy study is testing Anti-CD20 Antibody (Rituximab, Rituxan) for anti-B cell therapy in the treatment of SLE.
- Primary Sjögren's syndrome therapeutic: An open-label, one arm, Phase I safety study of anti-CD20 antibody (Rituximab, Rituxan) is being tested as a treatment for Primary Sjögren's syndrome.

Planned Clinical Trials and IND applications

- Stem cell transplantation for autoimmune disease therapy: Two clinical trials are evaluating stem cell transplantation for the treatment of scleroderma and SLE, two autoimmune diseases. These complex trials, expected to open in FY 2006, will also include studies of the underlying immune mechanisms of these diseases and treatments.
- Systemic lupus erythematosus therapeutic: A randomized, open label, Phase II multi-center study is testing non-myeloablative autolgous transplantation with auto-CD34+HPC versus currently available immunosuppressive/immunomodulatory therapy for treatment of SLE.

New Activities

- HLA Region Genetics in Immune-Mediated Diseases has the objective to define the association between human leukocyte antigen (HLA) region genes or genetic markers and immune-mediated diseases, including risk and severity of disease, and organ and cell transplantation outcomes. In FY 2005, NIAID, with co-sponsorship from NINDS, awarded five research cooperative agreements under the new program.

Ongoing Activities

- Autoimmune Diseases Prevention Centers co-sponsored by NIAID, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), NICHD, The Office of Research on Women's Health (ORWH), and the Juvenile Diabetes Research Foundation International (JDRF) conduct research on the development of new targets and approaches to prevent autoimmune diseases and evaluate these approaches in pilot and clinical studies. In FY 2005, the Prevention Centers supported 22 pilot projects to test innovative approaches that may lead to the development of novel targets for disease prevention or assays for biomarkers of disease progression.
- Autoimmunity Centers of Excellence (ACEs) co-sponsored by NIAID, NIDDK, and ORWH is a cooperative program that supports collaborative basic and clinical research on autoimmune diseases, including single-site or multisite pilot clinical trials of immunomodulatory therapies.
- Multiple Autoimmune Diseases Genetics Consortium (MADGC) is a repository of genetic and clinical data and specimens from families in which two or more individuals are affected by two or more distinct autoimmune diseases. This repository provides well-characterized material for use in research aimed at identifying the genes involved in autoimmune diseases.

- Stem Cell Transplantation for Autoimmune Diseases Consortium supports clinical trials to assess the efficacy of hematopoietic stem cell transplantation to treat several severe autoimmune diseases, including multiple sclerosis, SLE, and scleroderma.

Scientific Conferences, Symposia, and Meetings

- Eosinophil-Associated Disease: Approaches to Treatment was held May 25-26, 2005 in Bern Switzerland.
- Eosinophilia-Myalgia syndrome (EMS) was held October 22, 2004 in Bethesda MD.
- NIH Autoimmune Diseases Coordinating Committee (ADCC) has ongoing meetings to increase collaboration and facilitate coordination of research among NIH Institutes and Centers, other Federal agencies, and private groups interested in these diseases.

**Scientific Advances in Rare Diseases Research**

**Other Immune System-Mediated Diseases**

*Mastocytosis*

Mastocytosis is a group of disorders caused by the presence of too many mast cells, a type of immune system cell. Chemicals released by mast cells cause symptoms similar to allergic responses such as hives, itching, abdominal cramping, and even shock. Mastocytosis is often associated with organ involvement and hematological disorders. Previously it was shown that patients with mastocytosis had elevated levels of an immune system signaling molecule called interleukin 6 (IL-6). To determine the significance of these levels, NIAID researchers tested 29 patients with mastocytosis to see if the plasma levels of IL-6 and soluble IL-6 receptor (sIL-6R) correlated with disease severity. The severity of bone marrow pathology, enlargement of the visceral organs, and extent of skin involvement all correlated with plasma IL-6 levels, suggesting that IL-6 levels may be a useful surrogate marker of disease severity and that elevated IL-6 levels may contribute to disease pathogenesis.

**Ongoing Activities**

- Clinical Trials in Organ Transplantation co-sponsored by NIAID, NIDDK, and National Heart, Lung, and Blood Institute (NHLBI), is a clinical consortium established to improve the success of transplants for end-stage organ disease, e.g., end-stage renal disease (ESRD). ESRD is a frequent complication of many autoimmune diseases. The goals of the consortium are to identify genetic factors in patients that could help doctors predict transplant outcomes as well as responses to post-transplant therapy; develop diagnostic tests that enable early detection and ongoing monitoring of immune-related processes; and test the safety and effectiveness of new, less toxic immunosuppressive drugs.
- Clinical Islet Transplantation Consortium co-sponsored by NIAID and NIDDK performs studies of islet transplantation in patients with type 1 diabetes to improve treatment of this disease. This consortium will develop and implement single- and/or multi-center clinical

studies, accompanied by mechanistic studies, in islet transplantation with or without accompanying kidney transplantation, for the treatment of type 1 diabetes.

- Genomics of Transplantation Cooperative Research Program supports interdisciplinary, large-scale, broad-scope genomic studies in clinical transplantation, including solid organ, tissue, and cell transplantation. The goal of the program is to understand the genetic basis of immune-mediated graft rejection and differences in transplant outcomes and to provide a rational basis for the development of more effective treatment and prevention strategies to improve long-term graft survival and provide better quality of life for transplant recipients.
- Hyperaccelerated Awards for Mechanisms in Immunomodulation Trial co-sponsored by NIAID, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIDDK, National Institute of Neurological Disorders and Stroke (NINDS), supports immune-based mechanistic studies associated with clinical trials of infectious disease vaccines and immunotherapies for immune-mediated diseases.
- Immune Tolerance Network (ITN) co-sponsored by NIAID, NIDDK, and JDRF is an international consortium of scientists and clinicians dedicated to the clinical evaluation of promising tolerance induction therapies in four areas: autoimmune diseases, kidney transplantation, islet transplantation, and asthma and allergic diseases.
- National Center for Biotechnology Information co-sponsored by NIAID and the National Library of Medicine is a centralized public database of results from clinical blood and marrow stem cell transplants involving unrelated donors.
- Pathogenesis of Polyomavirus Associated Nephropathy supports projects that are focused on basic, preclinical, clinical, and epidemiological research projects on polyomavirus-associated nephropathy (PVAN).
- Sex-based Differences in the Immune Response research initiative co-sponsored by NIAID, NIAMS, NINDS, ORWH, and the National Multiple Sclerosis Society supports research to increase our understanding of the mechanisms underlying the differences in the immune response in males and females findings may allow more targeted approaches for the prevention and treatment of immune-mediated disease.
- Systems Approaches to Innate Immunity, Inflammation, and Sepsis supports a multidisciplinary team at Scripps Research Institute, which is employing a systems biology approach to create a comprehensive picture of innate immunity, an essential first line of defense against bacterial, viral, and fungal diseases.

## Scientific Conferences, Symposia, and Meetings

- Second Symposium on the Definition and Management of Anaphylaxis jointly sponsored by NIAID and the Food Allergy and Anaphylaxis Network (FAAN) was held July 2005 in Bethesda, MD.
- Adverse Reactions to Vaccination in Atopic Dermatitis course organized by the Atopic Dermatitis and Vaccinia Immunization Network was held March 2005 in San Antonio, TX.
- Lipid-based Elongated Microstructures: Delivery of Agents Mediated by Cochleates was held on September 14, 2005 in Bethesda, MD. The purpose of this forum was to present this enabling technology to NIH staff and discuss its potential application to preclinical/clinical drug development.

## NATIONAL INSTITUTE OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES (NIAMS)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is to support research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases; the training of basic and clinical scientists to carry out this research; and the dissemination of information on research progress in these diseases. NIAMS-supported researchers have made significant progress in broadening the base of knowledge related to many of the rare diseases within the Institute's scope.

### Recent Scientific Advances in Rare Diseases Research

*Muscular dystrophy*

One form of muscular dystrophy, Duchenne muscular dystrophy (DMD), is a severe form of the disease, and few people with DMD live past their early 20s. Researchers have recently shown that injecting a fragment of a protein called heregulin improves the structure and function of muscles of mice that develop a disease similar to DMD. After these mice were injected with the heregulin fragment for three months, the mechanical properties of their muscles were improved, and they showed fewer sites of muscle degeneration and less muscle inflammation than those injected with an inert saline solution alone. Previous research suggested that heregulin works by increasing the body's production of another muscle protein, utrophin, which is structurally and functionally similar to dystrophin. In laboratory animals, utrophin is produced in high levels before birth, but decreases to low levels by adulthood. By increasing utrophin production, scientists found they could halt muscle degeneration related to dystrophin deficiency in mice. It was suspected that utrophin would serve as a substitute for deficient dystrophin, and the latest research suggests that this is the case. Successful treatments for muscular dystrophy are being actively pursued through a number of research avenues, and additions to the candidate therapeutic approaches, such as this one, increase the chances of discovering ways to incrementally improve the lives of those affected by this devastating disease.

*Osteogenesis imperfecta*

Osteogenesis imperfecta (OI), or "brittle bone disease", leads to weak bones that fracture easily. OI is a genetic disease, most often caused by defects in the gene for a protein called type 1 collagen, which forms fibers that are crucial structural components of bone. Many severe cases of OI are caused by defects that do not simply reduce the amount of collagen available, but instead result in production of a defective collagen protein. Therapy for such forms of OI depends upon finding ways to reduce or eliminate the production of the defective protein without affecting the production of normal collagen in the same cell.

Some ribonucleic acids (RNAs), called ribozymes, act as molecular scissors, cutting the chemical links that hold other RNAs together. Researchers have now designed a ribozyme to cut

and inactivate the messenger from a defective collagen gene. They tested this ribozyme by introducing it into cultured bone cells that had been genetically modified to produce a defective collagen in addition to normal collagen, as happens in cases of OI. The ribozyme significantly reduced the production of the defective collagen. Importantly, the properties of the collagen fibers produced by the cells, which were abnormal when the ribozyme was not present, more closely reflected normal fibers when the ribozyme was present. This demonstrates that the ribozyme had the desired specificity, blocking production of the defective collagen, but allowing continued production of normal collagen.

*Paget's disease of bone*

Normal bone remodeling is essential for a healthy skeleton; however, in Paget's disease, the rates of bone resorption and bone formation at affected sites are so rapid that the new bone is both over-abundant and of poor quality. A key feature of the disease is excessive numbers and activity of the cells, called osteoclasts, that resorb bone. The large number of active osteoclasts, in turn, stimulates excessive bone formation by other cells. Osteoclasts arise from precursor cells that are present in bone marrow. Previous work has shown that osteoclast precursors from people with Paget's disease are unusually sensitive to Vitamin D3. The levels of D3 that are normally present in the body do not stimulate formation of osteoclasts from normal precursor cells. However, when precursor cells from people with Paget's disease are exposed to the same levels of D3, they readily form osteoclasts. One component of the molecular machinery responsible for this hyper-responsiveness to D3 is a protein called the vitamin D receptor (VDR). A number of drugs, called VDR antagonists, have been developed that block the action of the VDR in cells. Investigators have now tested one of these VDR antagonists in cultures of marrow cells from people with Paget's disease. They found that the VDR antagonist effectively prevented the stimulation of osteoclast formation by D3. This suggests that VDR antagonists could be useful in the treatment of Paget's disease, perhaps in combination with bisphosphonates, which block bone resorption by osteoclasts.

*Pseudoxanthoma elasticum*

Pseudoxanthoma elasticum (PXE) is a connective tissue disease that affects the skin, the eyes and the cardiovascular system. Several years ago, a consortium of investigators worked together to uncover the gene underlying this disease. It was found to be caused by a mutation in a gene termed ABCC6 which is a gene that encodes for a specific multidrug resistance-associated protein. However, despite the clear evidence that this is the gene underlying the disease, it was not clear how mutations in this gene would result in the manifestations of PXE. This is a gene that is primarily expressed in the liver and kidneys rather than in the tissues affected by PXE. In the disease, excess mineralization is deposited within these tissues. In recent work, investigators developed an animal model in which the ABCC6 gene was removed. They demonstrated that these mice did not express the protein normally encoded for by the gene in the liver or kidneys and there was profound mineralization of several tissues in these animals including the skin, arterial blood vessels, and the retina which parallels the findings in the human disease.

Only animals carrying two copies of the mutated gene showed these effects. Those animals having one normal and one abnormal gene were completely normal. This also parallels the human PXE situation. This animal model will be very useful in further investigating the pathophysiologic mechanisms in PXE, examining potential therapeutic interventions, and providing a general model for mineralization studies seen in other diseases with vascular and ocular degeneration.

**New/Planned Extramural and Intramural Research Initiatives**

*Muscular dystrophy*

NAIMS-supported researchers have recently established a new Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center that will explore new strategies for treating a variety of muscular dystrophies. Two laboratory projects at the Center are focused on ways to increase muscle growth, and another project on compounds that may be able to inhibit enzymes involved in breaking down muscle tissue. Clinical trials will determine the safety and feasibility of a potential drug treatment for Duchenne muscular dystrophy. The core facility, a muscle physiology lab, will analyze muscular dystrophy mouse models. This Center joins five others already funded by the NIH. The centers work individually and collaboratively, and are guided by a steering committee that includes representatives from each center. Each has both basic and clinical research projects, and one or more core facilities to support them. Centers must also make core resources or services available to the national muscular dystrophy research community.

NIAMS, along with the National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Child Health and Human Development (NICHD), and the NIH Office of Dietary Supplements (ODS), co-sponsored two program announcements in FY 2005 to encourage training of scientists in muscle disease research. The first, "Ruth L. Kirschstein National Research Service Awards for Postdoctoral Fellowships in Muscle Disease Research," encouraged postdoctoral fellows with diverse scientific interests to apply their expertise to enhance our understanding of the pathogenesis and treatment of muscle diseases and disorders, including the muscular dystrophies. Applicants were encouraged to develop innovative and novel approaches for studying and treating these diseases. The second program announcement, "Mentored Clinical Investigator Career Development Awards in Muscle Disease Research," was issued in recognition of the urgent need for highly skilled, interactive researchers who are able to integrate various disciplines and levels of expertise to successfully address the increasing challenges in the current research environment of muscular dystrophy and other muscle diseases. It is expected that these career development programs will increase the number of investigators in basic, translational, and clinical research on muscular dystrophy and other muscle diseases, and will also increase the quality of their research and training.

NIAMS, in collaboration with NINDS, NICHD, and the NHLBI, co-sponsored a third program announcement in FY 2005 entitled, “Muscular Dystrophy: Pathogenesis and Therapies,” to encourage investigator-initiated research grant applications for projects studying the pathogenesis of and therapies for the muscular dystrophies. Responses to this announcement

could include basic, translational, or patient-oriented studies of the muscular dystrophies. This program announcement was the third release and it updated two previous highly successful solicitations entitled "Therapeutic and Pathogenic Approaches for the Muscular Dystrophies," which was released in January 2001, and "Pathogenesis and Therapy of the Muscular Dystrophies," released in March 1998.

*Pediatric rheumatology training*

In a continuing effort to strengthen the nationwide supply of pediatric rheumatologists, NIAMS recently awarded a training grant for the training of pediatric rheumatology fellows in both basic research and clinical care. Nationwide, there are just over 200 board-certified pediatric rheumatologists. Consequently, many children have no access to the most recent treatments for juvenile rheumatoid arthritis and other rheumatic diseases. These diseases can be especially devastating in children, and can impact their well-being for life. Early diagnosis and treatment by a pediatric rheumatologist can make a significant difference in delaying or preventing lifelong disability.

*Scleroderma and lupus training*

A training grant in inflammatory and fibrosing diseases was recently awarded in order to encourage fellows to pursue academic careers in either basic science or health services research. Training new physician scientists and translational researchers is a critical need in rheumatology. Researchers in this program are focusing on defining the environmental, genetic, and societal factors that affect African American patients with scleroderma and lupus.

**Significant Ongoing Rare Diseases Research Initiatives**

*Juvenile rheumatoid arthritis*

NIAMS supports a state-of-the-art genomics project to uncover gene expression patterns that contribute to the development of pediatric arthritis. By using DNA microarrays, small silicon chips that contain tiny amounts of thousands of known genes, to carry out a technique called gene expression profiling, NIAMS-supported researchers are analyzing thousands of genes in the blood, fluids, and tissues of children newly diagnosed with various types of pediatric rheumatic diseases. Identifying gene expression patterns, groups of genes that are "turned on", for different types of childhood arthritis will help to improve diagnosis and to predict disease severity for affected children.

Two separate clinical trials are examining therapeutic interventions in children with juvenile rheumatoid arthritis (JRA). One trial is investigating osteopenia (reduced bone mass), a frequent complication of JRA. This clinical trial measures the effectiveness of daily oral calcium supplementation to increase total body bone mineral density. The long-term goal is to determine the safety and effectiveness of current and new biologic and pharmacologic treatments as alternative treatments to calcium in those JRA patients with osteopenia.

Scientists have hypothesized that juvenile rheumatoid arthritis is a complex genetic disease. In a continued effort to inform this hypothesis, NIAMS has renewed its commitment to the Research Registry for Juvenile Rheumatoid Arthritis which collects data on multicase families with affected sibling pairs. In addition to this information, researchers have also developed a related genomics program to identify susceptibility genes for this disease. To date, data from more than 524 families have been collected.

*Juvenile systemic lupus erythematosus*

In the area of childhood lupus, NIAMS-supported researchers are currently conducting a large, controlled study to assess the ability of statins (cholesterol-lowering agents) in preventing or delaying progression of cardiovascular disease in children with lupus. This research study involves 20 centers from the Pediatric Rheumatology Research Network in establishing the largest cohort of pediatric lupus patients ever prospectively studied. Approximately 15percent of patients have been enrolled and baseline data analysis is currently underway.

*Marfan syndrome*

Marfan syndrome is an inherited disorder caused by a mutation in the fibrillin gene. This mutation causes the tendons, ligaments, and other connective tissues in the body to weaken. Marfan syndrome can affect the heart, skeletal system, eyes, and other organs in the body and symptoms range from mild to severe. NIAMS-supported researchers have developed a multi-site translational research program in Marfan syndrome. The long-term goal of this program is to translate basic research in matrix biology into treatment strategies for individuals with Marfan syndrome and related disorders of connective tissue. The program is utilizing a comprehensive and multidisciplinary approach that integrates the scientific interest and expertise of four leading laboratories in this and related research fields. Researchers are studying genetically engineered mouse models of Marfan syndrome to uncover the abnormal cellular activities that contribute to this disorder and will translate this new knowledge into more effective therapies.

*Muscular dystrophy*

In addition to the new Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center mentioned previously, NIAMS continues to provide support for one of the original centers which is examining gene and stem cell therapies to treat muscle disease, in particular Duchenne muscular dystrophy. Researchers are attempting to deliver and engraft muscle stem cells into diseased heart tissue without causing an immune response. Other researchers are using herpes virus vectors in functional genomics studies to discover and characterize factors that guide stem cell maturation into muscle. In a third project, researchers are using adeno-associated viral vectors in preclinical studies in a dog muscular dystrophy model to seek the most effective ways to deliver gene therapy. Clinical disease outcomes will be carefully defined in patients with Duchenne and limb-girdle muscular dystrophies for the preparation of phase I gene therapy safety trials.

NIAMS continues to support the National Registry for Myotonic Dystrophy and Facioscapulohumeral Dystrophy Patients and Family Members. This registry aims to seek out and classify patients with clinically diagnosed forms of myotonic dystrophy and facioscapulohumeral dystrophy. The registry stores their medical and family history data and serves as a central information source where researchers can obtain data for analysis associated with these diseases. NIAMS recently renewed its commitment to this registry for an additional 5 years.

*Neonatal lupus*

NIAMS continues to support the Research Registry for Neonatal Lupus. Women with lupus and other related disorders produce certain antibodies in the blood. Some women have these antibodies even if they have not yet developed symptoms of lupus. When these women become pregnant, they may pass the antibodies to their infants. The infants may then develop a disease called neonatal lupus. This registry collects information on women and infants affected by neonatal lupus as well as other family members who may be healthy. The Research Registry is a central repository of patient information, sera, and DNA. The Registry provides blood samples (kept anonymous) to scientists studying neonatal lupus. Information from the registry forms the basis of family counseling and tracks important data such as recurrence rates in subsequent pregnancies and the effects of treatments. The Research Registry also serves as an educational resource for women who are eager to learn about this disease. NIAMS recently renewed its commitment to this registry for an additional 5 years. Currently, 412 children and 350 mothers have been registered.

*Neonatal onset multisystem inflammatory disease*

Neonatal onset multisystem inflammatory disease (NOMID) is a rare, chronic, inflammatory disease that leads to major disability in affected children. Intramural researchers at NIAMS have identified the genetic cause for this disease. Research suggests that a pro-inflammatory cytokine (a protein involved in the body's immune response), interleukin-1 (IL-1), could be contributing to the disease manifestations of NOMID. NIAMS has initiated a multicenter study to evaluate the safety and efficacy of anakinra, a drug that blocks the activities of interleukin-1. Results in children with NOMID indicate that IL-1 blockade is highly efficacious in improving clinical signs and symptoms of this disease including fevers, rash, joint pain, headaches, and conjunctivitis (inflammation of the eye), and in lowering inflammatory markers of disease activity. In addition, anakinra crosses the blood-brain barrier and has led to improvement in inflammation in the brain in a number of children. Intramural researchers at NIAMS have seen a significant expansion in their pediatric research population within the past year. Information obtained from these patients has provided additional insight into NOMID, as well as other areas of pediatric research.

*Scleroderma*

Scleroderma, often referred to as a single disease, is actually a symptom of a group of diseases that involves the abnormal growth of connective tissue, which supports the skin and internal

organs. In some forms of scleroderma, hard, tight skin is the extent of the disease. In other forms, however, the problem goes much deeper, affecting blood vessels and internal organs, such as heart, lungs, and kidneys. A NIAMS-funded project is using a unique sample set, lung tissue from scleroderma patients undergoing lung transplant surgery, as well as lung tissue from unused donor lungs, to facilitate investigation into the cellular changes that cause the hardening of the lungs. Other NIAMS-supported researchers are examining the cellular and molecular processes of scleroderma, cell transfer between mother and child, and the development of innovative therapies.

*Vasculitis*

NIAMS continues to provide support for the Vasculitis Clinical Research Consortium (VCRC), one of the consortia of the Rare Diseases Clinical Research Network. The clinical and research expertise of four vasculitis centers and the resources of the NIH-supported General Clinical Research Centers at each site comprise the core of the consortium. The VCRC has established a large longitudinal cohort of patients with at least one of the following diseases: Wegener's Granulomatosis, Microscopic Polyangiitis, Churg Strauss Vasculitis, Polyarteritis Nodosa, Takayasu's Arteritis, and Giant Cell Arteritis. The VCRC has an electronic Web site resource which has significant information for clinicians, researchers, and patients on the six VCRC diseases.

**Rare Disease-Specific Conferences, Symposia and Meetings**

*Muscular dystrophy*

NIAMS and the NIH Office of Rare Diseases sponsored a workshop on the burden of muscle diseases in January 2005. Speakers and attendees included muscle disease clinicians and researchers, health economists, epidemiologists, representatives of patient advocacy groups, and patients and their families. There were 100 registered attendees representing universities and institutions across the U.S., Canada and the United Kingdom. Also represented were several national and international voluntary health organizations and five agencies of the U.S. government. The participants in this workshop identified existing data on the economic and psychosocial burdens of muscle diseases on patients, families, and societies, with a focus on the muscular dystrophies, and recommended strategies for developing new information sources.

*Systemic autoinflammatory diseases*

In November 2005, NIAMS sponsored the Fourth International Congress on the Systemic Autoinflammatory Diseases. The goal of the Fourth International Congress was to foster a broad, synergistic approach to these illnesses through a variety of sessions including those on clinical features, natural history, and diagnostic criteria; novel advances in the treatment of these diseases; and animal models of autoinflammatory disease. A few of the rare diseases discussed at this meeting included Familial Mediterranean Fever, neonatal onset multisystem inflammatory disease, and Behcet's disease. Co-sponsors included the Foundation for the NIH, National Human Genome Research Institute, National Institute of Allergy and Infectious Diseases,

National Institute on Deafness and other Communication Disorders, National Institute of Diabetes and Digestive and Kidney Diseases, and the NIH Office of Rare Diseases.

**Activities with Voluntary Rare Diseases Organizations to Stimulate Research**

*Health partnership program*

The NIAMS Health Partnership Program (HPP) is a community-based medical research program operating as a collaborative effort between NIAMS and Washington, D.C. area community leaders and representatives. Through biomedical and behavioral research with underrepresented patients affected by arthritis and other rheumatic diseases, the HPP aims (1) to enhance our understanding of health disparities and their causes, and (2) to provide direction for improving the health status and health outcomes of the minority communities affected. The HPP community partners include individuals and organizations representing the local African American and Hispanic/Latino communities, including local government agencies, schools and universities, faith-based organizations, civic and community groups, voluntary and professional organizations, and private businesses. Community partners provide planning and promotion support for the HPP's research, training, and education activities. Through this partnership, the HPP has established the NIAMS Community Health Center which is located in a medically underserved minority community in Washington, D.C. To date, approximately 1300 individuals have received patient services at the CHC.

*Tuberous sclerosis complex*

NIAMS actively participates in the Trans-NIH Tuberous Sclerosis Coordinating Committee which includes representatives from other NIH components including the NIH Office of Rare Diseases. Other participating organizations include the Tuberous Sclerosis Alliance. Members of the Coordinating Committee have reviewed and discussed the research portfolios of the participating organizations, including relevant ongoing clinical trials, in order to identify potential research partnerships and opportunities for targeted initiatives on tuberous sclerosis complex. Recently, NIAMS and several other NIH components partnered with the Tuberous Sclerosis Alliance to release a program announcement focused specifically on tuberous sclerosis complex. The solicitation encouraged applications designed to broaden the base of knowledge related to the disease including the identification of new therapeutics.

**Education Activities on Rare Diseases for the Researchers, Public, and the Health Care Providers Communities**

*Information dissemination*

NIAMS is committed to a comprehensive program of information dissemination to patients and to their health care providers. Research advances are of limited value if they never reach the arena of health care, and they miss the goal of improving public health for all Americans. To this end, the NIAMS has published several new Web-based documents on rare diseases, including Behcet’s disease, Lichen Sclerosus, and Epidermolysis Bullosa. These documents are

part of the Institute's Fast Facts series which includes easy-to-read publications covering basic information on various diseases.

*Pediatric rheumatology CD-ROM*

NIAMS, in collaboration with the Arthritis Foundation, has produced a pediatric rheumatology CD-ROM to provide pediatric health care professionals with access to the latest information on pediatric rheumatic diseases and to encourage early diagnosis and treatment. The CD-ROM was launched at the November 2005 meeting of the American College of Rheumatology. Disease topics include scleroderma, juvenile rheumatoid arthritis, and Osteogenesis imperfecta.

*Osteogenesis imperfecta publications*

Through the NIH National Osteoporosis and Related Bone Diseases~National Resource Center (NIH~NRC), the Institute has produced the new fact sheet *Exercise and Activity: Key Elements in the Management of OI*, and updated additional fact sheets including the following:

- *OI Issues: Maintaining Health During the Adult Years*
- *Talking with Your Orthopaedist: A Guide for Persons With OI*
- *Talking with Your Primary Care Doctor: A Guide for Persons With OI*
- *What People With OI Need to Know About Osteoporosis*

Through the NIH-NRC, the Institute has also updated two publications for health professionals working with patients who have Osteogenesis imperfecta: *Therapeutic Strategies for Osteogenesis imperfecta: A Guide for Physical Therapists and Occupational Therapists,* and *Osteogenesis imperfecta: A Guide for Nurses*. These publications are available in print and included on the Institute's pediatric rheumatology CD-ROM.

## NATIONAL INSTITUTE OF BIOMEDICAL IMAGING AND BIOENGINEERING (NIBIB)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) is to improve human health by leading the development and accelerating the application of biomedical technologies. The Institute is committed to integrating the physical and engineering sciences with the life sciences to advance basic research and medical care. The Institute works to achieve this mission by supporting research that has broad applicability across disease or organ lines.

The NIBIB funds research on rare diseases through its extramural programs via grant solicitations, although the Institute's primary support of research is through unsolicited, investigator-initiated grant awards. Rare disease research funded in FY 2005 includes adrenal carcinoma, aortic valve disease, Barrett's syndrome, basal cell carcinoma, bladder cancer, bone dysplasia, brain tumor, congenital adrenal, congenital heart disorders hyperplasia, Cushing's syndrome, cystic fibrosis, glaucoma, glioblastoma, Huntington's disease, macular degeneration, Marfan syndrome, Niemann-Pick disease, renal disease, respiratory distress syndrome, and sudden infant death syndrome.

Highlighted below are some of NIBIB's activities related to treating and preventing rare diseases and conditions that fall within the purview of the Institute's mission.

### Recent Scientific Advances in Rare Diseases Research

*Niemann-Pick disease type C (NP-C)*

Niemann-Pick disease type C (NP-C) is a genetic pediatric neurodegenerative disorder, which causes progressive deterioration of the nervous system. This metabolic disorder leads to a series of neurological problems that are ultimately fatal. This disorder affects an estimated 500 children in the United States. The disease is autosomal recessive and is inherited when a child receives two mutant genes, one from each parent. In NP-C, cholesterol derived from low-density lipoprotein accumulates in cells of the brain, liver, spleen, lungs, and bone marrow. This leads to an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia. NIBIB researchers have recently used Magnetic Resonance Imaging (MRI) techniques to generate quantifiable images and data to discriminate NP-C mice from normal mice. Researchers now plan to investigate the two organ systems that are most affected by the disease, the brain and liver. The goal of the research is to develop MRI methods that monitor the progression of NP-C and drug therapies in mice which can then be applied for use in human patients.

*Bladder cancer*

Bladder cancer is the sixth most common cancer in the United States. Approximately 53,200 people are diagnosed with bladder cancer each year. Gene therapy, a technique for correcting defective genes responsible for disease development, has demonstrated some success in inhibiting bladder cancer tumor growth. The current method, intravesicular BCG therapy, augments local production of immune mediators of tumor clearance. However, BCG therapy has no effect on 20 percent of patients with bladder cancer. NIBIB researchers have found a less virulent gene vector, Mycobacterium *smegmatis*, that can deliver eukaryotic expression plasmids to mammalian cells, and for the first time, can deliver plasmids expressing the green fluorescent protein from a eukaryotic promoter to macrophages. This is a protein naturally occurring in some animals including jelly fish that spontaneously fluoresces. It can be used as a noninvasive marker in living cells by attaching to different proteins and then letting it fluoresce so as to track the cell (from www.bioethics.gov/reports/stemcell/glossary.html). Researchers now plan to use this discovery to engineer a new anti-tumor therapy for bladder cancer that is safer and more efficient than the current method.

One of the greatest challenges to successful treatment of bladder cancer is early detection and staging. Optical coherence tomography (OCT), a noninvasive imaging device, is used for imaging of *in vivo* bladder tissue structure and blood flow simultaneously. Research has shown that OCT can distinguish between normal and cancerous bladder cells. NIBIB researchers are now developing an improved OCT imaging device that integrates arrays of optoelectronic sources, detectors, and micro electronic processing and control systems. This will allow for more rapid, high quality imaging that can lead to improved diagnosis of bladder cancer.

*Glaucoma*

Glaucoma is the leading cause of blindness in the United States. It is a disease of the optic nerve, the part of the eye that carries images to the brain. When damage to the optic nerve fibers occurs, blind spots develop. Blindness can occur if the blind spots go undetected and significant damage to optic nerve occurs. However, medical advances have made it easier to diagnose and treat glaucoma. NIBIB researchers have developed new high-frequency ultrasound systems that provide new opportunities to advance tissue evaluation in the anterior segment of the eye. This technique allows research to develop a two-dimensional map of blood flow in the anterior segment of the eye, providing important information in the staging and diagnosis of glaucoma.

## NATIONAL CANCER INSTITUTE (NCI)

### Overview of Rare Diseases Research Activities

### Recent Scientific Advances in Rare Diseases Research

*Acute myeloid leukemia*

Acute myeloid leukemia (AML) is the most common type of leukemia in American adults. This disease typically strikes older adults, a group that may be less able to tolerate the aggressive therapies that are currently used to treat AML, such as combination chemotherapy and stem cell transplantation. Consequently, doctors are searching for new treatment approaches for older patients with AML.

- In an NCI-sponsored study, researchers are testing a new drug called tipifarnib (Zarnestra™) to treat patients aged 70 or older who have AML and who are not eligible for standard treatment. Tipifarnib belongs to a class of drugs called farnesyltransferase inhibitors, which inhibit the biochemical signals that tell cancer cells to grow.

  In a previous clinical trial, about 20 percent of AML patients treated with tipifarnib achieved a complete response, and an additional 15 percent achieved partial response. Those who responded to tipifarnib experienced better survival rates.

- AML is prevalent in the geriatric population in America, and as that population continues to grow, there will be an expected increase in the number of older Americans with AML. Although AML long-term remission rates in younger patients are better, the long-term remission rates for AML in patients over 60 years old are very low, about 10-15 percent.

  In another trial involving the elderly, researchers are trying to determine whether adding the drug oblimersen (Genasense) to chemotherapy will improve survival in patients aged 60 and older who have previously untreated AML Oblimersen blocks production of a protein called Bcl-2, which helps cancer cells survive. Bcl-2 is overexpressed in many types of tumors and contributes to cancer cell resistance to chemotherapy. By blocking production of Bcl-2, oblimersen may make cancer cells more susceptible to the cytotoxic effects of chemotherapy.

AML is one of the most aggressive forms of leukemia in adults. Initial treatment for AML usually involves sequential, combination chemotherapy designed first to induce or bring about a remission (induction chemotherapy) and second to keep the cancer in remission and prevent a relapse (consolidation or post-remission therapy).

In an ongoing trial, researchers are testing whether addition of an immunotoxin, gemtuzumab ozogamicin, to standard chemotherapy will improve the disease-free survival of patients with previously untreated AML. Gemtuzumab ozogamicin is a monoclonal antibody linked to a powerful bacterial toxin. The monoclonal antibody can locate and bind to leukemia cells and

deliver the toxin to them. Patients may receive standard therapy alone or gemtuzumab ozogamicin during induction chemotherapy, after consolidation chemotherapy (post-consolidation therapy), or both.

In phase II studies, treatment including gemtuzumab ozogamicin led to promising remission rates for patients with AML. There have not been any significant improvements in remission rates for AML in many years, so it is important that to confirm those findings with a large phase III trial.

*Ataxia-telangiectasia*

Breast Cancer Risk among Women with Ataxia-Telangiectasia. Epidemiological studies have consistently shown elevated rates of breast cancer among female blood relatives of patients with ataxia-telangiectasia (AT), a rare autosomal recessive disease. A large proportion of the members of AT families are carriers of AT-causing gene mutations in *ATM*, and it has been hypothesized that these otherwise healthy carriers are predisposed to breast cancer. In a follow-up study of blood relatives of patients with verified AT in Nordic families, 225 cases of cancer were identified through population registries, compared to 170 expected cases based on general population rates. Invasive breast cancer occurred among 34 female relatives and was diagnosed among 21 women before the age of 55 years, including seven mothers of AT patients. When the group of mothers was excluded, no clear relationship was observed between the allocated mutation carrier probability of each family member and the extent of breast cancer risk. The findings of breast cancer risk in mothers, but not other likely mutation carriers, in this and other studies raise questions about the hypothesis of a simple causal relationship with *ATM* heterozygosity. *Br J Cancer* 2005;93:260-265.

*B cell cancer*

Novel Antibodies Reveal Potential New Immunotherapy Target in B Cell Cancers. Recent clinical trials demonstrate the promise of recombinant immunotoxins as therapy for certain hematological malignancies, especially hairy cell leukemia (HCL). One potential new target for immunotherapy is a receptor protein called IRTA2, since the gene for this protein is implicated in B cell malignancies and its mRNA is selectively expressed in cells of B cell origin. Scientists from Laboratory of Molecular Biology, Center for Cancer Research have identified for the first time the intrinsic IRTA2 protein on the surface of many human lymphoma cell lines and on HCL cells from patients by the use of newly developed monoclonal antibodies specific for IRTA2. Their results indicate that these monoclonal antibodies may be useful for leukemia diagnosis and raise the possibility of IRTA2-targeted immunotherapy.
Ise, T., Maeda, H., Santora, K., Xiang, L., Kreitman, R.J., Pastan, I., Nagata, S. "IRTA2 Protein on lymphoma cell lines and hairy cell leukemia cells detected by novel monoclonal antibodies." *Clinical Cancer Research*; Vol. 11: 87-96, January 2005.

*Biliary tract cancer*

Aspirin Use and Risk of Biliary Tract Cancer. The associations of gallbladder and bile duct cancers with gallstones, cholecystitis, and cholangitis suggest that chronic inflammation contributes to the carcinogenic process. A population-based case-control study conducted in Shanghai, China, examined the relationship between aspirin use and the risk of biliary disease. Aspirin use was associated with a reduced risk of gallbladder cancer, and an inverse relationship was observed with frequency and duration of use and use starting at a younger age. In addition, there were non-significant reductions in the risk of bile duct and ampullary cancers associated with aspirin use, whereas no clear association was seen with biliary stones. *Cancer Epidemiol Biomarkers Prev* 2005;14:1315-1318.

*Bladder cancer*

Approximately 70 to 80 percent of the estimated 60,000 patients with newly diagnosed bladder cancer will present with superficial bladder tumors. Frequent surveillance of bladder cancer patients is critical because of the high recurrence rate and the potential for the recurrent tumor to become more aggressive. Urine cytology, which checks the number and appearance of cells in urine samples, often fails to detect early tumors. Cystoscopy -- examining the urethra and bladder with a thin lighted scope -- can give patients a false-positive result in addition to being invasive and unpleasant. Several NCI efforts are directed at improving the diagnosis, surveillance and treatment of early stage disease.

- A three-year study to validate a test to detect the recurrence of bladder cancer has been initiated by the National Cancer Institute (NCI), part of the National Institutes of Health (NIH), at 13 centers across the United States and Canada. This test was conceived and is being conducted by NCI's Early Detection Research Network (EDRN). By examining genetic changes in DNA obtained through urine samples, the test, if successfully validated, will provide a sensitive and non-invasive method of screening for bladder cancer recurrence.

- Gemcitabine has demonstrated activity in advanced, metastatic bladder cancer in randomized clinical trials. In 2006, a national phase III trial in patients with early disease -- superficial bladder cancer -- will test whether a single instillation of gemcitabine into the bladder at the time of local resection of the tumor, can prevent or delay time to development of another superficial bladder tumor. A companion study is being designed to evaluate whether a panel of biomarkers is (a) superior to single markers and, (b) if the panel could replace cystoscopy as a noninvasive surveillance tool. Pending availability of funds, this study will be embedded into the treatment study.

- There is no standard of care for superficial disease that has recurred after treatment with the sole approved regimen, intravesical Bacillus Calmette-Guerin (BCG). The Southwest Oncology Group is conducting a phase II study to determine whether repeated instillations of intravesical gemcitabine, an agent which has activity in metastatic disease, can reduce the number of recurrences in this population.

- In collaboration with industry, a number of novel targeted therapies are being tested for promising activity in advanced bladder cancer -- multitargeted tyrosine kinase inhibitors, antibody against HER-2 (herceptin), and an angiogenesis inhibitor. To identify other biologic targets which may be exploited for therapeutic intervention, the assessment of the frequency of EGFR is embedded as a substudy in the herceptin trial.

- A multicenter, randomized phase III trial is ongoing that will test whether the p53 gene status is predictive of which patients with organ-confined bladder cancer will benefit from chemotherapy after standard cystectomy and lymphadenectomy.

- Chemotherapy for advanced bladder cancer has been associated with significant toxicities, especially renal and bone marrow. A trial being planned will pilot a kidney-sparing triplet while SWOG is evaluating a doublet in two age groups to determine whether there are important differences in the elderly.

*NAT2*, *GSTM1* Genes and Bladder Cancer Risk. Polymorphisms in *NAT2, GSTM1, NAT1, GSTT1, GSTM3,* and *GSTP1* were investigated in 1,150 patients with transitional-cell carcinoma of the urinary bladder and 1,149 controls in Spain. Meta-analyses were conducted, which included over twice the numbers of cases observed in previous studies. The odds ratios for bladder cancer for individuals with deletion of one or two copies of the *GSTM1* gene were 1.2 and 1.9 respectively. Compared with *NAT2* rapid or intermediate acetylators, *NAT2* slow acetylators had an increased overall risk of bladder cancer that was stronger for cigarette smokers than for never smokers. No significant associations were found with the other polymorphisms. Meta-analyses showed that the overall association for *NAT2* was robust and case-only meta-analyses provided support for an interaction between *NAT2* and smoking. The overall association for *GSTM1* was also robust and was not modified by smoking status. Although the relative risks are modest, these polymorphisms could account for up to 31percent of bladder cancers because of their high prevalence. *Lancet* 2005;366:649-659.

Poorer Survival of Bladder Cancer Among African Americans Due to Extent of Disease and Histology. African Americans are less likely than whites to develop bladder carcinoma. However, once they are diagnosed, black patients experience poorer survival. The authors investigated which factors were related to survival differences in black patients and white patients with bladder carcinoma stratified by extent of disease. A population-based cohort of black patients with bladder carcinoma and a random sample of frequency-matched white patients with bladder carcinoma, stratified by age and gender, were identified through cancer registry systems in Atlanta, New Orleans, and San Francisco/Oakland. Patients had no previous cancer history and were ages 20-79 years at the time they were diagnosed with bladder carcinoma in 1985-1987. Medical records were reviewed at initial diagnosis, and 77 percent of patients were interviewed. Tumor grade, T classification, and other variables, including age, socioeconomic position, symptom duration, smoking history, and comorbidities, were recorded. Survival of black patients and white patients by extent of disease was modeled using Cox regression analysis. A greater proportion of black patients had histologic types of tumors that were associated with poorer survival. Among those with pure urothelial carcinoma, black patients had

greater extent of disease at the time of diagnosis. Within specific extent-of-disease categories, there was some evidence of poorer survival for black patients with T2 tumors and strong evidence of poorer survival among those with T3 tumors compared with white patients. Black patients with muscle-invasive carcinoma who died within 6 months of diagnosis tended to present with life-threatening symptoms. Black patients and white patients did not differ with respect to diagnostic tests performed or therapy given. Black patients with bladder carcinoma had poorer survival due to greater extent of disease at diagnosis and a higher proportion of more aggressive histologies compared with white patients. Within urothelial carcinomas, by extent of disease (clinical/pathologic stage) these black/white survival differences were limited to patients with muscle invasion (T2 and T3 tumors).
Prout, G.R., Wesley, M.N., McCarron, P.G., Chen, V.W., Greenberg, R.S., Mayberry, R.M., and Edwards, B.K. (2004). Survival experience of black patients and white patients with bladder carcinoma. *Cancer, 100(3), 621-30.*

*Blood cancers*

NCI has taken action the past year to strengthen its support for translational and clinical blood cancer research. The NCI has undertaken a comprehensive review of the NCI clinical trials program through the Clinical Trials Working Group. The report of this Working Group can be found on http://integratedtrials.nci.nih.gov/ict/overview. The Clinical Trials Working Group has made specific recommendations for the NCI to better coordinate activities across funding mechanisms and to leverage resources across Divisions/Offices of the NCI.

In addition to the national clinical trials cooperative groups supported by the U10 mechanism, the NCI support other infrastructures that perform translational and clinical research under the P01 and U01 mechanisms. Many of these entities are being recompeted in FY 2006. These include the AIDS Malignancies Consortium, the Blood and Marrow Transplant Clinical Research Network and the Chronic Lymphocytic Leukemia Clinical Research Consortium. Other potentially new infrastructures are at various stages of development or funding decision process including the Myeloproliferative Disorders Research Consortium and the Myelodysplastic syndrome Consortium. In FY 2005, a new P01 on Myelodysplastic syndrome was funded that involves collaboration between the MD Anderson Cancer Center and the Dana Farber Cancer Center. Furthermore, the R01 and the R21 portfolios also contain translational and clinical trials research in blood cancers that will improve treatment options and rapidly move discoveries from the laboratory bench to the patient's bedside.

Several meetings were held with investigators, patients and advocacy organizations about myelodysplastic syndrome and chronic lymphocytic leukemia in 2005 in the greater Washington, DC, area. A follow-up meeting to coordinate the activities of the national clinical trials cooperative groups and the Blood and Marrow Transplant Clinical Research Network on blood cancers was held in Chicago on October 10 to 11, 2005. Three protocols were identified for collaboration, one on multiple myeloma, one on follicular lymphoma and another on myelodysplastic syndrome.

*Brain tumors*

When a tumor disappears during treatment and later recurs, the question is always: Why? One theory to be tested in the coming years blames such recurrences on a small but hardy population of cells inside tumors that can withstand an attack by drugs and then reconstitute a tumor. These cells, known as cancer stem cells, resemble traditional stem cells in their ability to perpetuate themselves while giving rise to different types of cells.

Stem cells in tumors are not the same cells that, early in human development, give rise to all the tissues of the body. But tumors are like other tissues in that they develop according to certain rules. Research on cancer stem cells aims to understand how this process unfolds and the roles of stem cells in that process. At the moment, cancer stem cells appear to be the driving force behind the development of some tumors, including brain tumors.

If current notions about cancer stem cells are correct, then some chemotherapy and cancer drugs may be missing their most important targets, nearly wiping out whole tumors but leaving stem cells intact. Leading stem cell researchers believe that in order to cure cancer it is necessary to locate and kill tumor stem cells.

The hypothesis that stem cells may play a role in cancer is an old one, going back decades. But no one had been able to isolate the cells from tumors until 1994, when they were found in patients with acute myeloid leukemia. In recent years, cancer stem cells have been isolated in tumors of the breast and the brain and found in cancer cell lines, sparking new interest among researchers.

A new informatics initiative to create a public database that will house biological and clinical data from several thousand primary brain tumors has been launched. REMBRANDT, REpository of Molecular BRAin Neoplasia DaTa, was developed as a partnership between the NCI and NINDS. The data can be used for a variety of purposes, including the development of novel molecular classification systems, and will move us toward an era of individualized cancer treatment based on the molecular genetics of each patient's tumor.

To accomplish these goals, REMBRANDT will house two sets of valuable data. The first set of data will come from the prospective Glioma Molecular Diagnostic Initiative (GMDI). This study will collect tumor specimens from patients enrolled on NCI-sponsored clinical trials and will generate data from the tumors on gene expression, chromosomal alterations, and presence of single nucleotide polymorphisms (SNPs) as well as proteomic data from patient serum. The second type of data housed by REMBRANDT will be a wide array of molecular and genetic data regarding all types of primary brain tumors generated by NCI extramural investigators. REMBRANDT will allow huge amounts of disparate data types to be housed in a single place and will also supply the bioinformatics tools critically necessary for the useful analyses of such data.

NCI's Cancer Biomedical Informatics Grid, (caBIG, http://cabig.nci.nih.gov), is providing a library of tools and resources to REMBRANDT, in order to facilitate integrative analysis from

bench to bedside and back. The new molecular glioma classification system that will result from GMDI and REMBRANDT will be biologically based, giving insight into the pathology of glioma cells and helping physicians to predict responsiveness to specific therapies. The research community will be able to access REMBRANDT resources through an NCI-developed internet portal.

Anti-Inflammatory Drugs May Decrease Brain Cancer Risk. Use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with decreased risk of adult glioblastoma multiforme, which is the most common primary malignant brain tumor, in a study by Niccole Sivak-Sears, PhD, of the Ohio State University, and Margaret Wrensch, PhD, of the University of California at San Francisco, and colleagues. The population-based study included 236 adults with the cancer and 401 controls. Cases were less likely than controls to report use of at least 600 pills of all types of NSAIDs during the 10 years prior to diagnosis (odds ratio = 0.53). The findings were consistent for aspirin, ibuprofen, and naproxen and/or other NSAIDs. Cases also reported less use of acetaminophen than controls did.
Sivak-Sears, N.R., Schwartzbaum, J.A., Mike, R., Moghadassi, M., and Wrensch, M. (2004). Case-control study of use of nonsteroidal anti-inflammatory drugs and glioblastoma multiforme. *Epidemiology, 159(12), 1131-9.*

Human and Mouse Gliomas Inactivate Expression of the Growth Suppressor gene *SLC5A8* by Shared Epigenetic Mechanisms. Both genetic and epigenetic alterations contribute to tumorigenesis. Epigenetic alterations are changes in the genome that are heritable, but do not involve changes in the DNA sequence. This is different from genetic alterations, which do involve changes in the DNA sequence of a genome. In several mouse models of human tumors, the tumorigenic phenotype is reversible, suggesting that epigenetic mechanisms contribute significantly to tumorigenesis in mice. A gene on chromosome 12q23.1, named *SLC5A8*, was shown to be frequently affected by epigenetic alterations in human astrocytomas and oligodendrogliomas. These alterations were determined to be modifications of cytosine bases in DNA called cytosine methylation. A grouping of several methylatable cytosines next to guanosines in DNA defines a "CpG island." These CpG islands are often found in the regulatory region upstream of genes. Methylation of the cytosine bases in the CpG island results in the silencing of gene expression. The *SLC5A8* gene encodes a sodium monocarboxylate cotransporter, a protein that brings sodium into cells. It is highly expressed in normal brain, but its expression is significantly reduced in primary gliomas. The CpG island methylation pattern was found to be consistent with the tumor-specific loss of gene expression. A similar tumor-specific reduction in expression of the mouse *SLC5A8* gene, m*SLC5A8*, was shown in 9 of 10 murine oligodendroglial tumors. The data from these experiments suggest that *SLC5A8* functions as a growth suppressor gene and that its expression is frequently silenced by epigenetic mechanisms in primary gliomas. The shared epigenetic inactivation of m*SLC5A8* in mouse gliomas indicates an additional degree of commonality in the origin and/or pathway to tumorigenesis between primary human tumors and these mouse models of gliomas. [5 R01 CA094971-04, "Convergent Mechanisms Contributing to Cancer," Costello, Joseph F., P.I. Hong, C., et. al., "Shared epigenetic mechanisms in human and mousse gliomas inactivate expression of the growth suppressor *SLC5A8*," Cancer Res. 65: 3617-3623, 2005.]

*Cervical cancer*

Cervical Cancer Risk in Oncogenic HPV DNA-positive Women. Cervical cancer is the second most common cause of death from cancer in women worldwide. Human papillomavirus (HPV) infection causes virtually all cases of cervical cancer, and is also a major cause of other epithelial malignancies, including cancers of the anus, penis, vulva, and upper aerodigestive tract. Together, HPV infection accounts for close to 10 percent of cancers in women worldwide and somewhat less than 5percent of cancers in men. Among women participating in cervical screening programs, approximately two-thirds of those with high-grade cervical neoplasia had antecedent equivocal or mild cytologic abnormalities. Equivocal cytology, or ASCUS (atypical cells of undetermined significance), and LSILs (low-grade squamous intraepithelial lesions) are the most common abnormal cytologic findings of the 60 million Pap tests performed annually in U.S. Within the ASCUS LSIL Triage Study population, baseline specimens of women with equivocal or mildly abnormal cytology were tested for HPV DNA. Women who tested positive for HPV-16 had 38 times the risk for CIN3 or cervical cancer compared to women who were HPV negative, while those who tested positive for other oncogenic HPV types had seven times the risk. Patients with a positive HPV-16 diagnosis may require more aggressive management than those who test positive for other oncogenic HPV types or who are HPV negative. *J Natl Cancer Inst* 2005; 97:1066-71.

*Chronic lymphocytic leukemia*

Chronic lymphocytic leukemia (CLL) is a slowly progressing cancer in which too many white blood cells (lymphocytes) are found in the blood and bone marrow. This disease primarily affects middle-aged and older adults. Treatment for CLL depends upon the stage of the disease and may include chemotherapy, radiation therapy, surgery, stem cell transplantation, or some combination of therapies.

The CLL Research Consortium (CRC) funded by NCI has made tremendous progress since its initial funding in 1999. Over 165 papers have been published in outstanding journals, such as *Blood, Proceedings of National Academy of Sciences, New England Journal of Medicine, Nature Medicine, Journal of Clinical Oncology*. Seminal accomplishments include:

- The characterization of genetic lesions and patterns of specific gene expression in chronic lymphocytic leukemia (CLL) that have brought new opportunities for distinguishing patients who might have different tendencies for disease progression or response rates to a given therapy. For example, the Consortium was first to demonstrate that microRNA genes, a family of highly conserved non-coding genes, are found on a region of chromosome 13 that is deleted in more than half of CLL cases. These genes are involved in temporal and tissue-specific gene regulation and differences in their expression can be associated with differences in the clinical behavior of CLL as well as other cancers. Another gene identified on chromosome 13 that is a novel tumor suppressor gene, ARLTS1, is associated with development of familial CLL and familial solid-tissue cancers. Again, this information would enable physicians to categorize CLL patients into familial or sporadic origin. Finally, the over expression of ZAP-70, a tyrosine kinase, is a

risk factor for aggressive disease which may help identify patients in need of more aggressive treatment.

- The development of the first transgenic mouse model for CLL that enables investigators to study disease pathogenesis and perform preclinical evaluation of novel therapeutic agents *in vivo*.

- The identification and development of novel pharmacologic and biologic agents for CLL. These include molecules with novel mechanisms of action, small molecule inhibitors of proteins that block programmed cell death as well as biologic agents such as monoclonal antibodies and gene therapy reagents. The Consortium completed the first gene therapy phase I trial in CLL using an adenovirus-CD154 construct. The Consortium also rescued a novel compound, flavopiridol, from the junk pile of drug development. Adventis-Santofi had discontinued the development of this compound until investigators in the Consortium developed a new dosing schedule that resulted in tremendous activity against CLL in patients. The patients developed tumor lysis syndrome, a condition that is rare in cancer drug development thus indicating the drug was efficacious and very potent against CLL. Adventis-Sanofi has resurrected the development of flavopiridol and will attempt to obtain fast track approval from the FDA.

- Improved algorithms to assess disease progression risk and response to therapy

- Development of infrastructure that facilitates bench-to-bedside and bedside-to-bench research via the establishment of a national tissue bank, a CRC biomedical informatics system, and a familial CLL cohort

Despite this outstanding progress, the competitive renewal of this consortium failed to make the scoring range for funding consideration. The CRC is currently on $1 million total cost interim support to keep this outstanding research team together until further funding decisions can be made in fiscal year 2006.

NCI also supports CLL research activities through the Quick Trials mechanism, cooperative agreements, contracts, and through the intramural research program. On average over the last five years, 13 cooperative group CLL protocols and 17 phase I/II protocols were actively accruing patients at approximately 177 patients per year. In addition, there are at minimum 15 phase I/II CLL protocols that are ongoing.

*Esophageal cancer*

Tissue Zinc Levels and Esophageal Cancer Risk. The association between incident esophageal squamous cell carcinoma and zinc was examined using baseline esophageal biopsy specimens from residents of Linzhou, China who participated in a nutrition intervention trial in this high-incidence area. X-ray fluorescence spectroscopy was used to measure zinc, copper, iron, nickel, and sulfur concentrations from biopsies collected in 1985 from 60 eventual case and 72 control subjects, matched on baseline histology and followed for 16 years. The risk of developing

esophageal cancer was much lower for subjects with the highest esophageal tissue zinc concentration compared with those in the lowest quartile. Individuals with the highest sulfur concentration also had a lower risk of esophageal cancer than those in the lowest quartile, but the trend was not significant. There was no association between copper, iron, or nickel concentrations and risk of esophageal cancer. High tissue zinc concentration was strongly associated with a reduced risk of developing esophageal squamous cell carcinoma. X-ray fluorescence spectroscopy can be used to assess relationships among concentrations of both nutritional and toxic elements and disease risk in banked tissue specimens. *J Natl Cancer Inst* 2005;97:301-306.

*Head and neck cancer*

Head and Neck Cancer Survivorship Outcomes. Because effects of head and neck cancer and its treatment can compromise speech, swallowing, self-image and self-esteem, survivors often face significant functional and quality of life challenges after treatment. Despite this, survivors of head and neck cancer have rarely been the focus of survivorship studies. Reversing this trend, Campbell and colleagues report a series of studies on outcomes for this neglected population. Among their findings is the surprising discovery that almost half of long-term nonlaryngectomy head and neck cancer survivors demonstrated at least some degree of aspiration. The presence of aspiration is associated with substantial weight loss, advanced initial tumor stage, diminished oropharyngeal swallowing efficiency, and lower scores on a variety of quality of life scales. Many long-term survivors of head and neck cancer treatment reported that poor speech intelligibility adversely affected quality of life self-assessment, including distress about communication, eating, and recreation. In addition, pre-morbid pessimism was consistently the best predictor of quality of life measures. Of key importance in this research was the recognition that both psychosocial and physiologic effects together affect quality of life outcomes. These data have important implications for patient care. Campbell, B.H., Spinelli, K., Marbella, A.M., Myers, K.B., Kuhn, J.C., and Layde, P.M. (2004). Aspiration, weight loss, and quality of life in head and neck cancer survivors. *Archives of Otolaryngology - Head and Neck Surgery, 130(9), 1100-3.* Meyer, T.K., Kuhn, J.C., Campbell, B.H., Marbella, A.M., Myers, K.B., and Layde, P.M. (2004). Speech intelligibility and quality of life in head and neck cancer survivors. *Laryngoscope, 114(11), 1977-81.* Holloway, R.L., Hellewell, J.L., Marbella, A.M., Layde, P.M., Myers, K.B., and Campbell, B.H. (2005). Psychosocial effects in long-term head and neck cancer survivors. *Head and Neck, 27(4), 281-288.*

*Hodgkin's disease*

Cardiovascular Abnormalities in Long-term Hodgkin's Disease Survivors. With the majority of pediatric cancer survivors expected to be cured of their disease, understanding and reducing risk for life-threatening events related to treatment is more important than ever. One of the more worrisome among these is late cardiac failure. Lipshultz and colleagues have found that a variety of unsuspected, clinically significant cardiovascular abnormalities are common in long-term survivors of Hodgkin's Disease who are treated at a young age with mediastinal irradiation. Even though every patient described their health as good or better in this study, and none had symptomatic heart disease at screening, all but one of the survivors had cardiac abnormalities

when assessed. Similar risk for cardiac damage has been observed by these investigators in childhood cancer survivors exposed to doxorubicin as part of their curative therapy for leukemia. In an exciting pilot study, they found that dexrazoxane, a free-radical scavenger, prevents or reduces cardiac injury without compromising the antileukemic efficacy of doxorubicin. While longer follow-up will be necessary to determine the influence of dexrazoxane on echocardiographic findings at four years and on event-free survival, this work holds promise for reducing the human cost of cure in young survivors and potentially those treated with anthracyclines as adults. Adams, M.J., Lipsitz, S.R., Colan, S.D., Tarbell, N.J., Treves, S.T., Diller, L., Greenbaum, N., Mauch, P., and Lipshultz, S.E. (2004). Cardiovascular status in long-term survivors of Hodgkin's disease treated with chest radiotherapy. *Journal of Clinical Oncology, 22(15), 3139-48.*

*Hutchinson-Gilford progeria syndrome (HGPS)*

NCI Study Demonstrates That Cellular Defects in Premature Aging Disease are Reversible. Cells affected by Hutchinson-Gilford Progeria syndrome (HGPS) disease associated with premature aging, can be made healthy again, according to findings by Dr. Tom Misteli, Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research. Using specially modified segments of DNA, Dr. Misteli and his colleagues reversed the abnormalities seen in HGPS cells by correcting defects associated with the key protein, lamin A. By demonstrating that HGPS cellular characteristics are reversible, this study brings scientists one step closer to understanding this disease and may provide insights into the normal aging process. Less than two years ago, NIH-led researchers discovered that the genetic basis for HGPS is a single mutation in the gene encoding lamin A, a critical structural protein that acts as the scaffolding in a cell's nucleus. Lamin A is defective in HGPS cells because the mutation creates an aberrant splice site in the lamin A gene, resulting in synthesis of a truncated lamin protein. Without the lamin A scaffolding, the nuclei of progeria cells become wrinkled and misshapen and numerous other nuclear proteins show reduced expression. The NCI researchers designed a chemically stable DNA oligonucleotide that would bind to the mutant splice site and prevent the splicing machinery from cutting in the wrong place. One week after inserting these oligonucleotides into progeria cells, the mutant lamin A protein had been eliminated and more than 90 percent of progeria cells were restored to normal. Visually, the nuclei lost their wrinkles and lobes and returned to a natural ellipsoid shape, and the expression of other nuclear proteins was restored to normal levels. These results demonstrate a proof-of-principle that the cellular effects of progeria can be reversed. Paola Scaffidi and Tom Misteli, "Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford Progeria syndrome." *Nature Medicine*, vol 11, no 4; April 2005.

CAAX Protein Processing and Cancer Therapy. Restrictive Dermopathy (RD) and Hutchinson-Guilford Progeria syndrome (HGPS) are rare pre-mature aging syndromes where children suffer a variety of premature aging-related defects, including osteoporosis, alopecia and atherosclerosis. Children suffering from HGPS die at a mean age of 13, generally from myocardial infarctions or strokes. Mis-shapened cell nuclei and accumulation of a defective protein, progenin, in the nuclei of cells from these patients are characteristic of these diseases. Recent studies have shown that progenin is chemically modified in a way similar to the well-characterized Ras oncogene.

This modification, farnesylation, is responsible for localization of the Ras oncogene into cellular membranes. Numerous pre-clinical and clinical studies have accordingly developed farnesyl transferase inhibitors (FTIs) to disrupt oncogenic Ras membrane localization. Many of these inhibitors have already been approved for human use in trials to test their anti-tumor properties and have been shown to be well tolerated in human patients. Drs. Stephen Young and Loren Fong reasoned that these FTIs might also disrupt the localization of progenin to the nuclear membrane of RD and HGPS cells and correct the most obvious cellular defect associated with this class of premature aging syndromes. In 2005, using a mouse model of HGPS and cells derived from human RD and HGPS patients, Drs. Young and Fong showed that FTI treatment did disrupt progenin nuclear localization and reduced the characteristic nuclear abnormalities. These findings suggest a potential new strategy for treating these diseases that could be quickly developed (R01CA99506-04, Stephen G. Young, MD, University of California, Los Angeles).

*Kaposi's sarcoma*

Role of the Kaposin B Protein of Kaposi's sarcoma–associated herpesvirus. An emerging theme in virology and cancer biology is the mechanisms that viruses and tumor cells use to impede the host's normal immune response. A gamma herpesvirus, KSHV/HHV8 (Kaposi's sarcoma-associated herpes virus or human herpesvirus 8) is an essential causative agent present in all forms of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and Multicentric Castleman's disease (MCD). Herpesvirus genomes encode latent and lytic groups of genes. The role of the latent genes is maintenance of the viral genome within the host cell nucleus during cell division. Only a few genes are expressed during latency. When internal and/or external signals are received by the host cell, HHV8/KSHV lytic genes are switched on, replication of the lytic genes begins, and soon new virus particles are produced. One of the hallmarks of clinical disease pathologies associated with infection by KSHV/HHV8 is the high levels of inflammatory cytokines and factors that that encourage blood vessel growth in and around tumor sites. Inflammatory cytokines are the group of biological factors synthesized and released by specific cell populations that have specific interactions and communications with other cells when mounting a local immune response following infection or injury. A cell culture model that may explain the clinical disease pathology of KS and KSHV/HHV8-associated tumors was used by this NCI supported investigator. They have found that the latent KSHV/HHV8 viral protein kaposin B is at least partially responsible for the dramatic increase of inflammatory cytokines. The kaposin B protein prevents the normal degradation pathway of cytokine messenger RNA (mRNA) transcripts by binding to a cellular protein MK2 [mitogen-activated protein kinase (MAPK)-associated protein kinase 2], which allows translation of the cytokine transcripts in the absence of normal cellular signals. The initial inflammatory cytokine production through this mechanism may induce further cytokine production by short-circuiting the cell's normal regulatory pathways. Their results are the first to show that the kaposin B protein of KSHV/HHV8 can activate a cellular biochemical pathway and stabilize cytokine mRNA. This finding also suggests a method viruses can use for modulating mRNA turnover in a host cell. While the complete mechanism is still unknown, the important role of kaposin B may explain the association between KSHV/HHV8 infection and enhanced cytokine production observed in diseases associated with a KSHV etiology. These results are significant, because aberrant increases in inflammatory cytokines result in dysregulation of the normal immune response and

may create the disease pathologies observed in KS lesions, PEL, and MCD. The discovery of the interaction between the KSHV/HHV8 kaposin B protein and normal host cellular proteins provides a target for future development of therapeutic agents that may halt inflammatory cytokine production during the development of KS tumor lesions. "The Kaposin B Protein of KSHV Activates the p38/MK2 Pathway and Stabilizes Cytokine mRNAs" McCormick, C. and Ganem, D., 4 February, 2005, *Science* 307(5710):739-41 http://www.sciencemag.org/cgi/reprint/307/5710/739.pdf. R01-CA-073506 "Herpesviral Gene Expression in Kaposi's Sarcoma" and R01-CA-096491 "Pathophysiology of lytic reactivation and spread of KSHV"; Don E. Ganem, University of California, San Francisco, California.

*Kidney cancer*

Kidney, or renal, cancer is made up of several distinct cellular and genetic subtypes. The most common form of renal cancer, clear cell carcinoma, is related to loss of function of the tumor suppressor, von Hippel-Lindau (VHL). The absence of the functional protein product of the VHL gene leads to activation of several genes, including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and other mediators of tumor angiogenesis, growth, and metastasis. The identification of molecular targets in the VHL pathway, as well as other pathways relevant to kidney cancer (Ras-Raf-MEK-ERK and EGFR-MAPK-PI3K-Akt-mTOR), have led to partnerships with industries for the clinical development of targeted agents in kidney cancer.

- The Food and Drug Administration (FDA) approved sorafenib (Nexavar) for the treatment of advanced renal cell carcinoma in January 2006. In preclinical studies, sorafenib was shown to inhibit multiple targets associated with tumor angiogenesis, including RAF kinase, VEGFR-2, VEGFR-3, PDGFR-ß, KIT, and FLT-3. Through a clinical trials agreement, NCI and Bayer are cosponsoring a variety of phase II studies currently open or planned for advanced kidney cancer that will test whether adding targeted agents to sorafenib can improve its activity.
- Intellectual property, liability, and regulatory issues can slow progress in developing trials with agents from more than one company, either in trials with multiple single agent arms or those testing combination regimens. The NCI has played an important role in facilitating collaborations within the private sector without the need for additional bargaining between the parties. NCI developed standard language now used in all agreements with industry concerning how data is to be shared and how companies may benefit from any invention that may arise using drug combinations. Examples of NCI-facilitated research involving more than one company are cited below.

  → Trials of combinations of inhibitors of m-TOR, EGFR, and the multiple targets associated with angiogenesis have been designed and have either already opened or will in 2006.

  → There is currently no known effective adjuvant therapy for patients with localized kidney cancer -- that is, those who undergo resection but are at risk for relapse. Both sorafenib and sunitinib will be tested alone versus placebo in a

large, three-arm, randomized trial of adjuvant therapy conducted by NCI-sponsored cooperative groups, led by the Eastern Cooperative Oncology Group, and in collaboration with Bayer and Pfizer. The trial is slated to open in the first quarter of 2006. Furthermore, this trial is designed to collect and store the kidney tissue removed at surgery so that this rapidly expanding field will have high quality resources to conduct cutting edge research.

- Promising results from a small trial of a monoclonal antibody that inhibits VEGF (Avastin®, bevacizumab, Genentech, Inc.) conducted at the NCI intramural program led to a confirmatory national trial through the NCI cooperative groups and the Cancer Trial Support Unit. The trial, CALGB 90206, opened in October 2003 and accrued more quickly than anticipated, with the targeted enrollment of 700 patients reached mid-2005. The data is maturing to questions such as whether interrupting VEGF signaling will lead to prolongation in survival. Adjunctive studies address whether pre- and post-treatment plasma, urine, and/or baseline tumor tissue can select for patients likely to respond to anti-angiogenesis treatment.

- Novel agents under study in NCI-sponsored clinical trials include inhibitors of VEGF, PDGF, HSP-90 (stabilizer of HIF and EGFR), m-TOR and raf kinase. Other novel agents include SB-715992 (ksp inhibitor), epothilone B, and SAHA. Many of these clinical trials include companion translational research, facilitating bidirectional bench-to-bedside research to accelerate progress in renal cancer.

- Novel dendritic cell based tumor immunotherapy is a very promising approach for cancer treatment. NCI continues its funding support for a Phase I/II clinical trial for treating renal cell carcinoma by Dendritoma Vaccine. The novelty of this trial is that Dendritoma Vaccine is made from patient's own tumor cells, dendritic cells and autologous serum. This vaccine has been very effective in activating patient's functional cytotoxic T lymphocytes that efficiently lyse autologous tumor cells and reduce tumor burden. A total of 29 advanced renal cell cancer patients who have exhausted all available therapies will be recruited and evaluated in this trial, and correlative studies will be conducted to understand the molecular mechanisms of tumor specific immune responses and treatment effects. So far, 10 (1 females and 9 males) out of 29 patients have been recruited into this trial and eight patients' data were eligible for analysis. Seven of the eight patients showed increased INF-gamma expressing CD4+ T cells, and five patients had increased tumor eradicating and INF-gamma expressing CD8+ T cells respectively. Among those patients, there is one partial response and three stable diseases and four progressive diseases. No grade three or four toxicities were observed. The ongoing clinical trial and correlative studies are important for the future treatment of renal cell carcinoma which currently lacks effective treatments.

- Subsets of kidney cancer other than clear cell have distinct genetic profiles, comprise approximately 15 percent of diagnoses, and are more difficult to study due to their rarity. To find treatments relevant to these subtypes, three trials have opened nationally in the cooperative groups in papillary, collecting duct, and sarcomatoid renal cancers. Target

enrollment has been reached in the trial focusing on papillary carcinoma, and results are pending.

Second Mutations Implicate Novel Gene as Tumor Suppressor in Renal Cancers. Individuals with Birt-Hogg-Dubé (BHD) syndrome, an inherited disease characterized by hair-follicle tumors and pulmonary cysts, have a seven-fold increased risk over the general population of developing kidney cancer. These individuals have germline mutations in a novel gene, BHD. The BHD gene encodes a protein, folliculin, which gets its name from the disease's hallmark skin lesion. The high frequency of germline mutations in the BHD gene of BHD patients suggests that this gene may be a tumor suppressor gene. Identifying a somatic mutation in the copy of the BHD gene without a germline mutation, a so-called "second hit," would further implicate BHD as a tumor suppressor gene.

To determine the mutation status of the BHD gene in tumors from BHD patients, Dr. W. Marston Linehan, Urologic Oncology Branch, Center for Cancer Research, and colleagues at the National Cancer Institute conducted direct DNA sequence analysis of 77 renal tumors surgically removed from 12 BHD patients. The known germline mutation from each patient was detected in every tumor. Somatic point mutations were found in 53percent of renal tumors, and these mutations were distributed throughout the BHD coding region. The analyses also yielded evidence for deletion of the second copy of the BHD gene in an additional 17percent of tumors. These results indicate that renal tumors associated with BHD syndrome arise from cells in which both copies of the BHD gene are inactivated. The documentation of both a high frequency and wide spectrum of second mutations in the BHD gene in BHD patients strongly supports the hypothesis that the BHD gene acts as a tumor suppressor. Vocke, C.D., Yang, Y., Pavlovich, C.P., Schmidt, L.S., Nickerson, M.L., Torres-Cabala, C.A., Merino, M.J., Walther, M.M., Zbar, B., and Linehan, W.M. "High Frequency of Somatic Frameshift BHD Gene Mutations in Birt-Hogg-Dubé-Associated Renal Tumors." *Journal of the National Cancer Institute*; Vol. 97 (12), June 15, 2005.

*Liver cancer*

NCI remains dedicated to the development of drugs targeting liver cancer, with particular emphasis on the evaluation of new biologic agents that interfere with the cellular pathways of the disease. Over 90 percent of primary carcinomas of the liver are hepatocellular carcinomas (HCC). While HCC is a common cause of cancer and cancer-related mortality worldwide, until recently it has been rare in the U.S. New studies demonstrate that liver cancer is the most rapidly increasing cause of cancer in the U.S., resulting in at least 14,000 deaths annually. The recent upsurge in HCC in the U.S. may be attributable to chronic infection with hepatitis C virus (HCV), which is found in more than half of patients diagnosed with HCC.

The 2004 Conference, "Hepatocellular Carcinoma: Screening, Diagnosis, and Management," cosponsored by NCI and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), set out summary recommendations for HCC that were published in a special supplement to the November 2004 issue of Gastroenterology. The recommendations focused on the promotion of research in four main areas: surveillance, prevention, early detection, and

treatment. Based on the recommendations from this conference, NCI and NIDDK continue to discuss the potential development of a Request for Applications specifically designed to address these main areas of research. For treatment, the recommendations supported development and evaluation of innovative local ablative therapies, cytotoxic agents, and molecularly targeted therapies along with basic research to define key molecular pathways that contribute to the malignant transformation of liver cells.

NCI is currently sponsoring development of 17 new agents in 29 phase I and phase II clinical trials for patients with liver and hepatobiliary cancer; 23 of these trials are for patients with HCC. In addition, NCI is funding phase II trials evaluating radiofrequency ablation (the use of electrical current, passed through electrodes placed directly into a tumor, to destroy the tumor with heat) and chemoembolization (a procedure in which the blood supply to a tumor is blocked surgically or mechanically and anticancer drugs are administered directly into the tumor) in patients with HCC. During FY 2006, an NCI-funded trial will begin to test an investigational agent for the chemoprevention of hepatocellular carcinoma in patients chronically infected with HCV.

The NCI recently issued two program announcements soliciting research grant applications in etiology, prevention, and treatment of hepatocellular carcinoma. NCI anticipates receiving a number of applications to support research on primary liver cancer.

*Lymphoma*

The NCI is aggressively pursuing answers to the factors that contribute to lymphomas. By understanding different immune system components of lymphomas, NCI is creating the foundation for targeted therapy**.**

- Histone deacetylase (HDAC) inhibitors are promising anti-tumor agents. These drugs alter the structure of the histone proteins associated with DNA and contribute to regulating gene expression. Although not well-studied in B-cell lymphoma, NCI-supported research found that the drugs repressed lymphoma cell proliferation and promoted cell death in these cells, providing new rationale for using HDAC inhibitors in the treatment of B-cell lymphoma.

- The NCI is also supporting studies of proteins that alter gene expression in T-cell lymphoma. The proteins that regulate gene expression can also be affected by the addition or subtraction of methyl groups by DNA methyltransfersases (DNMTs). New experiments from these studies suggest that DNMTs can form complexes with HDACs and alter the expression of proteins specifically required for T-cell lymphomas to become malignant. Drugs designed to inhibit HDACs or DNMTs or combinations of these inhibitors are attractive candidates for novel anticancer therapies in T-cell lymphoma.

The NCI promotes studies that examine the simultaneous presence of infectious agents among individuals with lymphoma, specifically in the context of HIV infection.

- For example, the NCI supports the AIDS Malignancy Consortium (AMC), which conducts clinical trials on AIDS-associated lymphoma. The AMC has shown that combination chemotherapy can be safely administered along with Highly Active Anti-Retroviral Therapy (HAART) to patients with AIDS-associated lymphoma. Another AMC study showed that the addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy results in improved clinical outcome for individuals with AIDS-associated lymphoma. However, these patients are at an increased risk for death from infection related complications of therapy. The higher infectious death rate seen in these patients may be compounded by the immunodeficient state of these individuals. The safety and efficacy of rituximab in these patients will require further studies.

- Another important contribution from the AMC, is towards developing approaches targeted to EBV in management of EBV positive AIDS-associated lymphomas.

In order to follow changing trends in morbidity and mortality due to lymphomas and map the impact of HAART, NCI supports the AIDS Cancer Match Registry. Observations from these studies show that reduction in the incidence of NHL is lesser than that observed for other AIDS-defining cancers such as Kaposi's sarcoma and that there is an unexplained increase in Hodgkin lymphoma in the HIV population.

NCI also supports clinical trial protocols aimed at treating lymphoma. The Blood and Marrow Transplant Clinical Trials Network (BMT CTN), cosponsored by NCI and the National Heart, Lung and Blood Institute, has two active phase III lymphoma protocols.

- One compares two transplant strategies in relapsed follicular NHL patients to determine whether one type of stem cell transplant is better than the other in improving long-term progression-free survival.

- The other compares progression-free survival after a specific type of transplant called, autologous hematopoietic stem cell transplantation, for chemotherapy-sensitive diffuse large B-cell lymphoma using Rituxan/BEAM versus Bexxar/BEAM for pre-transplant conditioning.

To research outcomes for long-term survivors of both Hodgkin lymphoma and NHL, the NCI has supported the Childhood Cancer Survivor Study (CCSS) for the past 13 years. The CCSS has expanded the cohorts to be followed so that long-term effects of the newer cancer regimens can be determined. Hodgkin's and NHL patients are well represented in CCSS cohorts.

*Non-Hodgkin lymphoma*

Smoking and Non-Hodgkin Lymphoma. The International Lymphoma Epidemiology Consortium (InterLymph) provided an opportunity to analyze the relationship between cigarette smoking and non-Hodgkin lymphoma (NHL) with sufficient statistical power to consider NHL subtypes. In a pooled analysis of 6,594 cases and 8,892 controls from nine case-control studies

conducted in the U.S., Europe, and Australia, smoking was associated with a slightly increased NHL risk. Compared with nonsmokers, current smokers, particularly heavy smokers, had a higher odds ratio for follicular lymphoma than former smokers. Cigarette smoking may increase the risk of developing follicular lymphoma, but not the other subtypes examined. *Cancer Epidemiol Biomarkers Prev* 2005;14:925-933.

*Hodgkin lymphoma*

Hodgkin Lymphoma Susceptibility Gene. Hodgkin lymphoma (HL) has a strong familial component, although no genes have yet been identified. A genome-wide linkage screen was performed in 44 high-risk HL families, with a total of 254 individuals providing DNA samples. Among these families, 95 HL cases and four cases of NHL were informative for linkage. The strongest linkage finding was on chromosome 4p near the marker D4S394. Other locations suggestive of linkage were found on chromosomes 2 and 11. The number of independent regions identified was more than expected by chance, although no one region met genome-wide significance levels. These linkage findings represent the first step towards identifying one or more loci leading to susceptibility to HL and understanding its complex etiology. *J Med Genet* 2005;42:595-601.

*Melanoma*

Malignant melanoma continues to increase rapidly in incidence in the U.S. and worldwide. Despite the routine use of established clinicopathological criteria there is a need to improve accuracy of estimating prognosis in melanoma patients at the time of primary diagnosis using other strategies such as biomarkers whose use would be accepted in routine clinical practice.

Thus the major goals for the melanoma research and clinical community to reduce melanoma mortality are improved accuracy of diagnosis and prognosis to predict which patients will develop metastasis. In addition, there is a need for early evidence of patients who would benefit from alternative therapy and for new targets and new therapies. However, melanoma research has been hampered by the limited access to large numbers of melanoma tissue samples with associated clinical information that would permit discovery and validation of clinically useful new biomarkers and new assays.

NCI, recognizing the challenges in the translational research in melanoma and the need for melanoma tissue collection, convened the following activities.

- Melanoma Tissue Resource Workshop, February 2004. A two-day workshop brought together melanoma research experts to identify areas of common interest and to suggest new research resources needed to address important issues in melanoma diagnosis and prognosis. Developing tissue resources for melanoma is one of the most important goals in translational research in melanoma that will aid in identifying and validating biomarkers to aid with disease management.

- As a result of the workshop, in 2005 NCI supported the construction of melanoma tissue microarrays (TMAs). NCI provided administrative supplements to six institutions to

support collection of the requisite tissue including melanocytic nevi, primary melanomas and samples from metastatic lesions and associated clinical information to construct the TMA. The progression nevus > melanoma TMA has been produced by the NCI's Tissue Array Research Program (TARP) laboratory as a collaborative effort with the melanoma research community with support from the Cancer Diagnosis Program (CDP). TMAs will be available to members of the melanoma research community in 2006. Availability of TMAs as tissue resources should greatly accelerate progress in discovery of new markers and validation of various molecular profiles and pathways in melanoma.

- The Second Melanoma Resource Workshop was convened to address the need to identify more accurate strategies for prognosis and prediction in melanoma. The meeting was organized by the CDP and SPORE Programs at NCI in October 2005. The meeting brought together researchers and clinicians from the major melanoma research centers in the U.S. to discuss recent advances in translational research to identify biomarkers for the diagnosis, prognosis and prediction of response to treatment of melanoma. The discussions included availability of the tissue resources needed for these studies. Sessions also included discussions of TMAs as a tissue resource for melanoma research at the biomarker discovery stage and at the melanoma biomarker validation stage and for development of assays for clinical application.

- As a result of the meeting the investigators who have developed or are in the process of developing melanoma TMAs in their laboratories agreed to contribute to the melanoma TMAs "bank," which will include the TMAs that was developed by TARP and SPORE Program. CDP will help to coordinate this activity to provide valuable resources such as TMA to the melanoma research community.

- CDP is involved in NCI Melanoma Focus Group Activities, which include NCI staff, the Melanoma Research Foundation, and melanoma community investigators. The goal for these activities is to identify and come to consensus on the NCI's directions needed to make progress in melanoma cancer.

- Research supported by CDP in 2005 combined data on gene expression, DNA copy number, DNA sequence, immunohistochemistry etc. to elucidate comprehensive genetic changes, gene mutations, mRNA and protein changes that are relevant to melanoma. This study has resulted in developing a new molecular classification scheme for the disease that is based on the very significant differences in molecular pathways that could be targeted for treatment in the different types of melanoma. These studies provide groundbreaking discoveries for understanding melanoma that had previously been treated as one entity and for targeting specific melanoma subgroups with targeted. Certain subtypes of this disease may respond to available drugs such as BAY 43-9006, a BRAF kinase inhibitor.

A phase III clinical trial evaluating BAY 43-9006, or sorafenib, is underway. Researchers are testing chemotherapy with the drugs carboplatin and paclitaxel in combination with BAY 43-9006. This new drug works by blocking the activity of proteins important for cell proliferation

and for generating new blood vessels to tumors (angiogenesis). Many melanoma tumors carry a mutation in a gene called B-RAF, which in turn produces a protein called Raf kinase. This protein facilitates cellular processes that lead to tumor cell proliferation and survival. Sorafenib blocks the Raf kinase protein and interrupts these processes. It also inhibits a protein called vascular endothelial growth factor receptor (VEGFR), which helps tumors grow the blood vessels needed for nourishment. Researchers hope that sorafenib will weaken melanoma tumors and enhance the cell-killing effects of chemotherapy.

NCI continues its funding support for a Phase II melanoma clinical trial targeting a specific enzyme defect - argininosuccinate synthetase deficiency found in melanoma cells. Malignant melanoma is usually resistant to drug therapy which historically has been non-selective in action and often very toxic. This trial takes advantage of the fact that exposure of melanoma cells to arginine deiminase, an enzyme that catalyzes the hydrolysis of arginine to citrulline, results in melanoma cell arginine starvation and apoptotic cell death while minimizing toxicity. A total of 43 stage IV metastatic melanoma patients will be recruited and evaluated in this trial, and parallel molecular correlative assays will be conducted to elucidate the mechanisms of apoptotic cell death, drug resistance and treatment effect. So far, twelve advanced metastatic melanoma patients (5 females and 7 males) have been recruited into this study. Four patients had partial response ranging from 5 to 10 months. Excisional biopsy after five months showed melanosis but no residual melanoma cells. Sites of response include skin in two patients, lung metastases in one, and soft tissue masses in the abdomen in one patient. Two patients had mixed responses and one patient had minor response in liver metastases. No grade three or four toxicities were observed. The ongoing clinical trial and correlative studies are important for the future treatment of melanoma which is a disease without effective treatment. Results from this study will generate a new approach for the treatment of melanoma.

NCI Researchers Confirm the Effectiveness of Immunotherapy in Melanoma Treatment.
Dr. Steven Rosenberg and colleagues from Surgery Branch, Center for Cancer Research, have found that patients with advanced melanoma who had not responded to previous therapies experienced a significant reduction in the size of their cancers as a result of receiving a new immunotherapy. This immunotherapy consisted of a combination of chemotherapy and reintroduction of their own (autologous) activated lymphocytes. Autologous lymphocytes are white blood cells that have been removed from the patient, activated or re-educated to attack the tumor, then reintroduced into the patient. The promise of this therapy is that a patient's own immune system can be used to effectively treat existing tumors. Of the 35 patients in this study, 18 experienced an improvement in the amount of tumor present at various sites in the body. Eight other patients demonstrated a mixed or minor response. Of the 18 patients showing improvement, 15 had a partial response that lasted from two months to more than two years. It is noteworthy that there were three patients who continued to experience complete disappearance of tumors. This result is particularly significant because these patients had not responded to standard treatments or chemotherapies used in treating patients with melanoma. The results of this study prove that a combination of chemotherapy and infusion of autologous, stimulated, white blood cells can have an impact on metastatic melanoma tumors in patients who do not respond to other therapies. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones

SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastic melanoma. *Journal of Clinical Oncology*. April 1, 2005;23(10).

DNA Repair and Melanoma Risk. Melanoma risk factors include fair pigmentation, multiple nevi, low DNA repair capacity, and CDKN2A or CDK4 mutations. Variants of the melanocortin-1 receptor (MC1R) gene have been associated with fair pigmentation and melanoma risk, and a polymorphism of the Agouti Signaling Protein (ASIP) gene has been associated with dark pigmentation. Variants of the MC1R and ASIP genes were examined among 267 melanoma patients and 382 control subjects from a case-control study and a family study in northeastern Italy in relation to phenotypic characteristics, sporadic and familial melanoma risk, and melanoma thickness. MC1R variant alleles were associated with a two- to four-fold increased risk of sporadic and familial melanoma compared with wild-type MC1R, particularly among individuals with multiple variant alleles. This association was stronger among individuals with fewer additional risk factors. MC1R variant allele carriers were also three-to-four times more likely than non-carriers to have thick melanomas. The ASIP polymorphism was not associated with pigmentation, nevi, or melanoma risk. MC1R was associated with melanoma risk and progression in a Mediterranean population, particularly in the absence of other strong risk factors, such as freckling or many nevi. *J Natl Cancer Inst* 2005;97:998-1007.

*Lung cancer*

Potential lung cancer gene discovered. Lung cancer is a major cause of death in the U.S. and other countries. More people die from lung cancer than from breast, colon, and prostate cancer combined. The risk of lung cancer is greatly increased by smoking and by certain occupational exposures, but familial factors also clearly play a major role. To identify susceptibility genes for familial lung cancer, this study examined 52 families who had at least three first-degree family members affected by lung, throat, or laryngeal cancer. Results of this study found strong evidence that a lung cancer susceptibility gene (or genes) is coinherited with a genetic marker on chromosome 6. Markers on chromosomes 12, 14, and 20 also indicated possible linkage to lung cancer susceptibility, although the results were not as strong. Another discovery involved the effects of smoking on cancer risk for carriers and noncarriers of the predicted familial lung cancer gene. In noncarriers, the more they smoked, the greater their risk of cancer. In carriers, on the other hand, any amount of smoking increased lung cancer risk. The finding on the effects of smoking on risk for carriers and noncarriers suggests that smoking even a small amount can lead to lung cancer for individuals with inherited susceptibility. However, as a result of limited biospecimen availability, effective performance of a linkage study for a rapidly fatal disease such as lung cancer is difficult. Because of this, the authors intend to follow up the results of this study by fine mapping the most significant regions and will attempt to replicate these linkage findings in an independent set of families with familial lung cancer. Discovering lung cancer susceptibility genes will be important in improving our understanding of this life-threatening disease. Bailey-Wilson, J.E., Amos, C.I., Pinney, S.M., Petersen, G.M., De Andrade, M., Wiest, J.S., Fain, P., Schwartz, A.G., You, M., Franklin, W., Klein, C., Gazdar, A., Rothschild, H., Mandal, D., Coons, T., Slusser, J., Lee, J., Gaba, C., Kupert, E., Perez, A., Zhou, X., Zeng, D.,

Liu, Q., Zhang, Q., Seminara, D., Minna, J., and Anderson, M.W. (2004). A major lung cancer susceptibility locus maps to chromosome 6q23-25. American Journal of Human Genetics,75, 460-74. (Epub ahead of print, Jul 21). http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15272417&query_hl=22.

*Mesothelioma*

Malignant mesothelioma is a rare cancer of the lining of the lungs, the heart, or the abdomen (the pleura, pericardium, or peritoneum). If diagnosed at the earliest stage, mesothelioma can be cured by surgery and treatment with chemotherapy or radiation therapy. However, advanced mesothelioma is usually inoperable and is rarely curable.

In an ongoing study, researchers are adding a biological agent called bevacizumab (Avastin®) to chemotherapy to see if it can help delay the progression of mesothelioma in patients with advanced disease. Bevacizumab is a monoclonal antibody that blocks the action of a protein called vascular endothelial growth factor (VEGF). In mesothelioma, VEGF may stimulate both tumor cell growth and the formation of tumor blood vessels.

Bevacizumab has shown promise in several other types of cancer, and it is hoped that it will be particularly effective against mesothelioma because VEGF plays such a prominent role in the growth of this disease.

*Myelodysplastic syndrome*

Genetic mutations often get much of the credit for causing cancer, but another important factor is genes that turn on or off at the wrong times, despite being free of mutations. This abnormal gene activity often involves "epigenetic" changes to DNA and in some cases may be reversible. Epigenetic changes alter gene activity without modifying the genetic code, and are essential to normal development. But the changes can cause problems if they occur when they shouldn't or disable genes that suppress tumors and control growth.

Studies have suggested that epigenetic changes may be as common in some tumor cells as genetic mutations, and many researchers now say that epigenetic changes are critical to understanding, detecting, and treating cancer.

A recent study suggests that epigenetic changes in normal tissue can create the perfect environment for cancer to develop if genetic mutations arise later. The idea is that epigenetic and genetic factors may cooperate in causing tumors. Scientists are looking at what sorts of epigenetic changes might occur in normal tissue that set the stage for mutations that come along later.

To identify epigenetic changes that confer cancer risk, researchers first need to know which changes are normal. This presents a challenge because unlike genetic code, epigenetic changes are dynamic and vary by cell type, age, and sex, among other factors.

The most commonly observed epigenetic change is DNA methylation, in which chemicals called methyl groups attach to DNA, often silencing a nearby gene. DNA methylation can be detected in body fluids such as blood and urine, and new technologies can rapidly scan genomes for epigenetic changes.

At least a dozen drugs that target epigenetic changes such as methylation are in clinical trials and more are in development. In May 2005, the Food and Drug Administration (FDA) approved a methylation inhibitor to treat the rare bone marrow disorder myelodysplastic syndrome (MDS), which can lead to leukemia.

The drug, azacitidine (Vidaza), had nearly been abandoned two decades ago but found new life as researchers showed that it helps MDS patients when given at low doses.

*Multiple myeloma*

Novel Targeted Therapies for Multiple Myeloma.

**Background:** Multiple myeloma is a cancer characterized by the accumulation of plasma cells in the bone marrow and an abnormal protein made by these cells. More than 14,000 people are diagnosed with, and close to 11,000 die from, this disease each year in the United States. Although conventional and high dose therapies have achieved responses and improved patient survival, few, if any, patients are cured. Animal and laboratory studies have indicated that a new, targeted cancer therapy, a drug called Bortezomib, may be effective against multiple myeloma. SPORE-supported laboratory and animal studies showed that Bortezomib, a novel proteasome inhibitor, kills tumor cells resistant to all other therapies by blocking the breakdown of excess proteins made by myeloma cells. Clinical trials confirmed remarkable activity in patients with advanced myeloma, leading to FDA approval of this agent for treatment of recurrent myeloma. However, intrinsic or acquired resistance to this novel agent occurs in most cases.

**Advance:** Three mechanisms to overcome resistance to Bortezomib have been identified in SPORE-sponsored laboratory studies. First, Heat shock protein 90 (Hsp90) is an important component of the protein degradation process in myeloma cells. Preclinical studies have shown that inhibiting Hsp90 with 17-(allylamino)-17-demethoxygeldanamycin (17 AAG) augments myeloma cell killing by Bortezomib, and derived ongoing clinical trials are promising. Second, laboratory studies have also shown that Hsp27 mediates resistance to Bortezomib; and that SCIO469, a p38 mitogen-activated protein kinase inhibitor, overcomes this resistance. A derived clinical trial combining Bortezomib with SCIO469 is fully enrolled. Third, the investigators have identified a second system for protein degradation in myeloma cells, called the aggresome, and they have made a drug, tubacin, which can block this pathway in myeloma cells. Laboratory studies show that blocking both the proteasome and aggresome pathways of protein degradation with Bortezomib and tubacin enhances myeloma cell killing, providing the rationale for clinical testing.

**Implications:** The U.S. Food and Drug Administration has approved Bortezomib for treatment of relapsed myeloma. The drug is now being studied in clinical protocols combined with novel therapies as described above. In addition Bortezomib is being evaluated for its efficacy as an initial treatment for myeloma. (Specialized Program of Research Excellence in Myeloma; 1 P50 CA100707; Dana-Farber Harvard; Principal Investigator: Kenneth Anderson.)

**Citations**:

Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Kung AL, Davies FE, Morgan G, Akiyama M, Shringarpure R, Munshi NC, Richardson PG, Hideshima T, Chauhan D, Gu X, Bailey C, Joseph M, Libermann TA, Rosen NS: Anti-myeloma activity of heat shock protein-90 inhibition. *Blood*, in press.

Hideshima T, Podar K, Chauhan D, Ishitsuka K, Mitsiades C, Tai YT, Hamasaki M, Raje N, Hideshima H, Schreiner G, Nguyen AN, Navas T, Munshi NC, Richardson PG, Higgins LS, Anderson KC: p38 MAPK inhibition enhances PS-341 (bortezomib) –induced cytotoxicity against multiple myeloma cells. *Oncogene* 2004; 23: 8766-76.

Hideshima T, Bradner J, Wong J, Chauhan D, Richardson P, Schreiber S, Anderson KC: Small molecule inhibition of proteasome and aggresome function induces synergistic anti-tumor activity in multiple myeloma. *Proc Natl Acad Sci* 2005; 102: 8567-72.

Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Blade J, Boccadoro M, Cavenagh J, Dalton WS, Boral A, Esseltine DL, Porter JB, Schenkein D, Anderson KC: A randomized comparison of Bortezomib with high-dose dexamethasone in relapsed multiple myeloma. *N Engl J Med* 2005; 352: 2487-98.

*Neurofibromatosis*

In collaboration with NINDS, NCI provided funding to the National Neurofibromatosis Foundation to support the meeting entitled "The National Neurofibromatosis Foundation International Consortium for the Molecular and Cell Biology of NF1 and NF2 and Schwannomatosis" at the Hotel Jerome in Aspen, CO, on June 5-8, 2005. This meeting brought together scientists and clinicians to share their latest research findings and experiences and to form new collaborative ventures. Critical gaps in knowledge relating to NF1 and NF2 and schwannomatosis were discussed and strategies to address them were suggested. These discussions will play an essential role in developing improved therapies for the complications of these disorders with broad implications to the fields of developmental neurobiology and cancer research.

*Ovarian and endometrial cancer*

In January 2006, NCI issued an announcement encouraging treatment with anticancer drugs via two methods, after surgery, for women with advanced ovarian cancer. The combined methods,

which deliver drugs into a vein and directly into the abdomen, extend overall survival for women with advanced ovarian cancer by about a year. The clinical announcement, the first issued by NCI since 1999, was made with the support of six professional societies and advocacy groups. The announcement coincided with publication in the *New England Journal of Medicine* of the results of a large clinical trial by an NCI-supported research network known as the Gynecologic Oncology Group (GOG). This was the eighth trial evaluating the use of chemotherapy delivered into the abdomen for ovarian cancer. Together, these trials show a significant improvement in survival for women with advanced ovarian cancer. The two treatment methods are called intravenous, or IV, for chemotherapy delivered into a vein and intraperitoneal, or IP, for chemotherapy delivered into the abdominal, or peritoneal, cavity. IP therapy is not a new treatment approach, but it has not been widely accepted as the gold standard for women with ovarian cancer. There has been a prejudice against IP therapy in ovarian cancer because it's an old idea, it requires skill and experience for the surgery and for the chemotherapy, and it's more complicated than IV chemotherapy. But now there are firm data showing that a combination of IP and IV chemotherapy should be used in most women with advanced ovarian cancer who have had successful surgery to remove the bulk of their tumor.

Molecular Differences Identified between Low-Grade and High-Grade Ovarian Cancers. A new study led By Dr. Michael Birrer, Cell and Cancer Biology Branch, Center for Cancer Research, in collaboration with scientists from the Dana-Farber Cancer Institute and the M.D. Anderson Cancer Center, suggests that ovarian tumors classified as serous borderline or low malignant potential (LMP) are not early precursors in the development of aggressive ovarian cancer, but may instead be part of an entirely different class of tumors. Using a gene expression technique that reveals which genes are turned on or off in a cell, the researchers identified distinct differences between the gene expression profiles of LMP tumors and high-grade ovarian malignancies. The gene expression results suggested that serous low-grade ovarian tumors are more similar to LMP tumors than to serous high-grade ovarian cancers, and that different biochemical pathways may be involved in the development of LMP and low-grade tumors compared to high-grade tumors. Patients with serous low-grade or high-grade ovarian tumors currently receive the same treatment. However, women with low-grade invasive tumors may benefit from therapies that are different from those given to patients with high-grade tumors. The biochemical pathways identified in this study may provide targets for more rational therapies for these different tumor types. Bonome T, Lee J, Park D, Radonovich M, Pisemasison C, Gardner G, Hao K, Wong W, Barrett C, Lu K, Sood A, Gershenson D, Mok S, Birrer M. Expression profiling of serous low malignant potential, low-grade, and high-grade tumors of the ovary. *Cancer Research* November 15, 2005; Vol. 65, Issue 22.

Proteomic Evaluation of Epithelial Ovarian Cancer to Uncover New Biomarkers. Advanced-stage ovarian cancer has a high likelihood of returning within three years of initial treatment even when there are no signs of cancer being present. Current tests, such as CA-125 and transvaginal ultrasound, are not able to predict reliably whether cancer will return. New biomarkers, such as those found in blood, are urgently needed. Dr. Elise Kohn, Laboratory of Pathology, Center for Cancer Research, is directing a multi-institutional study designed to build a repository of blood samples in order to develop an accurate means of detecting ovarian cancer soon after the disease returns. The trial will enroll 400 women over 24 months. Researchers are

looking for women who have advanced-stage ovarian cancer, have completed their initial chemotherapy, and show no evidence of cancer following completion of their first treatment program. The women will have a physical exam and routine laboratory tests performed every three months and a CT scan of the abdomen and pelvis every six months. Research samples will be frozen to create a repository for analysis of blood proteins. The study will also compare the blood protein test that is developed with CA-125. A blood protein test is a blood test that measures the total amount of protein in blood serum as well as the amounts of two major groups of proteins, albumin and globulin (from http://www.bchealthguide.org/kbase/topic/medtest/hw43614/descrip.htm). Additional blood samples will be stored to create a repository so that other promising blood tests for ovarian cancer may be studied. The first site opening for this trial is at NCI's clinical facility on the NIH campus in Bethesda, Maryland. Ten other sites will begin enrolling patients later in 2005 or in 2006.

Microarray Data Suggest Distinct Profile for Clear Cell Cancers. Similar histological subtypes exist for both ovarian and endometrial cancer, including serous, endometrioid and clear cell cancer. While these subtypes share certain clinical characteristics, treatment for ovarian and endometrial cancers generally is determined by the organ of origin. In an effort to further define the molecular "signatures" of ovarian and endometrial cancer and to determine whether the presence of parallel subtypes reflects common pathogenic processes, Dr. Michael Birrer, Cancer and Cell Biology Branch, Center for Cancer Research, and collaborators at Memorial Sloan-Kettering Cancer Center compared the gene expression profiles of serous, endometrioid and clear cell cancers from both ovary and endometrium. They also analyzed renal clear cell cancer specimens. For serous and endometrioid tumors, gene expression profiles reflected the organ of origin. In contrast, clear cell tumors demonstrated remarkably similar expression profiles across organs, even when renal clear cell tumors were included in the comparison.

These results suggest that certain molecular events may be common to clear cell tumors regardless of where they originate. Present chemotherapy regimens for clear cell cancer are not very effective, and the clear cell subtype of both ovarian and endometrial cancer is associated with decreased survival relative to ovarian and endometrial cancer as a whole. If therapies can be designed based on the specific set of genes that is differentially expressed in clear cell cancers, then treatment choices for at least this small subset of ovarian and endometrial cancers may be driven by histological subtype rather than organ of origin. Zorn, K.K., Bonome, T., Gangi, L., Chandramouli, G.V.R., Awtrey, C.S., Gardner, G.J., Barrett, J.C., Boyd, J. Birrer, M.J. "Gene Expression Profiles of Serous, Endometrioid, and Clear Cell Subtypes of Ovarian and Endometrial Cancer." *Clinical Cancer Research*; Vol. 11, 6422-6430, Sept. 15, 2005.

Role of the Tubal Fimbria in the Pathogenesis of Pelvic Serous Carcinoma**.**

**Background**: Fundamental to ovarian cancer prevention is a clear understanding of the mechanisms by which ovarian cancer develops. Traditionally, it has been thought that ovarian cancer arises from the surface of the ovary or from the epithelial lining of cysts that may be remnants of ovulation. Essentially two forms of cancer were believed to come from these cells, with one form developing within the ovary and often confined to it when diagnosed, and another

more lethal form, found very early to be on the surface of the ovary. Because of its location on the surface of the ovary, the latter tumor is much more likely to spread to peritoneal surfaces. Studies of women with hereditary "ovarian" cancer have recently suggested that this second and more aggressive tumor type may come from the fallopian tube, as evidenced by the finding of very early carcinomas arising in tubes removed prophylactically before the development of a cancer involving the ovary. On the premise that the end of the fallopian tube closest to the ovary (the fimbriated end) is the most susceptible site, the investigators devised a protocol for sectioning and extensively examining the fimbriated end of the tube (the SEE-FIM protocol). Using this approach, they sought to determine how frequently this part of the tube gave rise to early cancers in high-risk women, as well as to determine how often it was involved in well-developed ovarian cancers in non-high risk women.

**Advance**: Three preliminary findings support further research on the role of the tubal fimbria in ovarian cancer development. The first is the finding of fimbrial involvement in 4 of 5 cases of early cancer in women with mutations in the BRCA (ovarian cancer susceptibility) gene. The second is the identification of possible early "cancer precursor" changes in the epithelium of the tube, which are more commonly identified in the fimbriated end. The third is the finding that, in well-developed ovarian cancers, the lining of the tubal fimbria is frequently involved, raising the possibility that the "ovarian" tumor actually arose from this site and subsequently spread to the ovarian surface.

**Implications**: If the more lethal "ovarian" carcinomas are actually arising from the distal end of the fallopian tube, the implications for ovarian cancer prevention are substantial for two reasons. First, a specific location with a defined epithelial surface could be a focus of attention for ovarian cancer prevention efforts. A combination of histological and molecular studies would permit the characterization of the earliest changes that precede and mediate the development of cancer in the tubal epithelium and could lead to new means of prevention or early detection. Secondly, it is conceivable that current approaches to preventing ovarian cancer in susceptible women would be revised by more selective removal of susceptible tissue. Whether the ovary could be spared is unknown, but more detailed examination of the fimbriated end in prophylactic salpingo-oophorectomy specimens will make it possible to determine the relative risk of tubal versus ovarian involvement in the early stages of pelvic cancer. (Dana-Farber/Harvard Cancer Center Ovarian Cancer SPORE; 1-P50 CA105009; Principal Investigator: Daniel Cramer.)

**Citations**:

Medeiros F, Muto M, Lee Y, Elvin J, Callahan M, Feltmate C, Garber J, Cramer D, Crum, CP. The Tubal Fimbria is a Preferred Site for Early Adenocarcinoma in Women with Familial Ovarian Cancer syndrome. *Am J Surg Pathol*, in press, Fall, 2005

Lee Y, Medeiros F, Muto M, Cramer D, Crum CP. P53 immunostaining identifies a putative "early" intraepithelial lesion in the tubal mucosa. *Abstract, submitted to the USCAP meeting (February 2006)*

Medeiros F, Elvin JA, Lee Y, Crum CP. A protocol for sectioning and extensively examining the fimbriated end of the fallopian tube (SEE-FIM): implications for the detection of early pelvic serous carcinoma. *Abstract, submitted to the USCAP meeting (February 2006)*

Kindelberger D, Lee Y, Muto M, Cramer D, Crum CP. Frequency of mucosal involvement of the fimbria in sporadic ovarian carcinoma. *Abstract, submitted to the USCAP meeting (February 2006)*

Collaborators:
Christopher P. Crum, MD (PI of this project)
Yonghee Lee, MD
Judy Garber, MD
Michale Muto, MD
Daniel Cramer, MD
Alexander Miron, PhD
Samuel Mok, PhD

*Pancreatic cancer*

Pancreatic cancer is the fourth leading cause of cancer death in the United States. Patients with pancreatic cancer are usually diagnosed with advanced disease because this type of cancer often spreads before symptoms develop. Current treatments may extend survival slightly or relieve symptoms in some patients, but they rarely produce a cure. Researchers in an NCI-sponsored trial are adding a biological agent called bevacizumab (Avastin®) to standard chemotherapy with the drug gemcitabine to see if the combination can help improve the survival of pancreatic cancer patients whose disease has spread to nearby lymph nodes (locally advanced) or to other sites in the body (metastatic). Bevacizumab is a monoclonal antibody that blocks the action of a protein called vascular endothelial growth factor (VEGF). VEGF stimulates the growth of new blood vessels (angiogenesis), which tumors need to survive, and it may also act as a growth factor for pancreatic cancer cells, stimulating them to multiply. Researchers hope they can cause pancreatic tumors to shrink or die by blocking VEGF activity. In a phase II study conducted with this combination, the observed a time to progression and survival was far better than expected. This randomized trial seeks to confirm these observations, and laboratory studies being performed should help reveal more about the biology of pancreatic cancer.
Smokeless Tobacco Causes Oral and Pancreatic Cancer, Nitrosamines Classified as Human Carcinogens. An International Agency for Research on Cancer (IARC) monograph reports that smokeless tobacco, including snuff and chewing tobacco, causes oral and pancreatic cancer in humans. In addition, two tobacco-specific N-nitrosamines (TSNA), N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3pyridyl)-1-butanone (NNK) were classified as human carcinogens. The chemicals occur in all smokeless tobacco products and are formed during the curing and processing of tobacco and during storage of manufactured smokeless tobacco products. Many studies in animals have shown that different routes of exposure to NNN and NNK cause benign and malignant tumors. Results of epidemiological studies of smokeless tobacco users and studies of the mechanisms of action of TSNA plausibly associate NNN and NNK with cancer in humans. The monograph's working group reaffirmed that the use of

smokeless tobacco causes oral cancer in humans, and concluded that it causes pancreatic cancer as well. These findings reinforce that tobacco use in not safe in any form. Cogliano,V., Streif, K., Bann, R., Grosse,Y., Secretan, B., and Ghissassi, F.E. (2004). Smokeless tobacco and related nitrosamines. *The Lancet Oncology, 5, 708.*

*Pituitary tumors*

Mutant Thyroid Hormone Receptor Contributes to Certain Pituitary Tumors. Thyroid-stimulating hormone (TSH) tumors typically go undiagnosed until relatively late in the natural course of the disease, and surgery to remove them is curative in less than half of patients. While the molecular genetics underlying this disease remain unclear, recent findings that several patients with TSH tumors harbor mutations in TRß gene, one of the two thyroid hormone receptor genes, raise the possibility that such mutations play a role in the development of TSH tumors. Dr. Sheue-yann Cheng, Laboratory of Molecular Biology, Center for Cancer Research ,and scientists from the National Human Genome Research Institute, NIH, used a genetically engineered mouse harboring a TRß mutation to examine the role of TRß mutations in the development of TSH tumors. They compared tumor development and gene expression profiles among the TRß-mutant mice, their wild-type littermates, and mice lacking all thyroid hormone receptors. Both the TRß-mutant and the thyroid-hormone-receptor-deficient mice had extraordinarily high serum concentrations of TSH despite highly elevated thyroid hormone levels, revealing a severe dysfunction of the normal negative feedback regulation of TSH by thyroid hormone. Gene expression profile comparisons revealed that the thyroid receptor mutation, but not the thyroid hormone receptor deficiency, led to overexpression of the oncogene cyclin D1 mRNA and activated the cyclin D1/cyclin-dependent kinase/retinoblastoma protein/E2F pathway. This study provides the first direct evidence *in vivo* to support the oncogenic functions of TRß mutants. The results suggest that the loss of the negative feedback regulation of TSH alone is not sufficient to induce the development of TSH tumors and that mice with the TRß mutations are much more deleteriously affected than are mice lacking thyroid hormone receptors altogether. Finally, the study identifies cyclin D1 as one oncogene that mediates the tumorigenesis of TSH tumors, although there may be others. Furumoto, H., Ying, H., Chandramouli, G.V.R., Zhao, L., Walker, R.L., Meltzer, P.S., Willingham, M.C., and Cheng, S. "An Unliganded Thyroid Hormone ß Receptor Activates the Cyclin D1/Cyclin-Dependent Kinase/Retinoblastoma/E2F Pathway and Induces Pituitary Tumorigenesis." *Molecular and Cell Biology*; Vol. 25 (1); 124-135; January 2005.

*Retinoblastoma*

Follow-up of Retinoblastoma Survivors. Many children who develop retinoblastoma (Rb) survive into adulthood and are prone to subsequent cancers, particularly persons with germline *Rb-1* mutations. Follow-up of 1,601 Rb patients diagnosed during 1914–1984 at two U.S. medical centers was extended through the year 2000 to provide new information on the risk of cancers in long-term survivors. Subsequent cancer risk in hereditary patients exceeded the risk in nonhereditary Rb patients. Radiation further increased the risk of subsequent cancer in hereditary patients by 3-fold. Hereditary patients continued to be at increased risk for sarcomas, melanoma, and cancers of the brain and nasal cavities. The cumulative incidence for developing

a new cancer at 50 years after diagnosis of Rb, adjusted for competing risk of death, was 36percent for hereditary and 5.7percent for nonhereditary patients. Hereditary Rb predisposes to a variety of new cancers over time, with radiotherapy further enhancing the risk of tumors arising in the radiation field. *J Clin Oncol* 2005;23:2272-2279.

*Salivary gland cancer*

Fusion Oncogene Deregulates Cell-Signaling Pathway in Salivary Gland Cancer. Recently, scientists from Genetics Branch and Cancer Therapeutics Branch, Center for Cancer Research discovered that the most common type of malignant salivary gland tumor, mucoepidermoid carcinoma, can arise from a chromosomal translocation that fuses portions of the *Mect1/Torc1* gene on chromosome 19p and the *Mam12* gene on chromosome 11q. Tumor-specific expression of the *Mect1-Maml2* fusion gene has been detected in 75 percent of primary and derived mucoepidermoid tumor specimens. The biological role of the fusion gene product in the development of this salivary gland cancer is not yet understood.

The intact *Mect1/Torc1* gene has been shown to be a coactivator of a cell-signaling pathway initiated by cyclic AMP/cyclic AMP-responsive element binding proteins (CREB). In contrast, the normal *Maml2* gene has been linked to a different cell-signaling pathway, which is triggered by certain proteins known as NOTCH receptors. The study found that induction of the fusion oncogene in a variety of cell lineages strongly activated the expression of a group of known cAMP/CREB-regulated genes, but not NOTCH-regulated target genes. While overexpression of components of the cAMP pathway has been associated with some human cancers, the data from this study provide a direct genetic link between deregulation of cAMP/CREB pathways and epithelial tumorigenesis and suggest future therapeutic strategies for this group of salivary gland tumors. Coxon, A., Rozenblum, E., Park, Y., Joshi, N. Tsurutani, J., Dennis, P., Kirsch, I.R. and Kaye, F.J. "Mect1-Maml2 Fusion Oncogene Linked to the Aberrant Activation of Cyclic AMP/CREB Regulated Genes." *Cancer Research* 65, 7137-7144, August 15, 2005.

*Testicular cancer*

The Ter mutation and Testicular Cancer. *Background*: Testicular cancer usually strikes men between the ages of 15 and 39 and is the most common form of cancer in men between the ages of 20 and 34. Although it accounts for only about 1 percent of all male cancers, the rate of testicular cancer among white men has more than doubled in the past 40 years.

In mice, testicular germ cell tumors (TGCTs) originate from primordial germ cells (PGCs) and develop within the testis during fetal development. Ter is a single gene mutation that causes progressive loss of PGCs on all inbred strains of laboratory mice and dramatically increased susceptibility to spontaneous TGCTs only in the 129 strain. This suggests strong genetic component and identifies the 129 strain as an ideal model system to dissect TGCT pathogenesis.

*Advance*: A group of investigators, led by Dr. Angabin Matin at M.D. Anderson Cancer Center have recently reported the positional cloning of Ter, revealing a point mutation that introduces a termination codon in the mouse dead end gene (*dnd1*). They showed that inactivation of *dnd1,*

causes the Ter phenotype in the 129 strain and that the Ter phenotype could be rescued by genomic fragments that included *dead end*. Testicular tumors and germ cell deficient testes from *Ter/Ter* mice show loss of expression of *Ter* encoded protein. Dnd1 is the first gene to be directly linked to heritable testicular cancer. The gene is expressed in fetal gonads during the critical period when TGCT originate. Analysis of the protein encoded by dead end indicates it is homologous to factors involved in RNA binding and editing, suggesting that Ter may disrupt essential aspects of RNA biology during PGC development.

*Implications*: TGCT development in the 129-Ter inbred mouse strain models testicular cancer in humans. The identification of Ter as dead end and the elucidation of its function in PGCs will help clarify the role of nucleic acid editing in PGC biology and have important implications for our understanding of the development of testicular cancer.

**Citations:**

1. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. Kirsten K. Youngren, Douglas Coveney, Xiaoning Peng, Chitralekha Bhattacharya, Laura S. Schmidt, Michael L. Nickerson, Bruce T. Lamb, Jian Min Deng, Richard R. Behringer, Blanche Capel, Joseph H. Nadeau and Angabin Matin. 2005 *Nature*: 435:360-364.

2. Search for Testicular Cancer Gene Hits Dead-End. Matin, A. Nadeau, J. H. 2005 *Cell Cycle* 4:1136-1138.

*Waldenstrom's macroglobulinemia*

Despite recent identification of a recurrent chromosome 6q21 deletion in sporadic Waldenstrom's Macroglobulinemia (WM), uncovering the molecular pathogenesis of WM remains challenging. In contrast to the growing body of cytogenetic studies in sporadic WM, there have been virtually no informative studies of familial WM. In this study, conventional and molecular cytogenetic evaluation was conducted in 18 patients with familial WM and 3 patients with immunoglobulin (Ig) M monoclonal gammopathy (IgM-MG) from 15 families to determine the nature and extent of chromosomal abnormalities associated with familial WM. The frequency and distribution of chromosomal changes in familial WM resembled those in sporadic WM, including lack of IgH rearrangements and t(9;14); however, the evaluation detected del6q21 in only 1 patient. Occasional findings appeared to be novel; however, none were recurrent, and their significance remains unclear. Only one abnormality found in bone marrow specimens was detected in parallel peripheral blood lymphocyte studies, suggesting that most abnormalities represented somatic changes. The results suggest that further progress in delineating the genetic determinants of WM susceptibility might be gained from alternative approaches such as candidate gene or linkage analysis. *Clin Lymphoma* 2005;5:230-234.

*Other*

Modifying Immune System Response to Cancer Chemotherapy Could Lead to New Treatment Approaches. Researchers at the Pediatric Oncology Branch, Center for Cancer Research, have

discovered a mechanism by which cancer patients' immune systems respond to chemotherapy. The new finding changes the current understanding of how the immune system responds to chemotherapy and could lead to opportunities for new treatments based on enhancing the body's immune response to the disease. Chemotherapy for cancer severely depletes the number of immune system T-cells, creating a condition known as lymphopenia. Paradoxically, lymphopenia leads to increased immune system response. This new study indicates that even though chemotherapy depletes T-cells, it does not selectively destroy suppressor or regulatory T-cells, as previously assumed. Instead, the study showed that the lymphopenia caused by chemotherapy actually provided a good environment for proliferation of suppressor T-cells that are believed to contribute to the ability of tumors to evade the body's immune system. The mechanism by which this occurs is not entirely clear, but could involve interleukin-2 (IL-2), a cytokine which was not previously associated with suppressing immune responses. Researchers examined immune recovery in 26 young cancer patients with pediatric sarcomas who received cyclophosphamide-based chemotherapy, which depleted lymphocytes. The patients were then infused with their own frozen lymphocytes, which had been stored before chemotherapy had begun. The impact of this treatment on the patients' immune recovery was examined, with or without recombinant IL-2, an agent that has been considered capable of restoring an immune system weakened by chemotherapy. The patients who received IL-2 showed a marked increase in suppressor T-cells after chemotherapy. The study has important implications for developing future immunotherapies against cancer and manipulating suppressor cells to make the immune system more effective in responding to cancer. Zhang H, Chua KS, Guimond M, Kapoor V, Brown MV, Fleisher TA, Long LM, Bernstein D, Hill BJ, Douek DC, Berzofsky JA, Carter CS, Read EJ, Helman LJ, Mackall CL. Lymphopenia and interleukin-2 therapy alter homeostasis of CD4+CD25+ regulatory T cells. *Nature Medicine*, Vol. 11, No. 11.

*Sleeping Beauty* Plays a Significant Role in Identifying Cancer Genes. Drs. Nancy Jenkins and Neil Copeland, of the Mouse Cancer Genetics Program, Center for Cancer Research, along with researchers at the University of Minnesota Cancer Center have discovered a new method that could accelerate the way cancer-causing genes are found. The gene identification method was developed in genetically modified mice and utilized a piece of jumping DNA, called *Sleeping Beauty*. Jumping genes, or transposons, insert themselves into or between genes and can activate or inactivate a gene's normal function. Related transposons are natural to the genetic makeup of humans, animals and fish, but, through millions of years of evolution, most transposons became inactive dead-ends. In this study, specially designed *Sleeping Beauty* transposons were introduced into mouse DNA and made to jump around in the nucleus of mouse cells. Eventually the transposons jumped into cancer-causing genes and caused a tumor to form. By isolating and studying the genes from tumors that contained *Sleeping Beauty*, researchers were able to efficiently find genes linked to cancer by seeing whether *Sleeping Beauty* turned them on or off—in effect, uncovering the fingerprint of each tumor's cancer genes. Although the discovery was made in laboratory mice, the technology will reveal new insights into human cancer that could be translated for clinical use. Cancer genes and their pathways associated with tumorigenesis can be rapidly identified, providing insight into human cancer through the use of mouse models. Jenkins and Copeland plan to generate and analyze a large number of tumors induced in mice using *Sleeping Beauty*, studying genes for tumors in the brain, melanoma, breast, leukemia and lymphoma. Dupuy A., Akagi K., Largaespada D., Copeland N., Jenkins N.

"Mammalian mutagenesis using a highly mobile somatic *Sleeping Beauty* transposon system," *Nature*, Vol. 436, No. 7047.

NCI Creates Gene Expression Database of Normal Human Organ Tissue. Dr. Javed Khan and colleagues from Pediatric Oncology Branch, Center for Cancer Research, have developed a genome-wide database of mRNA expression across a large number of human organs. They have shown that the data are internally consistent and the global pattern of gene expression reflects the function of the organs. The expression profiles for 18,927 genes, which include most of the genes that are known to help direct basic activities of the human body, are open to the public at http://home.ccr.cancer.gov/oncology/oncogenomics/. This database allows investigators to compare the gene expression results for their own tissue or genes of interest to a baseline standard that represents a generic picture of normal gene activity, organ by organ, in the human body. Genes identified by the database as abnormally active in a particular disease could become potential targets, guiding researchers to better candidates for new drug therapies, immune-based vaccine treatments, and potential biomarkers to help with diagnosis. To illustrate the kind of useful data that can emerge from using this tool, Khan's team analyzed 100 samples of the most common pediatric solid tumor, neuroblastoma (NB). Even though the tumor samples were taken from a variety of patients with different stages of cancer, the database identified a list of 19 genes that were consistently overexpressed compared to normal brain tissue. All of these genes are involved with activities associated with the development of cancer - apoptosis, growth, proliferation and transcription. These results provide scientists studying and treating NB with a focused set of genes to explore. Son, C.G., Bilke, S., Davis, S., Greer, B.T., Wei, J.S., Whiteford, C.C., Chen, Q.-R., Cenacchi, N., Khan, J. 2005. Database of mRNA gene expression profiles of multiple human organs. *Genome Research* 15: 443-450. (http://www.genome.org/)

Causes of Infertility as Predictors of Subsequent Cancer Risk. Although studies have found elevated risks of certain cancers linked to infertility, the underlying reasons remain unclear. In a retrospective cohort study of 12,193 U.S. women evaluated for infertility between 1965 and 1988, 581 cases of cancer were identified through 1999. Infertility patients demonstrated a higher cancer risk than the general population, with nulligravid (primary infertility) patients at even higher risk. Particularly elevated risks among primary infertility patients were observed for cancers of the uterus and ovaries. Analyses within the cohort revealed increased relative risks for colon, ovarian, and thyroid cancers and melanomas associated with endometriosis. Melanomas were also linked with anovulatory problems, whereas uterine cancers predominated among patients with tubal disorders and primary infertility. The effects of infertility may extend beyond gynecologic cancers. Thyroid cancers and melanomas deserve specific attention, particularly with respect to endometriosis. *Epidemiology* 2005;16:500-507.

Survivors of Childhood Soft Tissue Sarcoma. This study evaluated the risk of developing soft tissue sarcoma (STS) among 1,499 children (age < 18 years) who survived for at least one year after diagnosis and who were reported to the SEER population-based cancer registries from 1973 to 2000. Twenty-seven children developed 28 subsequent primary malignancies, compared with 4.5 expected cancers based on general population rates. The risk of developing a subsequent malignancy was increased among children with rhabdomyosarcoma, fibromatous neoplasms, and other STS. Initial therapy with radiation and chemotherapeutic agents was associated with a

significantly higher risk compared with surgery alone. Elevated risks were observed for acute myeloid leukemia, cutaneous melanoma, female breast cancer, and sarcomas of the bone and soft tissue, with generally higher risks among patients who initially received combined modality therapy. For several children, the pattern of multiple malignancies was consistent with a genetic syndrome, particularly neurofibromatosis type 1 and Li-Fraumeni syndrome. *Cancer* 2005;103:2391-2396.

Significant Ongoing Initiatives

*HPV Vaccine Trial*

NCI is conducting the HPV Vaccine Trial, a multi-year effort currently underway in Costa Rica, which is testing the ability of virus-like particle vaccines, originally developed at NCI, to protect against HPV-16/18 infection. The pivotal Phase III trial, which involves investigators from the Division of Cancer Epidemiology and Genetics, the Center for Cancer Research, and the Division of Cancer Prevention, is being carried out in close collaboration with Costa Rican investigators. It represents the culmination of several decades of work by NCI investigators to demonstrate that HPV is causally linked to the development of anogenital tumors and to develop prophylactic vaccines.

In this blinded study, either the experimental HPV vaccine or a hepatitis A vaccine is given to all healthy young women who enroll in the trial. By the end of December 2005, nearly 7,500 women participants were recruited, and all vaccinations will be completed by the late summer or early fall 2006. These women will be followed for the development of HPV infection and cervical lesions, which are treated according to state-of-the-art standard-of-care guidelines. Women in both arms of the study benefit from excellent cervical cancer screening throughout the trial and, at its end, cross-over vaccination and vaccination against hepatitis B will be offered to all participants.

It is anticipated that the results from the trial will support licensure of the GlaxoSmithKline-manufactured prophylactic vaccine as well as provide a wealth of information on the mechanisms of action of the vaccine and on the natural history of HPV infection and cervical neoplasia post-vaccination. The vaccine is likely to have its major impact on the prevention of cervical cancer in developing nations where medically underserved women are especially at risk for this devastating disease and in underserved areas within the U.S. The current vaccine targets HPV-16 and -18, which together account for about 60-70percent of cervical cancer worldwide. If a vaccine is 90percent effective against these HPV types, it will have the potential of reducing the incidence of cervical cancer by more than 60percent. An effect of this magnitude could translate to a reduction of about 150,000 deaths per year worldwide from cervical cancer.

*International Lymphoma Epidemiology Consortium*

NCI intramural and extramural investigators have joined forces in a coordinated series of ongoing case-control studies focused on non-Hodgkin lymphoma (NHL). The NHL collaboration, known as InterLymph, represents a new generation of large-scale molecular

epidemiology, with investigators pooling data from North America, Europe, and Australia to identify reasons for the increasing incidence of this tumor around the world. Each case-control study includes a detailed review of the pathological and genetic characteristics of the NHL cases. The investigators share data in order to test for genetic and environmental causes that cannot be addressed in individual studies with smaller sample sizes. Because the consortium involves essentially all major on-going epidemiologic studies of NHL, it represents a model for the study of many malignancies.

The first breakthrough finding from the consortium was the demonstration that the TNF-alpha gene plays a key role in diffuse large B-cell lymphoma, the most common form of the disease. A recent meta-analysis of 12 single nucleotide polymorphisms (SNPs) in genes that play a role in regulating the immune system revealed a statistically significant association between a gene variant of TNF-alpha and increased risk of developing diffuse large B-cell lymphoma. Individuals heterozygous for this SNP were at 30percent increased risk, while homozygous individuals were at 60percent increased risk of developing the disease.
*Lancet Oncol* 2006;7:27-38.

NCI continues to screen new synthetic and natural compounds for antitumor activity using the automated cancer cell line screen. Over 87,000 defined chemical structures have been evaluated since the screen became operational in April 1990. More than 9,200 compounds have demonstrated *in vitro* antitumor activity, of which more than 5,200 agents have been selected for *in vivo* evaluation for assessment of therapeutic activity.

Obviously, there are more compounds to test/develop than current resources would allow. The Drug Development Group (DDG) oversees the decision-making process regarding the development of new drugs and relies on extramural review of proposed activities. A complete description of this process is available on the Developmental Therapeutics Program Web site (dtp.nci.nih.gov).

Including vaccines and other biologicals, as well as chemotherapeutic agents, 4 agents are in DDG level 1B (early preclinical testing), 14 are in DDG level 2A (GMP production and late preclinical testing), 5 agents are in DDG level 2B (IND-directed toxicology), and 17 are in DDG level 3 (ready for human testing subject to obtaining an IND). Table 1 lists the agents in the DDG process. As the agents move through the different levels of the decision process, the level of NCI's financial commitment increases.

The NCI cooperates on the development of novel anticancer therapies with commercial as well as institutional entities ranging from fresh startups to the multinational biopharmaceutical firms. NCI currently holds 39 Cooperative Research and Development Agreements (CRADAs), 57 Clinical Trials Agreements (CTAs), 19 Clinical Supply Agreements (CSAs), and 399 Material Transfer Agreements (MTAs) with its collaborators. These are shown in Table 2.

Table 3 shows the number of investigational new anticancer agents in early clinical trials. NCI's Cancer Therapy Evaluation Program (CTEP) is sponsoring trials of many novel agents, from "first-in-man" through large, randomized comparisons of these new therapies with standard

treatments. With the emerging understanding of the importance of different signaling pathways in cancer, and the availability of therapeutic agents that target those pathways, CTEP has prioritized the exploration of rational combinations of these novel investigational agents. These agents include small molecule receptor tyrosine kinase inhibitors, signal transduction inhibitors, agents that target tubulin and the mitotic spindle, agents that modulate gene transcription, DNA-interactive agents, vaccines, monoclonal antibodies, antisense agents, and gene therapy. Increasingly, NCI's industry collaborators have recognized the importance of these combination studies, which may not have be carried out without NCI sponsorship.

To expedite the movement of academic discoveries from the laboratory to proof of principle clinical trials, NCI initiated the program Rapid Access to Intervention Development (RAID) in 1998. RAID makes resources available, on a competitive basis, to the academic research community that are necessary to convert a new molecule into a drug candidate suitable for clinical testing and are generally not available to academic investigators who lack a corporate partner. These resources include 1) GMP synthesis, formulation, range finding, and IND-directed toxicology and pharmacology; 2) clinical trial planning; and 3) regulatory assistance so that FDA requirements may be satisfied by any investigator who seeks to put a new molecule into the clinic. As of December 2005, 305 applications have been received, 108 of which were approved for NCI support. A description of the successful applicants and the projects can be found at dtp.nci.nih.gov/docs/raid/raid_index.html (HTTP://dtp.nci.nih.gov/docs/raid/raid_index.html). Table 4 lists current RAID projects pertaining to rare diseases.

**Table 1. Compounds That Passed Drug Development Group (as of December 2005)**

**Drug Development Group 1B**

**NSC Number**

735210 JPM free acid
726850 PIA5
726852 PIA23
624244 Discreet

**Drug Development Group 2A**

**NSC Number**

703939 RN321
680410 Adaphostin
711516 Chimeric antiamyloidosis MAb
724910 Discreet
721782 1-methyltryptophan
722134 Discreet
736512 Discreet
737186 Discreet

729280 Discreet
740281 Discreet
740282 Discreet
740283 Discreet
740480 Discreet
740481 Discreet
740482 Discreet

**Drug Development Group 2B**

**NSC Number**

281612 Dimethane sulfonate
729746 HA22
740377 $^{111}$In-CHX-A-Herceptin™
678515 FAU and $^{18}$F-FAU
678516 $^{18}$F-FMAU

**Drug Development Group 3**

**NSC Number**

716976 BNP7787
724770 VEGF-Trap
711193 CDDO
731636 SGN-30
737664 ABT-888
735464 AZD0530
737754 VEGFR 034
729968 Reolysin
732084 PCLUS 6.1-18MN and E1M184V HIV peptides
729280 GX-015-070
720735 PPI-2458 Fumagillin Analog
740102 2C4-pertuzumab
320846 Batracylin
740377 Herceptin Scan
678515 & 678516 19F-FAU & 18FMAU
741078 AZD6244
736511 Sutent (Sunitinib Malate, SU11248)

**Table 2: Active Research and Development Agreements (as of January 9, 2006)**

| Agent | Company | Type |
|---|---|---|
| 17-AAG (NSC#330507) | Kosan Biosciences Inc | CRADA |
| | | |
| 17-DMAG (NSC#707545) | Kosan Biosciences Inc | CRADA |
| 280-446 | Novartis Pharmaceuticals Corporation | CTA |
| 2-Methoxy Estradiol (NSC#659853) | Entremed Inc | CRADA |
| 506U78 (NSC#686673) | GlaxoSmithKline | CTA |
| ABT-888 (NSC#737664) | Abbott Laboratories | CTA |
| Adeno-p53 (Ad5CMV-p53); Advexin (NSC#683550) | Introgen Therapeutics Inc | CTA |
| AE-941 (Aeterna Shark Cartilage Extract) (NSC#706456) | Aeterna Laboratoires | CTA |
| Alemtuzumab (Campath) (NSC#715969) | Berlex Inc | CTA |
| ALL-TRANS RETINOIC ACID | F Hoffmann-La Roche Ltd | CTA |
| | | |
| ANTI-CTLA4 ANTIBODY | Medarex Inc | CTA |
| Arsenic trioxide (Trisenox) (NSC#706363) | Cephalon Inc | CTA |
| ARTEMISININ | Elsohly Laboratories, Inc. | M-CRADA |
| Azacitidine (NSC#102816) | Pharmion Corporation | CTA |
| AZD0530 | AstraZeneca Pharmaceuticals LP | CRADA |
| AZD2171 (NSC#732208) | AstraZeneca Pharmaceuticals LP | CRADA |
| AZD6244 | AstraZeneca Pharmaceuticals LP | CRADA |
| BAY 43-9006 tosylate (BAY 54-9085; sorafenib tosylate) (NSC#724772) | Bayer Corporation | CTA |
| Bevacizumab (rhuMAb VEGF) (NSC#704865) | Genentech Inc | CRADA |
| BMS 214662 (NSC#710086) | Bristol-Myers Squibb | CTA |
| BMS 275291 (MMPI) | Bristol-Myers Squibb | CTA |

| | | |
|---|---|---|
| (NSC#713763) | | |
| BMS-354825 (NSC#732517) | Bristol-Myers Squibb | CTA |
| BNP7787 (NSC#716976) | BioNumerik Pharmaceuticals Inc | CTA |
| BPU (Benzoylphenylurea) (NSC#639829) | Ishihara Sangyo Kaisha Ltd | CTA |
| Carboxypeptidase G2 (CAMR) (NSC#641273) | Protherics, Inc. | CTA |
| CC-5013 (lenalidomide, Revlimid) (NSC#703813) | Celgene Corporation | CTA |
| CCI-779 (rapamycin analog, temsirolimus) (NSC#683864) | Wyeth Pharmaceuticals | CRADA |
| CDDO (NSC#711193) | Reata Pharmaceuticals Inc | CRADA |
| Clodronate (Bonefos) (NSC#713466) | Schering OY | M-CRADA |
| | | |
| Cytochlor (NSC#371331); THU | Halogenetics Inc | CTA |
| Decitabine (5-aza-2'-deoxycytidine) (NSC#127716) | MGI Pharma Inc | CRADA |
| E7389 (Halichondrin B Analog) (NSC#707389) | Eisai Inc | CRADA |
| EMD 121974 (Cilengitide) (NSC#707544) | Merck KgaA | CRADA |
| Epothilone-B BMS 247550 (NSC#710428) | Bristol-Myers Squibb | CTA |
| Exemestane (Aromasin) (NSC#713563) | Pfizer Inc | M-CRADA |
| FK228 (Depsipeptide) (NSC#630176) | Gloucester Pharmaceuticals Inc | CRADA |
| Flavopiridol (alvocidib) (NSC#649890) | Sanofi Aventis | CRADA |
| Fumagillin analog (PPI-2458) (NSC#720735) | Praecis Pharmaceuticals | CTA |
| G3139 (oblimersen; Genasense) (NSC#683428) | Genta Inc | CRADA |
| GM-CSF | Berlex Inc | CTA |
| gp100 cDNA / gold (Plasmid Vector pWRG1644) (NSC#708477) | Powderject | CTA |
| GTI-2040 (NSC#722929) | Lorus Therapeutics Inc | CTA |
| GW572016 (lapatinib) (NSC#727989) | GlaxoSmithKline | CTA |

| | | |
|---|---|---|
| GW786034 (NSC#737754) | GlaxoSmithKline | CTA |
| GX015-070 (NSC#729280) | Geminx Biotechnologies | CTA |
| HALOFUGINONE I.V. | Collgard Biopharmaceuticals Ltd | CRADA |
| Hu 14.18 / IL-2 Fusion Protein (EMD 273063) (NSC#721298) | EMD Pharmaceuticals | M-CRADA |
| In2B8/Y2B8 Radiolabeling Kit (Ibritumomab tiuxetan, Zevalin) (NSC#710085) | Biogen Idec | CTA |
| IPI-609 | Infinity Pharmaceuticals | CRADA-LOI |
| Irinotecan (CPT-11, Camptosar) (NSC#616348) | Pfizer Inc | CTA |
| Irofulven (MGI-114) (NSC#683863) | MGI Pharma Inc | CTA |
| KRN5500 (NSC#650426) | Kirin Brewery | CTA |
| Lymphoma IG vaccine-KLH (NSC#659770) | BioVest International Inc | CRADA |
| Medi 522 (Vitaxin) (NSC#719850) | MedImmune Inc | CRADA |
| MLN 518 (NSC#726292) | Millennium Pharmaceuticals Inc | CRADA |
| Motexafin gadolinium (Xcytrin) (NSC#695238) | Pharmacyclics Inc | CRADA |
| Motexafin lutetium (NSC#695239) | Pharmacyclics Inc | CRADA |
| MS-275 (NSC#706995) | Schering AG | CRADA |
| O-6-Benzylguanine (NSC#637037) | AOI Pharmaceuticals | CRADA |
| OSI-774 (erlotinib; Tarceva) (NSC#718781) | OSI Pharmaceutical Inc | CTA |
| OXALIplatin (Eloxatin) (NSC#266046) | Sanofi Aventis | CRADA |
| Perifosine (NSC#639966) | AOI Pharmaceuticals | CRADA |
| | | |
| PROSTVAC-F/TRICOM [Recombinant Fowlpox-PSA(L155)/TRICOM] (NSC#717171); PROSTVAC-V/TRICOM [Recombinant Vaccinia-PSA(L155)/TRICOM] (NSC#717170) | Therion Biologics Corporation | CTA |
| Proteinase 3:PR1 Peptide (NSC#698102) | Vaccine Company | CTA |
| PS-341 (bortezomib; Velcade) (NSC#681239) | Millennium Pharmaceuticals Inc | CRADA |

| | | |
|---|---|---|
| PSC-833 (NSC#648265) | Novartis Pharmaceuticals Corporation | CTA |
| PXD 101 (NSC#726630) | TopoTarget | CRADA |
| PXD 101 (NSC#726630) | Curagen | CTA |
| R115777 (tipifarnib, Zarnestra) (NSC#702818) | Johnson & Johnson | CTA |
| Reolysin (NSC#729968) | Oncolytics Biotech Inc | CTA |
| rF-B7.1 (Recombinant Fowlpox-B7.1) (NSC#679008) | Therion Biologics Corporation | CRADA |
| RF-TRICOM; rF-TRICOM (Recombinant Fowlpox-TRICOM) (NSC#710658) | Therion Biologics Corporation | CRADA |
| Rituximab (MoAb C2B8 anti CD20, chimeric) (NSC#687451) | Biogen Idec | CRADA |
| SB-715992 (NSC#727990) | GlaxoSmithKline | CTA |
| SC-55494 | Searle | CTA |
| SGN-30 (NSC#731636) | Seattle Genetics | CTA |
| SJG-136 (NSC#694501) | Ipsen | CRADA |
| SMART 1D10 (HU1D10) | Protein Design Labs | CTA |
| STI571 (imatinib, Gleevec) (NSC#716051) | Novartis Pharmaceuticals Corporation | CRADA |
| Sunitinib malate (SU011248 L-malate; Sutent) (NSC#736511) | Pfizer Inc | CTA |
| Thalidomide (Thalomid) (NSC#66847) | Celgene Corporation | CTA |
| Tirapazamine (NSC#130181) | Sanofi Aventis | CTA |
| Topotecan (NSC#609699) | GlaxoSmithKline | CTA |
| Trastuzumab (Herceptin) (NSC#688097) | Genentech Inc | CRADA |
| Triapine (NSC#663249) | Vion Pharmaceuticals Inc | CTA |
| Tumor Necrosis Factor (TNF-alpha) (Boehringer Ingelheim / RandD Systems) (NSC#697068) | Boehringer Ingelheim Pharmaceuticals Inc | CTA |
| UCN-01 (NSC#638850) | Kyowa Pharmaceuticals Inc | CTA |
| VEGF-Trap (NSC#724770) | Sanofi Aventis | CTA |
| Vorinostat (suberoylanilide hydroxamic acid; SAHA) (NSC#701852) | Merck and Company Inc | CTA |
| XK469 | Bristol-Myers Squibb | CTA |

| | | |
|---|---|---|
| XL119 (becatecarin, rebeccamycin analog) (NSC#655649) | Exelixis Inc | CTA |
| ZD1839 (gefitinib, Iressa) (NSC#715055) | AstraZeneca Pharmaceuticals LP | CTA |

**Table 3: Investigational New Anticancer Agents in Early Clinical Trials (as of January 2006)**

| Phase I | Phase II |
|---|---|
| **Biologic Agents** | |
| Adenovirus p53 (Advexin) | Adenovirus p53 (Advexin) |
| Anti-idiotype-KLH Myeloma Vaccine | Anti-idiotype-KLH Myeloma Vaccine |
| Antisense GTI-2040 | Antisense GTI-2040 |
| Apolizumab + Rituxan | Avastin® (bevacizumab, MoAb: anti-VEGF) |
| Avastin™ (bevacizumab, MoAb: anti-VEGF) | Avastin® PAP-pulsed Dendritic Cells |
| G3139 | Avastin® + Erbitux™ |
| Gp100-reactive autologous cells + rFgp100P209 | FGF-5 peptides |
| Herceptin® (trastuzumab; MoAb: humanized Her2) | G3139 |
| IL-12 | gp100 Peptides A,C,F Vaccine |
| IL-12 + IL-2 | Gp100-reactive autologous cells + rFgp100P209 |
| LBM 2 Immunotoxin | Gp100 retroviral vector-transduced CD8 cells + IL-2 |
| MART-1 retroviral vector-transduced cells + IL-2 + MART-1 peptide | Herceptin® (trastuzumab; MoAb: humanized Her2) |
| MDX-010 (Human Anti-CTLA4 MoAb) | IL-12 + IL-2 |
| MEDI-522 | LMB-2 Immunotoxin |
| MoAb: CAMPATH-1H (Anti-CD52) | MDX-010 (Human Anti-CTLA4 mAb) |
| MoAb: HeFi-1 (Anti-CD30) | MoAb: CAMPATH-1H (Anti-CD52) |
| MS275 | Mutated VHL Peptides Vaccine |
| PANVAC-VF® | PANVAC-VF® |
| PR-1 Peptide Vaccine | PR-1 Peptide Vaccine |
| PSA Tricom Vaccine | PSA Tricom Vaccine |
| ras/p53 Vaccine | ras/p53 Vaccine |
| Recombinant Fowlpox GM-CSF Vaccine | Recombinant Fowlpox- CEA(6D)/TRICOM + Vaccinia- CEA(6D)/TRICOM Vaccine |
| Recombinant Fowlpox- CEA(6D)/TRICOM + Vaccinia- CEA(6D)/TRICOM Vaccine | Recombinant Fowlpox-gp100:ES209-217(210M) Vaccine |
| Recombinant Fowlpox-TRICOM and Vaccinia-TRICOM Vaccine | Recombinant Fowlpox-PSA Vaccine |
| Rituxan® (rituximab, MoAb: IDEC- | Recombinant Fowlpox-TRICOM and Vaccinia- |

| | |
|---|---|
| C2B8)/Chemotherapy | TRICOM Vaccine |
| SAHA | Rituxan® (rituximab, MoAb: IDEC-C2B8)/Chemotherapy |
| SB-IL2 + autologous T cells | SAHA |
| | SB-IL2 + autologous T cells |
| Sodium Phenylbutyrate (IV) | SGN-00101 (HspE7) Vaccine |
| SS1(dsFv) PE38 | Thalidomide |
| Zevalin®(MoAb: Y2B8) | Zevalin®(MoAb: Y2B8) |

**Chemotherapeutic Agents**

| Phase I | Phase II |
|---|---|
| 17-AAG | 17-AAG |
| 17-DMAG | Arsenic Trioxide |
| 2-ME | AZD2171 |
| Arsenic Trioxide | BAY 43-9006 |
| BAY 43-9006 | BMS 247550 (Epothilone B Analog) |
| BMS 247550 (Epothilone B Analog)) | Bryostatin 1 |
| BPU | CAI |
| Bryostatin 1 | CCI-779 (Rapamycin Analog) |
| CAI | COL-3 |
| Camptosar® (Irinotecan, CPT-11) | Compound 506U78 |
| CCI-779 (Rapamycin Analog) | Decitabine |
| Cytochlor + Tetrahydrouridine | Depsipeptide |
| Decitabine | EF5 |
| Depsipeptide | EMD 121974 |
| E7389 (Halichondrin B Analog) | Fenretinide |
| EMD 121974 | Flavopiridol |
| Flavopiridol | Gleevec® (Imatinib Mesylate, STI571) |
| Gleevec® (Imatinib Mesylate, STI571) | GW572016 |
| GW572016 | Halofuginone (Topical) |
| Iressa (ZD1839) | Iressa (ZD1839) |
| $O^6$-BG | Irofulven (MGI-114) |
| OSI-774 | $O^6$-BG |
| Oxaliplatin | OSI-774 |
| Pyrazoloacridine | Oxaliplatin |
| R115777 | Perifosine |
| Rebeccamycin Analog | R115777 |
| SB-715992 | Rebeccamycin Analog |
| SJG-136 | SB-715992 |
| Suramin | Suramin |

| | |
|---|---|
| Tirapazamine | Tirapazamine |
| Triapine® | Topotecan (Hycamtin®) |
| UCN-01 | Triapine |
| Velcade™ (Bortezomib, PS341) | UCN-01 |
| XK469 | Velcade® (Bortezomib, PS341 |

**Table 4. Current RAID Projects for the Treatment of Rare Diseases (as of December 2005)**

| Compound NSC | Name | Disease | Investigator | Pediatric Use |
|---|---|---|---|---|
| 710292 | Lipopeptide | Cytomegalovirus | Dr. Don Diamond; City of Hope Medical Center | Yes |
| 715816 | Tropism-Modified Adenoviral Vector | Ovary | Dr. Glenn Peters; University of Alabama, Comprehensive Cancer Center | |
| 718877 | Psuedomonas Exotoxin Construct | Glioblastoma Multiforme, Neoplastic Meningitis | Dr. Darrell Bigner; Duke University Comprehensive Cancer Center | Yes |
| 719277 | Nonpathogenic Oncolytic Poliovirus Chimeras | Glioma | Dr. Matthias Gromeier; Duke University Medical Center | Yes |
| 720836 | IL-6 plus Interferon | Multiple Myeloma | Dr. Richard Jones; Johns Hopkins University | |
| 723253 | Allogeneic multiple myeloma vaccine | Multiple myeloma | Ivan Borrello; Johns Hopkins University | |
| 723256 | d-24-RGD oncolytic virus | Chronic lymphocytic leukemia | Alfred Yung; University of Texas, M.D. Anderson Cancer Center | |
| 725178 | Targeted CRAd | Pancreatic adenocarcinoma | Selwyn Vickers; University of Alabama | |
| 726189 | SHetA2 | Ovary | Doris Benbrook; University of Oklahoma | |
| 723254 | hsp70-targeted E7 recombinant DNA vaccine | Cervical | Connie Trimble; Johns Hopkins University | |
| 731413 | Ad5/3-delta24 | Ovary | Akseli Hemminki; Helsinki University Hospital | |
| 731414 | MV-NIS virus | Refractory multiple myeloma | Stephen Russell; Mayo Clinic | |
| 731442 | KSR antisense | Pancreatic | Richard | |

## Cohort and Case-Control Consortia

Among NCI's top priorities is to understand how genes that make individuals susceptible to cancer are influenced by environmental factors such as chemicals, diet, and pharmacologic agents. DCCPS and the Division of Cancer Epidemiology and Genetics (DCEG) are collaborating to facilitate the development of consortia of cohort and case-control studies in order to accelerate research on gene-gene and gene-environment interactions in the etiology of cancer. The creation of such consortia is part of the revolutionary shift to big science, where studies of the future will be conducted on a much larger scale by multidisciplinary teams of scientists who pool their resources.

NCI is also fostering development of case-control consortia. Investigators may come together informally at first to discuss shared interests, for example, as has already occurred for brain tumors. In time, a formal structure may evolve, as with the International Consortium of Investigators Working on Non-Hodgkin's Lymphoma Epidemiologic Studies (InterLymph Consortium). Another advantage of consortia arrangements is the potential for advancing study of less common cancers and highly lethal cancers, for which it is difficult, if not impossible, for individual investigators to recruit sufficient study participants.

*Breast/Ovarian Cancer Family Registries*

The Breast/Ovarian and Colon Cancer Family Registries (CFR) studies support research to identify genetic changes that predispose to breast, ovarian, and colon cancers, and to explore gene-gene and gene-environment interactions that may contribute to the development of cancer among families with these cancers. These registries provide the tools and resources needed to clarify gene-environment interactions in cancer risk. They have identified thousands of families at high risk for breast, ovarian, and colorectal cancers who have agreed to be part of this research. Of particular interest are potential collaborations aimed at identification and characterization of cancer susceptibility genes; definition of gene-gene and gene-environment interaction in cancer etiology; and cooperative research on the translational, preventive, and behavioral aspects of such findings. The outcome will be a clearer understanding of the genes that affect the development of cancer, and how environmental factors may modify these genes.

*Trans-NCI R21 Pancreatic Cancer Program Announcement*

Pancreatic cancer is a highly lethal disease marked by pain, anorexia, sleep problems and weight loss. It has the worst prognosis among all of the major malignancies. Despite efforts over the past century, conventional treatment approaches such as chemotherapy, radiation surgery, or combinations of these modalities have had little impact on the course of this disease. It is clear that a better understanding of the molecular biology and biochemistry of pancreatic cancer is urgently needed to effectively diagnose, prevent, and treat this malignancy. Estimates by the American Cancer Society (ACS) indicate 31,860 new cases and 31,270 deaths from pancreatic cancer for 2004. The Trans-NCI Pancreatic Cancer program announcement would support a

variety of research areas across multiple disciplines. Brief descriptions of extramural research across the cancer continuum identified by NCI division are provided here.

- Cancer Biology - NCI is interested in expanding research in the biology of pancreatic cancer. Examples of appropriate research areas include, but are not limited to, how variations in cells combine with the microenvironment in the development of pancreatic cancer, development of experimental models for human pancreatic cancer, and exploration of molecular pathways important in cancer biology, particularly those that could lead to novel targets for therapeutic development.
- Clinical Chemoprevention Trials - NCI is interested in smaller phase II trial involving persons at risk for familial pancreatic cancer or patients with pancreatic intraepithelial neoplasia (PANIN) lesions. Such cohorts could be treated with a COXIB, FTI, statin, etc, and then be followed with endoscopic ultrasound. These smaller exploratory clinical trials could be complemented by additional epidemiological or preclinical studies, designed to identify candidate surrogate biomarkers and/or candidate chemopreventive or dietary compounds.
- Candidate Biomarkers - Biomarkers are needed to assess: 1) pancreatic cancer risk, i.e., factors modifying pancreatic etiologic events and/or exposures, 2) response modification, i.e., response to a putative chemopreventive drug or dietary factor, and 3) pancreatic cancer advancement or stage, i.e., ability to predict pancreatic cancer outcome.
- Cancer Control and Population Sciences - Identify genetic combinations that lead to pancreatic cancer, identify 'new' environmental exposures that contribute to pancreatic cancer, determine the relationship between inflammation and pancreatic cancer, develop a biofluid-based test for pancreatic cancer, and determine what combination of 'two hits'—genetic and/ or environmental—are needed for pancreatic cancer to develop.
- Cancer Treatment and Diagnosis - The Clinical Grants and Contracts Branch as well as the clinical trials sponsored by the Investigational Drug Branch and the Clinical Investigations Branch at CTEP would benefit greatly from grant support specifically directed toward: Development of early-stage clinical trials in pancreatic cancer, translational research associated with early-stage clinical trials in pancreatic cancer, imaging studies associated with clinical trials in pancreatic cancer, exploratory studies to identify and evaluate biomarkers (with associated assay development) to determine prognosis and predict response to therapy in pancreatic cancer, and Correlative studies using specimens from multi-institutional prevention and treatment trials.

**New/Planned Extramural or Intramural Research Initiatives**

*Coordinated Consortium Approach to the Molecular Epidemiology of Pancreatic Cancer*

DCCPS and DCEG are developing a concept for a Coordinated Consortium Approach to the Molecular Epidemiology of Pancreatic Cancer. The proposal will be to focus on pancreatic cancer via two-pronged approach. One approach is for a population-based consortia nested within very large cohorts that have accrued enough person-years of follow up to allow in-depth study in a manner that allows assessment of the temporality of observed associations. The other approach is for a hospital-based consortia nested within cancer centers that specialize in the treatment of pancreatic cancer cases. The key is that by studying gene-environment interactions

in the etiology of a complex, poorly understood tumor will require very large numbers of patients – more than can be accrued by one study acting independently. Having two consortia working in parallel will allow comparisons of findings across the two consortia, and hasten replicability, important for both positive and negative findings.

*Epidemiology Leadership Workshop on Rare Cancers to be Held in 2006*

This workshop is in response to a recommendation made by the rare cancers working group that took place during the 1st NCI Epidemiology Leadership Workshop last September, 2004 in Chicago, IL. The rare cancers working group focused on rare adult tumors, those with incidence rates of less than 15/100,000. Rare adult cancers include cancer of the adrenal gland, thyroid, bone, eye, testes, esophagus, kidney, stomach, blood, pancreas, head and neck. They account for 27percent of cancers diagnosed each year and 25percent of all cancer related deaths. Although these percentages seem small, the morbidity and mortality caused by these cancers is rising each year. Significantly, some of these cancers are more prevalent among specific ethnic populations, for example nasopharyngeal carcinoma among Chinese people and male breast cancer among Zambian men. The mortality rate for all rare adult cancers combined is 185/100,000. This is in stark contrast to the mortality rate of cervical cancer, 3.1/100,000. However, in 1950, the mortality rate of cervical cancer was 8.2/100,000. Due to significant research on this rare cancer, an etiologic agent was identified and screening programs were developed. These advances resulted in the 60 percent drop in mortality over the last 50 years. We hope for such advances for the aforementioned rare cancers. Scientifically, rare cancers, those with a lower incidence in the population, frequently have a strong genetic component. Therefore, with the increase in our knowledge of the genome and the accompanying advances in technology, today's cancer researchers are better poised to identify the underlying genetic components and fundamental etiology of these rare tumors.

*Interagency Workshop on the Science and Practice of Informal Caregiving to be Held*

All rare diseases are represented in our effort to better understand the state of the science and practice of informal caregiving. The informal caregivers of people with rare diseases must care for their loved ones within the context of extreme uncertainly; with little or no evidence based standards regarding diagnosis, prognosis, treatment or symptom management. We hope that advancing the science and practice of informal caregiving will help ease the extraordinary burden experienced by those providing informal care to those with rare diseases.

## NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (NICHD)

### Overview of Rare Diseases Research Activities

The National Institute of Child Health and Human Development (NICHD) mission is to ensure that children are born wanted, timely, and healthy and that they develop their full physical, emotional, and cognitive potential. The Institute achieves its mission in part by conducting and supporting a broad range of innovative research, outreach and education activities, including those that address rare diseases and conditions in human reproduction and in infants and children. Below are examples of these activities.

### Recent Scientific Advances

*New understanding of a devastating disease of preterm infants.* Each year, about 9,000 preterm infants in the United States develop a devastating intestinal disease, necrotizing enterocolitis (NEC). Surgical procedures can help some infants with NEC, but 20 to 40 percent of them die. Characteristics of NEC include injury to infants' fragile intestinal tissue, inflammation and infection. The mechanism responsible for these symptoms is not understood and there is no generally accepted preventive treatment for NEC. Recently, scientists with NICHD support found a promising lead in their study of NEC and a type of cell death, known as apoptosis. Apoptosis refers to a normal process in which cells are "programmed" to self-destruct in a controlled manner, to rid the body of unneeded cells. Irreparable injury, however, can set off uncontrolled self-destruction. In their research on apoptosis and the role of a small protein (epidermal growth factor, EGF) in NEC, the scientists found that feeding newborn rats a formula containing EGF altered the expression of apoptotic genes and proteins in a way that promoted cell survival and reduced NEC. Better understanding of the molecular processes underlying the effect of EGF could be the basis of new treatments to cure or prevent NEC.

*A clue to early detection of a dangerous complication of pregnancy.* Preeclampsia is a dangerous complication of pregnancy that occurs rarely, but is a leading cause of maternal and fetal mortality and morbidity. The clinical symptoms of preeclampsia, a sudden spike in a pregnant woman's blood pressure and protein in her urine, emerge suddenly and without warning after 20 weeks of pregnancy. Unless treated immediately, the woman's condition can progress quickly to life-threatening seizures, stroke, kidney failure, and death, and fetal growth may be restricted, or the fetus may die. The only "cure" is immediate delivery, regardless of the duration of the pregnancy, and surviving preterm infants may have life-long disabilities. Decades of research have identified certain risk factors for preeclampsia, but not its cause, and clinicians are not able to identify those women at risk who will develop the disease. A new clue, that could lead to a simple preeclampsia screening test, emerged recently from a study by NICHD researchers and extramural colleagues. They found that 5 weeks before preeclampsia symptoms appeared, pregnant women with the disorder had had abnormal serum levels of an important enzyme and two essential growth factors. A longer study, following women throughout pregnancy, could confirm the clinical usefulness of this clue. If confirmed, the enzyme and/or

growth factor abnormalities could be the basis of a simple test that would enable clinicians to refer women for specialized care early, before the onset of preeclampsia.

*Clinical and basic science advances against a disease that causes bones to break easily.* Pursuing both clinical and basic research in Osteogenesis imperfecta (OI) enabled the NICHD's intramural program to determine the subtle effects of a drug that was being prescribed for OI children, without adequate data on its safety and efficacy. OI is a rare genetic disorder that affects the body's production of collagen, the major protein of connective tissue, and that causes bones to break easily. The intramural OI program supports an ongoing, longitudinal study of pediatric OI. This study functions as a research "clinic" that enables researchers to follow OI children as they grow while providing the children with needed medical care and opportunities to participate in clinical trials of promising interventions.

Recently, working with the NIH Clinical Center rehabilitation program, NICHD scientists tested an osteoporosis drug (bisphosponate pamidronate) in a controlled trial in OI children and in transgenic mice. One the basis of positive reports from uncontrolled OI studies, clinicians had begun prescribing the drug for OI children, without adequate data on whether it was safe or effective. The NICHD's controlled trial of this drug did not replicate the previously-reported improvements in childrens' walking, muscle strength and bone pain. The mouse study, however, yielded unexpected mixed results. In the experimental animals, the drug made a critical type of bone material in long bones such as the femur, more brittle. But the drug also improved these bones' resistance to direct force and strengthened vertebral bones in the mice. The NICHD scientists concluded that the drug could be used long enough to gain its strengthening effects in children with OI, but stopped before brittle effects occurred. In addition, the intramural program recently created a new transgenic mouse, which will be used to test a potential gene therapy to suppress the abnormal action of the OI gene mutation.

**Significant Ongoing Rare Diseases Research Initiatives**

*Pediatric drug trials*

Few drugs that have been tested in adults for safety and efficacy have also been tested in children, partly because market disincentives discourage research on drugs for pediatric conditions, whether rare or common. Under the 2002 Best Pharmaceuticals for Children Act (BPCA), the NICHD developed, implemented, and currently administers and supports a major initiative to increase the number of drugs for which adequate pediatric safety and efficacy data exist. The BPCA initiative consists of an ongoing process for identifying priority drugs that should be studied for pediatric use, administering contract studies of the drugs, and a data coordinating center.

In the prioritizing process, the NICHD works closely with other Institutes, the Food and Drug Administration, and outside experts to select drugs on the basis of: (1) frequency of use in the pediatric population; (2) severity of the condition to be treated by a drug; and (3) the potential for providing a health benefit to the pediatric population. These criteria allow drugs for rare as well as common conditions, and drugs that would not otherwise be within the NICHD's research

mission, to be selected as priority drugs for research. Among the priority drugs for which the NICHD issued contracts or interagency agreements in FY 2005 are several for rare diseases, i.e. sickle cell disease and four types of pediatric cancer. The sickle cell trial is co-funded by the National Heart, Lung, and Blood Institute and the cancer trials are co-funded by the National Cancer Institute. In a related activity, the NICHD in FY 2005 began organizing a meeting of government, industry and academic representatives to discuss how further to stimulate research in pediatric drug formulations.

*Neural tube defects*

The NICHD supports a broad portfolio of research in the prevention of spina bifida and related neural tube defects and in the treatment and management of these complex conditions. NICHD research encompasses basic, clinical, and genetic epidemiological studies of the genetic and environmental factors associated with the susceptibility, health disparities and variability of human malformations associated with these defects. One current NICHD-funded project is the multicenter Management of Myelomeningocele (MOMs) study, which is a clinical trial comparing the safety and efficacy of traditional postnatal repair of open neural tube defects and fetal surgical repair. The children of trial participants will be followed to age three, to determine treatment effects of both procedures on their subsequent neurological and mental development, and on their need for shunting. Another project will link nutritional factors, folate status, and genetic information from a large number of affected families to define risk factors that are commonly thought to be associated with spina bifida. Information from this study can be used to inform the basic scientific investigations needed to clarify underlying developmental mechanisms and to define gene function, with the ultimate goal of improving prevention and treatment options.

*Rett, Prader-Willi and Angelman's syndrome research consortia*

Under the leadership of the NIH National Center for Research Resources and Office of Rare Diseases (ORD), the NICHD participates in the multi-IC Rare Diseases Clinical Research network. Each research center in this network focuses on a cluster of particular types of diseases, particular organ systems, or other grouped topics. Each consortium includes clinical investigators and institutions and patient support organizations, and each center must including training of investigators, to expand research expertise in the center's disorders. One NICHD-funded center focuses on three rare conditions, Rett, Prader-Willi and Angelman's syndromes, which cause mental retardation and other severe symptoms in children. This consortia's research encompasses new diagnostics, new therapies, and natural history protocols for these disorders. The consortia also seek to facilitate the use of "state-of-the-art" technologic advances to detect individuals with the disorders, both prenatally and after birth.

## New/Planned Extramural Or Intramural Research Initiatives

*Newborn Screening Initiative*

The NICHD leads a major, multi-Institute initiative to expand the number of rare genetic conditions that can be identified at birth, and to develop treatments for such conditions. Expanding knowledge of the genetic and molecular bases of various disorders and advances in testing technologies have paved the way for expansion of current state newborn screening programs, but also heightened the need for new treatments for potentially screenable conditions. The early detection of genetic conditions in newborn infants allows extra time for therapies, such as the dietary intervention for phenylketonuria (PKU), can have a dramatic impact on the clinical severity of the condition of an affected child. If left undiagnosed and untreated until the first clinical signs of the disorder appear, these conditions can cause irreversible mental retardation, physical disability, neurological damage, and even death. The goal of one component of the initiative is to develop one technology, or an integrated group of technologies, that would enable newborn screening programs at the state level, and large commercial laboratories, to rapidly test very large numbers of specimens from newborn infants for groups of genetic disorders, such as chromosomal abnormalities and metabolic conditions. The complementary goal of the other component is to develop new, effective therapies for such conditions, especially those that are identified as high priority conditions by the report *Newborn Screening: Toward a Uniform Screening Panel an System.* Joining with the NICHD in the solicitation for new treatments are the National Institute on Deafness and Other Communication Disorders and the National Institute of Diabetes and Digestive and Kidney Diseases.

*Fragile X syndrome*

The NICHD recently joined the National Institute of Mental Health, the National Institute for Neurological Disorders and Stroke and other public and private sector partners to sponsor a major new research initiative, *The Shared Neurobiology of Fragile X and Autism.* Non-NIH partners in this effort include components of the Canadian Institutes of Health Research, the Health Research Board of Ireland, the FRAXA Research Foundation, Cure Autism Now, and Autism Speaks. The 2005 solicitation for this initiative sought studies focusing on topics related to understanding neural pathways, circuits, systems and molecules that play a role in the origins of Fragile X and its disease process, and that may also be implicated in autism spectrum disorders and Rett syndrome. The solicitation also emphasized the need for studies to identify targets for new drugs to treat these conditions. Scientifically meritorious applications that fall just short of the NIH pay line will be distributed, for possible funding, to other partners in the initiative.

## Rare Disease-Specific Conferences, Symposia, or Workshops

*Osteogenesis imperfecta*

Among the multiple conferences on rare diseases that the NICHD has co-funded with the Office of Rare Diseases, was the triennial International Conference on Osteogenesis imperfecta in

FY 2005. Conference presentations ranged from basic studies with mouse models of the disorder to clinical research, including drug therapy, and orthopedic and rehabilitation trials, to issues of concern to patient support groups. The conference proceedings provided an important stimulus for research ideas.

*Primary immunodeficiency diseases*

The NICHD also actively supported conferences on a variety of topics related to primary immunodeficiency (PI) diseases in FY 2005, in collaboration with NIAID colleagues. The First Clinical Immunology Society (CIS) Primary Immune Deficiency Consortium Conference capitalized upon a mentoring program to train young investigators in the diagnosis and treatment of PI. This training effort, a collaboration between the CIS and the USIDNet Consortium (U.S. Immunodeficiency Network Consortium), advances the skills of both seasoned physicians and fellows in the diagnosis, molecular defects, complications, and treatment of these complex diseases. Investigators at the Bi-Annual Meeting of the International Union of Immunological Societies Expert Committee on PI presented cutting-edge basic research in immunology, descriptions of novel gene defects in patients with PIs, and potential new therapies. The meeting, organized under the auspices of the Jeffrey Modell Foundation, provided an important venue for updating the classification of identified PIs.

*Premature ovarian failure and Fragile X syndrome*

With the National Fragile X Foundation, the NICHD hosted the research conference, *Premature Ovarian Failure and Fragile X syndrome*, in April, 2005. Premature ovarian failure (POF), also referred to as ovarian insufficiency, is a condition in which the ovaries stop functioning normally in a woman younger than age 40. Women who carry the permutation FMR1 gene are at risk for POF, which can cause infertility. (The FMR1 gene causes Fragile X syndrome when it has mutated to the extent that it is fully "turned off" and fails to make an adequate amount of a normal brain protein. Individuals with the permutation FMR1 gene do not have the syndrome but may have a child with the disorder.) As a result of this conference, the Foundation posted information about premature ovarian failure on its Web site (http://www.fragilex.org/html/pof.htm), including NICHD-approved information about the condition, its relationship to Fragile X syndrome, and links to NICHD clinical studies on POF and the FMR1 mutation.

**Activities with Rare Diseases Patient Advocacy Groups to Stimulate Research**

*Strategic planning for mental retardation and developmental disabilities research*

The NICHD has adopted a new process to broaden and enhance the Institute's research reporting and planning processes, by involving patient advocacy groups and others in considering possible future research directions at the Institute branch level. The results of this process are incorporated into the comprehensive reports that NICHD branches present every four years to the National Advisory Child Health and Human Development Council (NACHHD). The first branch to use the process was the Institute's Mental Retardation and Developmental Disabilities

Branch (MRDD), which is the locus for extramural research on the many rare disorders that cause mental retardation and developmental disabilities.

The branch invited an expert panel of scientists, advocates, members of the public, and two members of the NACCHD to participate with MRDD staff in an analysis of the branch research portfolio. Branch staff considered the panel's recommendations in developing and informing the "Future Directions" section of its June, 2005 report to the NACCHD. As in earlier reports, the MRDD document also reviewed the research supported by the branch in the previous 4 years and described important scientific advances resulting from that research. The report was presented in draft to the Council for its comment and then posted on the Institute's Web site for a 2-week period of public comment. Members of the Friends of the NICHD were notified of the availability of the draft report for comment. The final report—*The Mental Retardation and Developmental Disabilities (MRDD) Branch, NICHD, Report to the NACHHD Council, June 2005*—is available at both the Institute's Information Resource Center and its Web site: http://www.nichd.nih.gov/publications/pubs/MRDD_Council_Report_2005.pdf.

Other NICHD activities with patient advocacy groups included inviting a representative of FRAXA, the Fragile X Research Foundation, to participate in peer review of research grant applications. The Institute's intramural Osteogenesis imperfecta (OI) research laboratory was also one of three NICHD laboratories to host a 2005 briefing of Congressional staffs and outside groups. This visit included a meeting with a group of families that participate in the ongoing longitudinal OI study. As noted above, the Institute also worked with the National Fragile X Foundation to host a research conference on premature ovarian failure and Fragile X, and with the Jeffrey Modell Foundation on a research meeting on primary immunodeficiency disease.

**Education Activities on Rare Diseases**

The Institute's educational activities on rare diseases included dissemination of information on the Fragile X and Rett syndromes. During 2005, the NICHD distributed more than 3,000 copies of the booklet, *Families and Fragile X syndrome*. and nearly 1,500 copies of its fact sheet, *Rett* syndrome*: Autism Research at the NICHD.* Also during 2005, the Institute worked with the internationally-recognized experts in Rett, Drs. Huda Zoghbi and Alan Percy, to revise its fact sheet on the disorder, for publication in 2006. With NICHD support, Dr. Zoghbi had discovered the gene that causes Rett.

## NATIONAL INSTITUTE ON DEAFNESS AND OTHER COMMUNICATION DISORDERS (NIDCD)

### Overview of Rare Diseases Research Activities

NIDCD conducts and supports research and research training on normal mechanisms and diseases and disorders of hearing, balance, smell, taste, voice, speech, and language. This mission is achieved through a wide range of research performed in its own laboratories, a program of research grants, individual and institutional research training awards, career development awards, center grants, cooperative clinical trials, and contracts to public and private research institutions and organizations. The Institute also conducts and supports research and research training that is related to disease prevention and health promotion. NIDCD addresses special biomedical and behavioral problems associated with individuals who have communication impairments or disorders. NIDCD also supports efforts to create devices that substitute for lost and impaired sensory and communication functions.

### Recent Scientific Advances in Rare Diseases Research

*Acoustic neuroma*

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder that occurs in about one out of every 40,000 Americans. This mutation on chromosome 22 is strongly associated with the development of bilateral vestibular schwannomas, which then results in damage to both auditory nerves. Treatment of these acoustic neuromas often requires bilateral removal of the auditory nerves, which usually renders the individual deaf. Electrical stimulation of the residual neural pathways within the cochlear nucleus can provide a sense of hearing after this surgery. NIDCD-supported scientists are working to optimize the design of a neural implant used for electrical stimulation of the cochlear nucleus in these individuals, with the goal of providing a device equal in performance to the cochlear implant used in individuals with profound hearing loss.

*Auditory neuropathy*

A small but substantial number of individuals with bilateral hearing loss have normal cochlear function. These individuals have severely abnormal central neural processing of auditory sensory input as evidenced by poor or absent auditory brainstem responses. Standard treatment strategies for bilateral hearing loss, such as hearing aids, are of little use to these individuals. When this disorder strikes young children or infants, it can cause severe disruption of normal language and speech development. The most likely cause of hearing loss is a disorder of the auditory nerve or central auditory pathway, hence the term “auditory neuropathy.” This disorder is rare but more common than previously estimated. Investigation of the physiologic mechanisms, the genetic basis, and possible treatments for this disorder is ongoing.

*Enlarged vestibular aqueduct (EVA) and pendred syndrome (PS)*

EVA is characterized by progressive childhood sensorineural hearing loss in association with enlarged vestibular aqueducts. NIDCD intramural scientists have determined that these cases can be clinically and genetically classified into one of the three groups. Approximately one third of individuals have PS, which causes hearing loss in combination with, in some cases, enlargement (goiter) of the thyroid gland (due to a defect in the ability of the thyroid gland to organify iodine in the synthesis of thyroid hormone), and two mutations of the PS gene (*PDS*). Another one third of individuals have a single *PDS* mutation, but do not have a thyroid organification abnormality and do not have PS (their EVA is nonsyndromic). NIDCD intramural scientists are working to identify the genetic basis of EVA, including several cases where it is clearly not caused by mutations in *PDS*. Family studies indicate that there must be one or more other etiologic cofactors that cause EVA, which is inherited as a complex trait, in these individuals. Congenital cytomegalovirus (CMV) infection was shown not to be an etiologic cofactor in these cases. The other one third of EVA individuals have no *PDS* mutations, normal thyroid glands (i.e., nonsyndromic), and these cases are sporadic. Current and future studies are oriented toward identifying the causes of EVA in these latter two groups of individuals, as well as generating a mouse model for this condition. Recent research supported by NIDCD discovered new developmental interactions between important growth regulatory factors called BMPs (bone morphogenetic proteins) and their antagonists. In particular, blocking the activity of a gene for a particular antagonist labeled 'DAN' can produce enlarged endolymphatic ducts and sacs, suggesting that these compounds regulate BMP spatial and temporal activity during development of the pattern and partitioning of otic tissue into distinct structures of the inner ear.

*Fanconi anemia (FA) and squamous cell carcinoma (SCC)*

FA is a rare autosomal recessive syndrome associated with chromosomal instability that predisposes affected individuals to squamous cell carcinomas (SCC) of the head and neck, cervix and vulva. NIDCD and NCI intramural scientists have collaborated in studies to ascertain genotypes associated with development of head and neck SCC; and determine whether FA pathway related gene expression is altered in sporadic head and neck SCC.

*Hereditary cerebellar ataxia syndrome of early onset*

Several abnormal genes that are associated with inherited cerebellar syndromes that cause disorders of balance and coordination have been identified. Relatively little is known about how different mutations lead to specific types of the disorder. There are typically great differences in the clinical signs and symptoms within families that segregate the same mutation, and across families with mutations in the same gene. NIDCD-supported scientists have previously demonstrated linkage to chromosome 19p in four families with episodic vertigo and the inability to coordinate muscle movement (ataxia). The scientists have identified a missense mutation in the calcium channel gene on chromosome 19p in a family with severe progressive cerebellar ataxia of early onset involving the trunk, the limbs, and speech function.

*Kallmann syndrome*

Kallmann syndrome is a rare genetic disorder with two main symptoms: an absence of the ability to smell and failure of the gonads to mature. There is a five- to seven-fold increased chance that this syndrome occurs in males in comparison to females, suggesting that the X-linked form of the disease is the most frequent. NIDCD-supported research has led to the identification of a common developmental defect in nerve migration, which links the two major disease symptoms. A unique family of proteins and their receptors that regulate nerve migration and direction during development are under investigation by NIDCD-supported scientists. Additional research is focused on isolating and cloning an X-linked gene responsible for Kallmann syndrome.

*Mitochondrial genes and deafness*

Mitochondria are specialized structures within cells that play a crucial role in metabolism and energy production. Mitochondria contain their own genes, which replicate during cell division. All of the mitochondria present in individuals are derived from the mother's egg. Therefore, diseases that appear to be passed exclusively through the maternal lineage are often linked to defective mitochondrial genes.

NIDCD-supported scientists have identified several specific mitochondrial mutations that predispose an individual to hearing damage resulting from toxicity from the aminoglycoside class of antibiotics to the inner ear hair cells. These investigators have determined that genetic loci in the nucleus of the cell act to modify the effects of the mitochondrial mutations. Most recently, a specific gene was identified in mice, which modulates the severity of mitochondrial deafness, which is implicated in age-related hearing loss. This mouse model will be extremely valuable for detailed studies of the molecular mechanisms by which mitochondrial mutations contribute to deafness. These findings could be used to develop genetic tests to determine whether an individual has an increased risk for aminoglycoside-induced hearing damage.

*Olfactory function*

NIDCD-supported scientists are investigating relationships between decreased olfactory function and a number of rare diseases. Studies have shown that olfactory loss appears to be among the first signs of such common neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Recent psychophysical studies have evaluated the prevalence and magnitude of olfactory loss in subtypes of Parkinson's disease, Down syndrome, schizophrenia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and the rare amyotrophic lateral sclerosis/Parkinsonism/dementia complex of Guam. Better understanding of the associations between olfactory function and rare diseases may lead to earlier diagnosis and improvement in monitoring of these rare diseases.

*Usher syndrome*

Usher syndrome (USH) is recessively inherited and characterized by hearing loss, retinitis pigmentosa (RP) and, in some cases, a vestibular disorder. About 5 percent of individuals who are deaf have USH, and more than half of the deaf and blind individuals (>10,000) in the United States have USH. The severity of the hearing loss and the presence of vestibular dysfunction distinguish two major clinical subtypes of USH, types 1 and 2. Individuals who have USH type 1 are congenitally deaf, and have a balance deficiency at birth, while RP has an onset at about the time of puberty. Individuals with USH type 2 are distinguished from USH type 1 in having a less severe hearing loss. A third form of USH (type 3) is characterized by progressive loss of hearing and retinal function. Mutations can cause USH in more than eleven different genes. NIDCD intramural scientists have identified and characterized some of the genes responsible for USH and two common recessive mutations that cause USH in the Ashkenazi Jewish population. They have discovered that the genes for Usher syndrome type 1D and type 1F, both encode cell adhesion proteins cadherin 23 and protocadherin 15, respectively. In addition, several NIDCD-supported scientists reported cloning the gene for Usher syndrome type 2A. The *USH2A* gene encodes a protein, Usherin, that has structures similar to other proteins involved in assembling cells and tissues into functional organs. NIDCD-supported scientists also have identified the genes responsible for Usher type 1C. These advances are critical steps towards developing strategies to treat this devastating disease that causes deafness and blindness.

*Waardenburg syndrome (WS)*

WS is an autosomal dominant disorder, which is characterized by pigmentary disturbances and deafness. NIDCD-supported scientists are seeking to determine the loci for WS type 2 by utilizing a high-density genome scan coupled with linkage analysis to identify candidate genes mutations that could be the cause of this disorder in three large, multigenerational families and several smaller families with WS2. Other scientists are studying the Dalmatian as an animal model for understanding the genetics of pigment-associated deafness in the dog and human. The relationship between pigmentation and deafness is not unique in Dalmatians and this model offers a unique opportunity to conduct genetic analysis of hereditary deafness.

**Conferences, Symposia, Meetings**

A Research Planning Workshop on Spasmodic Dysphonia was held in June 2005. The conference was cosponsored by NIDCD, the National Institute of Neurological and Stroke Disorders (NINDS), the Office of Rare Diseases (ORD), the (NSDA) National Spasmodic Dysphonia Association, and the Movement Society. Spasmodic dysphonia (or laryngeal dystonia) is a voice disorder caused by involuntary movements of one or more muscles of the larynx. Dysphonia is a broad term, referring to voice impairments. Severity of the spasmodic dysphonia may vary from mild to severe and speakers may experience sufficient difficulty with communication.

## NATIONAL INSTITUTE OF DENTAL AND CRANIOFACIAL RESEARCH (NIDCR)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Dental and Craniofacial Research is to improve oral, dental and craniofacial health through research, research training, and the dissemination of health information. NIDCR's programs encompass basic, translational and clinical studies of the broad range of diseases, disorders, conditions and syndromes involving the oral cavity and craniofacial structures. NIDCR's section of this report highlights selected scientific advances within the Institute's intramural and extramural programs and other related program activities relevant to rare diseases that fall within NIDCR's mission.

### Recent Scientific Advances in Rare Diseases Research

*Alzheimer's disease and amyotrophic lateral sclerosis*

Alzheimer's disease and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases characterized by selective loss of motor neurons in the brain and spinal cord. Neurotoxicity mediated by glutamate is believed to be one of the causative factors for these dreaded diseases. Dysregulation of cyclin dependent kinase5 (Cdk5) expression has been implicated to play a critical role in the underlying disease processes for neurodegenerative diseases. In order to analyze the precise role of Cdk5 in such disorders, NIDCR investigators have developed many mouse models in which Cdk5 expression is genetically altered in the whole brain or specific areas of the brain during or after embryonic development. Earlier studies revealed unique neuronal defects associated with abnormal migration, neurodegenerative changes, and defective formation of facial nuclei in Cdk5-null mice. Additional studies revealed context-dependent effects of Cdk5 on neurodegenerative processes typically seen in ALS. Specific elimination of Cdk5 in the frontal brain cortex resulted in drastic neurodegeneration similar to that observed in patients with advanced Alzheimer's disease. Current studies continue to elucidate the various roles of Cdk5 in ALS.

*Amelogenesis imperfecta*

Amelogenesis imperfecta (AI) is the most common hereditary disease affecting tooth enamel. AI is a heterogeneous group of rare inherited disorders that affect the formation of dental enamel and result in thin, malformed enamel that is easily abraded. As a tooth develops from its bud, an organic matrix forms that gradually crystallizes into tooth enamel. This process destroys nearly all traces of the matrix, along with clues to key information in understanding tooth formation.

- A team of NIDCR scientists reported for the first time that the proteins amelogenin and ameloblastin interact in the matrix before mineralization proceeds. Understanding molecular interactions in the developing organic matrix of the tooth is necessary for understanding the causes of mineralization disorders such as AI.
- A team of NIDCR grantees and colleagues has uncovered intriguing clues about structural processes that might orchestrate enamel deposition. They found in laboratory studies that the protein amelogenin forms into spherical balls which, in turn, assemble into long beaded

chains. Because of their thin, ribbon-like appearance, the scientists called the chains "microribbons." They next dipped the microribbons into a mineralizing calcium-phosphate solution to determine whether they possess the structural integrity to serve as protein scaffolds for the mineralization process. The result: the microribbons yielded ordered hydroxyapatite crystals with similar orientation to those formed in enamel. This marks the first time that scientists have assembled amelogenin into ordered structures, mineralized them, and shown that the spherical balls, called nanospheres, are responsible for the ordered crystal growth that is essential to proper enamel formation, information that will be absolutely essential to understanding how enamel is formed in the tooth bud, and furthering the NIDCR's ongoing goal to engineer the human tooth, which may provide therapies for addressing some of the oral manifestations of AI and similar disorders.

- NIDCR researchers created a line of mice that lack the protein amelogenin. The mice display a typical X-linked amelogenesis imperfecta phenotype characterized by chalky white teeth, enamel hypoplasia, a lack of prismatic crystals, and cuspal attrition. The availability of the new mouse line made possible subsequent investigations yielding a number of discoveries concerning the role of amelogenin in tooth formation and in the development of AI. For example, elemental analysis indicated that amelogenins are essential for the organization of the crystal pattern and enamel development but are not required for initiation of mineral crystal formation. Crossing these mice with other mouse lines yielded results demonstrating the importance of functional differences in amelogenin splice variants. In addition to their enamel-specific roles, amelogenins were also implicated in the formation of root cementum, and implied additional roles for amelogenins. In order to determine the precise role of amelogenins in tooth roots, they carefully analyzed tooth roots of aging amelogenin-null mice. This analysis unexpectedly revealed progressive cementum defects in the null mice. Currently, the functions of these isoforms in cementum biology are being characterized.
- NIDCR scientists also have recently created mice deficient in the enamel matrix protein ameloblastin. The mutant mouse is characterized by severe enamel hypoplasia, a disease similar to AI in humans. Although a mutation of ameloblastin has not been identified in the human disease, AI is a candidate for disease caused by ameloblastin mutations. In the mutant mice, ameloblasts, cells responsible for production of enamel matrix proteins, continue to proliferate. A significant number of mutant mice develop oral tumors in the maxilla with age. These tumors are of dental epithelial origin and are likely derived from mutant ameloblasts defective in ameloblastin. These ameloblast-like epithelioid cells are surrounded by multiple focal areas consisting of enamel matrix and some calcospherites. Deficiency of ameloblastin is also likely one of the causes of ameloblastoma, the most common human odontogenic tumor.

*Cleft lip/cleft palate*

- Some birth defects show a strong association with older maternal age, but the effect of older age of the father is less certain. A recent study evaluated the degree to which maternal and paternal age influence the risk of having a child with an oral cleft. The samples were derived from the Danish Registries and included 1920 children born with cleft lip with or without cleft palate (CLP), and 956 children born with isolated cleft palate. The investigators found parental age demonstrates differing effects on the two cleft conditions. The influence of

maternal and paternal age on the risk of CLP increases with advancing age of the other parent and that risk vanishes if the other parent is young. In contrast, for isolated cleft palate, there is increased risk with paternal age regardless of the age of the mother. While maternal age is known to be associated with many genetic disorders, this study emphasizes the importance of taking paternal age into account when analyzing the effect of maternal age.

- A multicenter international epidemiology study of oral clefts using a study design of family based tests is being conducted in four sites: Baltimore, Taiwan, Singapore, and Beijing. Recruitment of families has begun in each center and DNA collection has begun. Preliminary analysis of genetic diversity between Asian and US populations will define the range of haplotype and allelic diversity among the participating sites, increasing our understanding of the role of genetics in the development of cleft lip and/or palate.

*Cystic fibrosis*

Cystic fibrosis (CF) is an inherited disease of mucous and sweat glands. It affects mostly the lungs, pancreas, liver, intestines, sinuses, and sex organs. The incidence of CF in the US is 1 in 2,500 births. Over the past 5 years scientists have worked to develop gene transfer vectors able to introduce the CFTR gene into columnar epithelia cells in the lung as a treatment for CF. Adeno-associated viruses, such as a virus known as AAV5 are attractive vectors for gene transfer in CF. However, their utility has been limited because of the difficulty of packaging sequences of viral DNA larger than 5,000 bases. To partially circumvent this size constraint, the investigators recently focused on shortening the other elements in the AAV expression cassette. They found that some could be deleted without abolishing activity. Then they packaged the shortened elements into AAV5 and applied them to the apical surface of differentiated CF airway epithelia. Two to 4 weeks later, the AAV5 vectors partially corrected the CF chloride transport defect. These results demonstrate that a single AAV vector can complement the CF defect in differentiated airway epithelia and thereby further the development of effective CF gene transfer.

*Dentinogenesis imperfecta*

Dentinogenesis imperfecta (DI) is an inherited disorder that primarily affects dentin mineralization. It is classified into three subtypes: type I is the least severe, and type III is the most severe. Type I is also associated with Osteogenesis imperfecta, while the more severe forms are restricted to dentin. Several mutations have been identified in the dentin sialophosphoprotein (DSPP) gene in patients with DI. DSPP is predominantly expressed in dentin-producing odontoblasts and transiently in enamel-producing ameloblasts. Low levels have also been detected in several other tissues like bone, inner ear, salivary glands, kidney, etc. NIDCR scientists generated DSPP gene knockout mice to characterize the molecular roles of DSPP in tooth development and found that these mice developed a DI-III-like phenotype, which resembles the teeth from patients with rickets. They found elevated levels of biglycan and decorin in both the affected teeth of DSPP-null mice and human rickets patients. In order to understand the molecular mechanism underlying this phenotype, two mouse models (double gene knockouts) were generated. Detailed analysis of these two mouse models will indicate the potential gene regulatory function of DSPP gene products. NIDCR researchers also plan to characterize the precise roles of DSPP in osteogenesis.

*Dentin dysplasia*

Dentin dysplasia is a rare hereditary disorder resulting in dentin defects such as thin and broken dentin, resulting in chipped teeth susceptible to infection. NIDCR scientists previously generated transgenic mice overexpressing the TGF-beta1 growth factor. These mice displayed significant dentin defects similar to those seen in dentin dysplasia and dentinogenesis imperfecta. A more detailed examination of the enamel in these mice revealed that altered TGF-beta1 expression in the tooth seems to trigger detachment of ameloblasts and abnormal secretion and deposition of minerals in the cyst-like structures adjoining the dentin. NIDCR researchers are examining the mechanisms underlying the detachment of ameloblasts in these mice.

*Dyssegmental dysplasia, Silverman-Handmaker type*

Dyssegmental dysplasia, Silverman-Handmaker type (DDSH) is a rare inherited skeletal disorder characterized by short limbs and abnormal vertebral bodies. Individuals with DDSH also have a flat face, abnormally small jaws, cleft palate, and reduced joint mobility, with defects in the cartilage. NIDCR scientists identified mutations of the perlecan gene (*HSPG2*) in three patients with DDSH. These results indicate that perlecan is essential for cartilage development. NIDCR researchers are studying the role of perlecan in cartilage development by identifying more mutations of DDSH through the creation of additional animal models.

*Fabry disease*

Fabry disease is a familial sex-linked disorder of lipid metabolism in which glycolipid accumulates in many tissues. Major disease manifestations include pain in the extremities, angiokeratomas, corneal dystrophy, oral and dental abnormalities, and vascular disease of the heart, kidney, and brain, leading to premature death. In collaboration with NINDS investigators, NIDCR scientists reported a high prevalence of oral and dental abnormalities in Fabry patients, including malocclusion, diastemas, and developmental anomalies. In order to better understand the disease and its possible treatments, they have generated and characterized a line of AGA-deficient mice, which show similarities to patients with Fabry disease. Recent studies revealed malocclusions in the teeth of Fabry mice and subtle changes in the density of long bones. The scientists hope that continued study of the Fabry mice will speed the understanding and development of treatments for people who are born with this devastating disease.

*Growth hormone deficiency (Adult)*

Growth hormone deficiency (GHD) is a disorder most commonly caused by frank pituitary disease, often the presence of nonfunctional pituitary adenomas, or as a result of surgery or radiotherapy for pituitary adenomas. NIDCR scientists previously demonstrated the considerable value of salivary glands as gene transfer target sites for the treatment of rare systemic single protein deficiency disorders, such as growth hormone (GH) deficiency in adults, a disorder with a prevalence of ~1/10,000. When the target gene encodes a biologically potent secretory protein, such as GH or erythropoietin (EPO), it is critical that the expression of that protein be tightly controlled so that it is expressed only when therapeutically required. NIDCR

scientists have evaluated the potential ability to utilize the rapamycin-inducible transcriptional regulation system for long-term control of transgene expression in mouse salivary glands *in vivo*. These studies have employed a "third generation" viral vector known as AAV2 to encoding human erythropoietin (hEPO) under the control of a rapamycin inducible promoter. The vector was administered to mouse salivary glands through the salivary duct. Rapamycin induced elevation of serum hEPO levels, as well as concomitant hematocrit changes, that were dose-dependent, completely reversible and relatively stable over the course of this study (6 months), with no appreciable change in rapamycin responsiveness. These results suggest that the rapamycin transcriptional regulation system delivered in a single AAV2 vector to salivary glands may be valuable for systemic protein replacement applications. This finding, if reproduced in a large animal model, suggests that rapamycin-controlled gene transfer could be a useful clinical approach for patients with rare systemic single protein deficiency disorders.

*Kaposi's sarcoma*

Kaposi's sarcoma (KS) is the most common cancer arising in HIV-infected patients and the most frequent oral neoplasm in immunosuppressed patients. KS has also emerged as one of the most prevalent cancers among children and adult men in the developing world. The Kaposi's sarcoma-associated herpesvirus (KSHV; HHV-8) has been recently identified as the infectious cause of Kaposi's sarcoma. Of interest, compelling evidence now supports a critical role for the oral cavity as the primary source of infectious HHV-8 in both immunocompetent and immunosuppressed patients. The sequencing of the full KSHV genome revealed a candidate gene known as vGPCR, which promoted the development of visible dermal and internal vascular tumors that resemble KS lesions. The results implicated vGPCR in both the initiation and promotion of Kaposi's sarcoma. Because only a fraction of the cells in advanced KS lesions express vGPCR, the contribution of this gene to KS progression is still unclear. Studies were therefore set out to determine whether the few cells that express vGPCR in established tumors represent an appropriate therapeutic target for the treatment of patients with pre-existing KS. Endothelial cell lines were engineered to stably express vGPCR or key KSHV latently-expressed proteins. Mixed cell populations that approximate the ratio of the gene and KSHV latent gene-expressing cells formed fast growing tumors in mice. The pharmacological deletion of the vGPCR-expressing cells resulted in the apoptotic death of latent gene-expressing cells and rapid tumor regression. These findings indicate that vGPCR may play a key role in KS progression and provide experimental justification for developing molecular-based therapies targeting vGPCR for the treatment of KS-patients.

*Lysosomal storage diseases*

Lysosomal storage diseases (LSDs) represent a significant portion of inborn metabolic disorders. LSD therapies have been developed, but efficacy does not extend to the central nervous system, which is affected in over 60 percent of the known LSD. Viral vectors have demonstrated unique tropism for certain cells in the CNS known as ependymal cells, which could serve as a useful depot for therapeutic factors correct the CNS complications associated with LSD. Recombinant AAV4 viral vectors carrying genetic information necessary to encoding the enzyme glucuronidase were injected into mice with established disease. Subsequently, the mice were

found to produce high levels of the recombinant enzyme. The scientists conclude that ependymal cells can serve as a depot organ for enzyme secretion into the surrounding brain parenchyma and CSF and suggest that AAV4 based vectors could be useful for the treatment of the CNS complications associated with LSD.

*Systemic lupus erythematosus*

Systemic lupus erythematosus and Sjögren's syndrome are both systemic autoimmune diseases that are characterized by the presence of antibodies to proteins made in our bodies (autoantibodies) and known as Ro/SS-A and La/SS-B. The origin and development of autoantibodies to these intracellular proteins is not clear. In a new study, an NIDCR supported investigator and his colleagues have traced the accumulation of autoantibodies to the Ro antigen back in time to before the onset of autoimmune disease. While multiple anti-Ro antibodies were found in the blood from SLE patients, analysis of stored blood samples, which had been collected before the onset of disease, demonstrated progressively fewer autoantibodies. Their findings suggest that autoimmunity can be triggered when molecules mimic environmental antigens. These findings can also explain the proposed role of Epstein - Barr virus in the development of autoimmune disease. The identification of molecular triggers and pre-disease autoantibodies could serve to predict and diagnose early autoimmune disease. Additionally, the cross reaction of the earliest appearing antibody (i.e. Ro) with an environmental pathogen (i.e. EBV) suggests an intriguing possibility for the cause of the disease as an excessive response to viral infection.

*McCune-Albright syndrome*

The McCune-Albright syndrome is defined by the triad of fibrous dysplasia of bone (FD), café-au-lait skin spots, and endocrine gland hyperfunction. Some patients may have only a single focus of bone affected, and others may have severe disease affecting multiple endocrine glands and virtually the entire skeleton. NIDCR scientists have three active clinical research protocols under way studying various aspects of the disease, ranging from a study of the natural history of the disease to treatment studies for the bone and endocrine disorders. The natural history study continues to reveal various aspects of the disease that provide a better understanding of disease progression and long term quality of life of the patients. Clinical drug trials aimed to improve care for patients with this rare disease are ongoing, and studies are underway to develop new medicinal therapies. These findings, and those to come, have implications for the long-term prognosis and evidence-based changes in clinical management of patients with these rare disorders.

*Mucolipidosis-IV*

Mucolipidosis type IV (ML-IV) is a rare inherited metabolic disorder that leads to abnormal accumulation of certain fatty substances and complex carbohydrates in the cells of many tissues. It is generally characterized by mental retardation, impaired coordination of muscular and mental activities, and corneal opacity. Currently, there is no animal model available to develop effective therapies to treat this fatal disorder. However, in collaboration with NINDS investigators,

NIDCR scientists have generated a mouse model with an altered ML-IV locus. These mice are currently being characterized in detail to identify whether they mimic ML-IV disease. If so, the mice will provide new opportunities to study mechanisms and possible treatments for this serious disease.

*Papillon Lefevre syndrome*

Papillon Lefevre syndrome (PLS) is an inherited disorder characterized by hyperkeratosis of the palms and the soles of the feet, along with early onset, aggressive periodontitis. Affected individuals suffer from gingival inflammation that typically results in the premature loss of most of their teeth. The skin lesions are variable, but are very painful in many affected individuals. NIDCR scientists played a lead role in the initial identification of the underlying mutation that causes PLS. The scientists are continuing to study the mutations to better understand the underlying pathophysiology and develop new treatment strategies. Recent studies suggest that the severe periodontal tissue destruction in Papillon-Lefevre syndrome may be related to excess accumulation of inflammatory proteins know as LD78beta and LD78alpha and dysregulation of the microbial-induced inflammatory response in the periodontium. This work is helping to identify new treatment strategies for PLS.

*Schwartz-Jampel syndrome*

Schwartz-Jampel syndrome (SJS) is a rare inherited skeletal disorder. This disorder is characterized by short stature, muscle stiffness, a characteristic face with a "fixed" facial expression, pursed lips, and sometimes low-set ears and myopia. The electromyography shows persistent spontaneous activity, particularly in the face and thigh muscles, which tends to diminish at rest. NIDCR scientists and others have recently identified mutations of the perlecan gene (*HSPG2*) of patients with SJS. Based on clinical examinations, the SJS phenotype appears to encompass a wide spectrum of disorders. In fact, one of the patients that had the perlecan mutation originally was diagnosed as having the disease kyphomelic chondrodysplasia. NIDCR researchers are now examining for possible mutations of the perlecan gene in other skeletal dysplasias that were previously identified as chondrodysplasia. They have created a mouse model to study the mechanism of SJS myotonia by genetic manipulation. This mouse model will facilitate the development of strategies and reagents for therapy.

*Squamous cell carcinomas of the head and neck*

Although relatively rare (incidence is estimated at 38,500 annually), squamous cell carcinomas of the head and neck (SCCHN) are among the most fatal and morbid of cancers at any anatomic site. Head and neck cancer patients frequently fail to respond to standard therapies, and their 5-year survival rate is less than 50 percent, a number that has improved only marginally over the past three decades. These cancers result in approximately 11,000 deaths per year in the U.S. A number of scientific advances were realized in this field during the past year.

- Radiotherapy is commonly used to treat the majority of patients with head and neck cancers. Salivary glands in the radiation field are significantly damaged by this procedure. Currently there is no generally useful means to prevent this irreversible damage. The condition leads to

considerable morbidity, including difficulty eating and swallowing, chronic oral infections such as candidiasis and dental caries, and decreased mucosal wound healing. It would be ideal to prevent such radiation damage. NIDCR scientists and colleagues at NCI previously showed that a stable nitroxide compound known as Tempol, when administered intraperitoneally shortly before radiation was able to provide radioprotection of murine salivary glands. They next closely examined pharmacokinetic characteristics of Tempol to determine if it was potentially useful clinically. They found that Tempol significantly reduced radiation-induced salivary hypofunction (~50 – 60 percent) in a dose- and time-dependent manner. Intravenous or subcutaneous administration of Tempol offered a protection level comparable to intraperitoneal injection. Topical use of Tempol, either as a mouthwash or gel, also was radioprotective. These findings strongly suggest that Tempol is a promising candidate for clinical application to protect salivary glands in patients undergoing radiotherapy for head and neck cancers.

- Two promising advances in oral cancer diagnostics were achieved this year:
  - The ability to determine the profile of molecular changes in oral cancer will significantly improve early diagnosis, thereby reducing the mortality and morbidity associated with this devastating disease. While genomic approaches such as microsattelite markers have identified promising chromosomal regions that are predictive of oral cancer progression, novel approaches for a robust and high-throughput analysis of molecular markers have recently been explored. NIDCR-supported researchers have discovered that a large panel of human RNAs can be readily detected in saliva. Thus, they examined the utility of salivary transcriptome diagnostics for oral cancer detection. Microarray analysis of the salivary RNAs in cancer patients and normal controls showed that seven cancer-related genes exhibited enhanced expression in oral cancer patients, with a 91 percent specificity and sensitivity in distinguishing oral cancer patients from controls. This novel approach can have important clinical implications for early detection of oral cancer.
  - Oral pre-malignant lesions (OPLs) range from low-grade dysplasia to severe dysplasia and carcinoma *in situ*. Current prediction of malignant progression of OPLs is based on a combination of clinical and histological features. However, sensitive molecular and visual tools that can be used in conjunction with traditional methods are being investigated. A group of NIDCR grantees analyzed the use of the toluidine blue (TB) dye as an adjunct tool for identifying OPLs at risk for malignant progression. They monitored OPLs from 100 patients without an oral cancer history for an average of 44 months and found a strong association between TB positivity and clinicopathological/molecular risk factors as well as disease outcome. TB detected virtually all high-grade and low-grade dysplastic lesions that were at high risk for malignant progression. These results underscore a potential value of using TB as a screening tool for clinical diagnosis of high-risk OPLs by health professionals.
- Three studies advanced our knowledge regarding providers' knowledge and practices related to oral cancer prevention and diagnosis.
  - In the first study, medical primary care providers (PCPs) who worked in Federally-Qualified Health Centers (FQHC) in Michigan were surveyed to assess their knowledge level and practices related to screening and preventing oral cancer. Forty-four percent of PCPs had high knowledge level. Those who had high knowledge level were more likely

to be physicians, males, and more likely to perform screening for oral cancer than those with low knowledge level. There was no difference in age and race/ethnicity between high and low knowledge groups. Perceived barriers included (1) lack of education; (2) lack of specialists to refer patients; and (3) lack of reimbursement. The majority of PCPs in this survey had positive attitudes about performing screening for oral cancer. In conclusion, to involve PCPs in screening for oral cancer, oral health programs should focus on providing up-to-date education, setting up a referral system, and providing proper reimbursement.

- In the second study, researchers examined oral cancer prevention and early detection practice patterns in a population-based random sample of practicing oral health care professionals in New York State. The participants were assessed for their readiness to offer tobacco-use cessation and alcohol-abuse counseling and oral cancer examinations. In terms of readiness to perform oral cancer examinations for patients aged 40 years and older, the large majority were in the maintenance stage of behavior, indicating that oral cancer examinations were a routine part of their practice. In terms of readiness to offer tobacco-use cessation counseling, only 12 percent of dentists and 21 percent of dental hygienists were in the maintenance stage, and only 2 percent of dentists and 4 percent of dental hygienists were in the maintenance stage of offering alcohol-abuse counseling. These findings suggest that oral cancer examinations seem to have been adopted as a standard of practice by most oral health care providers in New York State, but cancer prevention services, such as counseling regarding cessation of tobacco use and alcohol abuse, are lacking.
- The last study examined the knowledge of oral cancer risk factors and diagnostic concepts in a random sample of dentists practicing in North Carolina using a pre-tested survey. Composite index scores for knowledge of risk factors and diagnostic concepts were created using previously developed scales. Only 31 percent of respondents had consistent medium-to-high levels of knowledge on both highly correlated indexes. Dentists who had higher risk factor and diagnostic knowledge scores were significantly more likely to have heard of one or more diagnostic aids, to have graduated from dental school within the previous 20 years, and to have performed biopsies or referred five or more patients with suspicious lesions per year than were less-knowledgeable respondents. The researchers concluded that more education is needed in dental schools, postgraduate programs and continuing education programs to enhance dental professionals' knowledge of OPC risk factors and diagnostic concepts. Such programs should include information about adjunctive diagnostic aids.

- Two new research techniques were developed to assist the fight against head and neck cancer:
  - The first direct proteome-wide analysis of laser-assisted microdissected tissue samples from normal oral epithelium and squamous carcinomas of the head and neck was conducted, as part of NIDCR's research program aimed at identifying molecules whose aberrant expression and activity promote the malignant progression of oral cancers. New techniques were developed and optimized for protein extraction and global proteolysis of whole cell extracts and the subsequent separation of complex peptide mixtures by reverse phase liquid chromatography and analysis by mass spectrometry followed by tandem mass spectrometry sequencing of selected peptides. In an initial analysis of

clinical samples from oral cancer patients, aminoacid sequence information was obtained from 94-105 proteins per tissue set, some of which correlated with the disease state. Further development of these techniques may soon allow a detailed proteome-wide profiling of oral cancer tissues.

- A novel method for studying protease activity was developed, which combines the sensitivity and specificity of zymography with the spatial resolution of immunohistochemistry to study the activity of tissue remodeling proteases in oral cancer. Indeed, laser capture microdissection combined with zymography was found to be excellently suited for analyzing the prognostic significance and causal involvement of the plasminogen activation system in oral cancer.

- Four advances that increase our understanding of the mechanisms by which this devastating carcinoma proliferates were achieved this year:
  - Emerging information suggests that the serine/threonine protein kinase, Akt, is a key regulator of normal and cancerous growth and cell fate decisions. NIDCR scientists reported last year that the aberrant activation of Akt is a highly frequent event in human head and neck cancers. Because Akt can promote cell proliferation, the NIDCR scientists focused their efforts on an atypical serine/threonine kinase, mTOR. This kinase is a downstream target of Akt that controls the synthesis of proteins required for cell proliferation, including one known as p70-S6 kinase (p70S6K). They found that aberrant accumulation of the phosphorylated and active form of S6 is a frequent event in clinical specimens from head and neck cancer patients and their derived cell lines. This enhanced level of p-S6 was rapidly reduced in head and neck cancer cells and tumor xenograft models by clinically relevant doses of rapamycin, a specific mTOR inhibitor. Furthermore, they observed that rapamycin displays a potent anti-tumor effect *in vivo*, as it halts the proliferation and promotes the apoptotic death of head and neck cancer cells, ultimately resulting in tumor regression. These findings identified the Akt-mTOR pathway as a potential therapeutic target for squamous carcinomas of the head and neck, and provide the rationale for the future clinical evaluation of rapamycin and its analogs for the treatment of oral cancer patients.
  - Matriptase is a recently identified proteolytic enzyme that consistently is found in increased amounts on the surface of oral cancer cells. Using transgenic mice that make matriptase in epithelial cells researchers at the NIDCR have now shown that increasing the amount of matriptase on the cell surface suffices to cause squamous cell carcinoma, the most frequent form of oral cancer. Moreover, the NIDCR researchers found that increased expression of matriptase strongly promoted the tumorgenic effects of chemicals found in tobacco products, suggesting that deregulation of the cell surface protease may increase the risk of smoking-related cancers. These findings provide the rationale for continued evaluation of matriptase as a target for oral cancer therapies.
  - Patients with SCCHN have depressed antitumor immune responses, and tumor progression or recurrence is associated with particularly severe immune dysfunction. Currently, a subset of CD4+ T cells referred to as regulatory T cells ($T_{reg}$), is considered to be responsible for the control of autoreactive lymphocytes. In the present study, the scientists investigated whether the circulating pool of such cells in patients with SCCHN was expanded relative to that in normal control. They also evaluated phenotypic and functional characteristics of $T_{reg}$. Their data suggest that SCCHN patients have higher

values of the cells which, by virtue of lowering antitumor immune functions, could contribute to the progression or recurrence of head and neck cancer. The study illustrates the importance of $T_{reg}$ in defining the immune profile of patients with SCCHN and emphasizes the role of these cells in downregulating functions of other T-cell subsets.

  - Angiogenesis is a process by which tumor cells induce new blood vessels to form and fuel their abnormal growth. Abundant information is available on how tumor-released factors can trigger angiogenesis in endothelial cells, cells that line the walls of the blood vessels. However, the role of physical interactions between tumor cells and endothelial cells in the induction of tumor angiogenesis has not been explored. A group of NIDCR-funded investigators have shown that head and neck tumor cells also physically attach to a protein displayed on the surfaces of endothelial cells. This physical interaction sends a signal within these cells to grow and sprout new capillaries. These results underscore the importance of a dynamic crosstalk and a bi-directional communication in the tumor microenvironment between the tumor cells and the neighboring endothelial cells and how this interaction can lead to tumor angiogenesis and subsequently tumor growth. In addition, the finding suggests a future strategy of blocking not only the secreted molecules but also the cell-to-cell contact.

- Salivary gland neoplasms account for 7 percent of the tumors arising in the head and neck region, affecting around 2,000 new patients each year in the United States of America. These tumors are often very aggressive and have poor prognosis. Using transgenic mice, researchers have created an animal model that can promote the rapid (7–20 days) development of a complex array of preneoplastic epithelial lesions and carcinomas affecting all major salivary glands. Current and future use of this animal model system will provide a novel insight into the molecular mechanisms responsible for salivary gland tumors, and aid in the search for new treatment modalities.

*Temporomandibular muscle and joint disorders*

Temporomandibular muscle and joint disorders (TMJDs) are a group of conditions causing pain and dysfunction in the temporomandibular joint and surrounding muscles. While there are no firm data on how many people are affected by TMJDs, orofacial pain is a major cause of poor quality of life. The prevalence of TMJDs is higher in women than men. Examination of several mouse models in which specific matrix molecules have been eliminated have revealed degenerative changes in the articular surfaces of the temporomandibular joint (TMJ). Such changes have been implicated in the development of TMJDs; current studies are focused on characterizing how these matrix molecules are involved in maintaining integrity of the TMJ.

*Tricho-dento-osseous syndrome*

Tricho-dento-osseous syndrome (TDO) is an autosomal dominant condition that typically affects the hair, teeth and bones of affected individuals. As a result of mutation of the DLX-3 gene, the bones of affected individuals get thicker and denser with age. NIDCR scientists are generating mice carrying the DLX3 mutation found in humans with TDO. The mice are physically smaller and have an altered craniofacial appearance. NIDCR scientists are studying the effect of mutant

DLX3 in developing bone. It is hoped that understanding the way mutant DLX3 increases the thickness and density of bones will help develop treatments to improve the quality of bones in humans.

**Education Activities on Rare Diseases**

The NIDCR Web site contains information, order forms for educational materials, and references, including pertinent news releases and links to advocacy groups. A number of rare conditions are addressed under the heading "genetics." Salivary gland disorders, temporomandibular joint disorders and oral cancers are listed individually. To learn more, please see: www.nidcr.nih.gov/HealthInformation/DiseasesandConditions/

**Planned Activities**

This year NIDCR will launch an initiative entitled: Temporomandibular Joint and Muscle Disorders--Pathophysiological Mechanisms Linking Comorbid Conditions. The objective of this initiative is to stimulate research on discovering etiological and pathophysiological mechanisms underlying a set of chronic, comorbid conditions associated with temporomandibular joint and muscle disorder (TMJD). Symptoms of TMJD occur together with other chronic illnesses such as fibromyalgia, atypical face pain, trigeminal neuralgia, chronic fatigue syndrome, multiple chemical sensitivity, irritable bowel syndrome, migraine headache, speech and hearing disorders, and certain cardiovascular diseases. This initiative will seek research applications that use state-of-the-art, multidisciplinary and interdisciplinary approaches to discover molecular, physiological, and behavioral mechanisms responsible for the overlapping symptoms manifested in the set of disorders that co-exist with TMJD. The outcome of this initiative will be a better understanding of how TMJD and these other comorbid disorders interact to influence the development, course, and persistence of TMJD. An additional outcome will be a better appreciation of the etiology and pathology of TMJD itself.

## NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (NIDDK)

### Overview of Rare Diseases Research Activities

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) supports basic and clinical research on many rare diseases. For example, although diseases such as type 1 diabetes, type 2 diabetes, and obesity are not rare, there are rare single gene defects that cause these conditions such as Maturity Onset Diabetes of the Young (MODY) and lipodystrophy. In addition, the NIDDK supports research on both common and rare causes of kidney, liver and gastrointestinal diseases. The Institute also supports research on genetic metabolic diseases such as cystic fibrosis; lysosomal storage diseases including Krabbe disease and Mucopolysaccharidoses; disorders of amino acid metabolism such as tyrosinemia and maple syrup urine disease; disorders of copper transport including Menkes and Wilson disease; and hematologic diseases such as Cooley's anemia and sickle cell disease. Many times research on rare diseases leads to insights into cellular processes relevant to the more common diseases.

### Recent Scientific Advances in Rare Disease Research

*Krabbe disease*

Researchers have tested the use of umbilical-cord blood cell transplantation to treat Krabbe disease. This disease is a rare, inherited degenerative disorder of the central and peripheral nervous systems that is uniformly fatal with many children dying before the age of two. It results from a deficiency in the enzyme that breaks down the molecule galactocerebroside, which is an important component of nerve tissue, leading to its accumulation and subsequent nerve damage. There is no cure for the disease, but replacing the missing enzyme in the brain may be therapeutic.

Researchers recently used umbilical-cord blood transplantation from closely-matched, but not identical, donors to combat the disease. The hope was that transplanted cells in the cord blood would migrate to the brain provide the missing enzyme and thereby halt nerve damage. The researchers treated two groups of newborns; one group was diagnosed with Krabbe disease before or at birth on the basis of family history and was transplanted in the first 6 weeks of life; the other was diagnosed at the onset of clinical symptoms of the disease after birth and was transplanted in the first 6 months to 1 year of life. After 3 years of follow-up when most untreated Krabbe patients would have died, survival was 100 percent for the newborns transplanted in the first 6 weeks of life, and 43 percent for infants transplanted after the onset of symptoms. Infants who underwent transplantation before the onset of symptoms did not develop the neurological impairment characteristic of the disease. Those who did not undergo transplantation until after the appearance of symptoms did not show neurological improvement. This research demonstrates that the greatest benefit results from early intervention before clinical manifestations of Krabbe disease. This research suggests that newborn screening should be considered for Krabbe disease to identify infants who would benefit from early transplantation.

*GM1-gangliosidosis*

GM1-gangliosidosis is a rare genetic lysosomal storage disease characterized by progressive neurodegeneration leading to death, usually within the first 2 years of life. It results from a deficiency in the enzyme that breaks down Gm1-ganglioside leading to its accumulation in the brain. NIDDK-supported investigators have elucidated the mechanism of the neurodegeneration in a mouse model of the disease. The accumulation of GM1-gangliosides in the neurons leads to a disruption of certain cell components (endoplasmic reticulum) and calcium depletion. This in turn activates the "cellular unfolded protein response" (UPR) leading to neuronal cell death. These results indicate activation of the UPR is the cause of the neuronal cell death in Gm1-gangliosidosis. A similar mechanism may occur in other neurodegenerative diseases.

*Focal segmental glomerular sclerosis (FSGS)*

Focal segmental glomerular sclerosis (FSGS) damages the filtering units of the kidneys, thereby allowing protein and sometimes red blood cells to leak into the urine. Many patients with FSGS progress to end-stage renal disease. In one recent study, researchers studying a large family with hereditary FSGS identified a mutation in the *TRPC6* gene, which encodes an ion channel in crucial cells within the kidney. The mutation produces a protein with altered subcellular distribution that is hypersensitive to stimulation. In a second study, researchers described in fine detail the subcellular localization of the normal *TRPC6* protein within the kidney filters and identified a number of important structural proteins that interact with this ion channel. They then identified five families with hereditary FSGS, and found each had a different mutation in the *TRPC6* gene. When expressed in cultured cells, two of these five mutations resulted in increased ion flow across the cell membrane—suggesting that the mutant proteins may alter normal functions in the kidney filters. These two advances identify a novel mechanism for the kidney damage seen in FSGS. The development of agents that target the mutated TRPC6 protein may be a useful strategy in the treatment of chronic kidney disease.

*Hemochromatosis*

An important connection has been identified between two molecules involved in maintaining the delicately balanced metabolism of iron. Hemochromatosis is a disease in which abnormal iron metabolism results in the accumulation of toxic iron levels—termed iron overload—that eventually damage the liver, heart and other organs. Recent studies to combat this problem have focused on the hormone hepcidin, which is known to be a key player in the regulation of iron metabolism. Although deficiency in hepcidin has been implicated in some forms of hereditary hemochromatosis, the precise mechanism for hepcidin regulation of iron levels was not known. Scientists recently identified the protein ferroportin (Fpn), an iron exporter on the surface of some cells, as a receptor for hepcidin. In cell cultures, the binding of hepcidin to Fpn resulted in internalization and degradation of the complex, thereby preventing iron export by Fpn. Because Fpn exports iron absorbed by intestinal cells into the circulation, hepcidin-mediated destruction of Fpn may be key to regulating the dietary iron equilibrium. Researchers then studied several mutations in the Fpn gene that are linked to one type of hereditary hemochromatosis, and found that they either produced a protein that never arrives at the cell surface or one that does not

internalize and degrade in the presence of hepcidin. Taken together, these findings suggest that loss of hepcidin regulation of Fpn levels—caused either by Fpn mutations or by deficiency in hepcidin—could explain the abnormal iron accumulation observed in hemochromatosis patients. A fuller understanding of the hepcidin-Fpn pathway in iron regulation will help to provide the foundation for future research aimed at treating or preventing iron overload disorders.

*Porphyria*

Researchers studying a protein that helps maintain core body temperature have found an important link to understanding porphyria. Porphyria is a rare but serious condition characterized by acute attacks of abdominal pain, severe psychiatric and neurological problems and sensitivity to sunlight. The attacks occur in susceptible people as a result of fasting or of taking certain drugs or hormones. Susceptibility is caused by any of several rare mutations that interfere with biosynthesis of an essential iron-containing compound called "heme." Heme is a central ingredient in hemoglobin, a major component of red blood cells, required for ferrying oxygen throughout the body. Heme is also used in every cell of the body as part of the energy-utilization machinery; in the liver, where it plays a vital role in neutralizing toxins, as well as many drugs; and in a variety of other proteins throughout the body. Since too much heme is toxic, its production is tightly controlled. Researchers studying PGC-1$\alpha$, a protein that helps the body respond to cold temperatures by boosting energy utilization, noted that the protein is also up-regulated in the liver in response to fasting. Because fasting is also known to trigger attacks of porphyria, the scientists tested whether PGC-1$\alpha$ is also responsible for up-regulating heme biosynthesis—and found that it is. These results not only help explain what triggers porphyria attacks, they also clarify how the attacks can be blunted by treatment with glucose, and represent another piece in the complex puzzle of how the body maintains energy balance.

*Cystic fibrosis (CF)*

Cystic fibrosis is the most common fatal genetic disease in Caucasians, affecting approximately one in 3,000 newborns. Patients are diagnosed in early childhood with symptoms such as failure to thrive. With management of the nutritional problems and infections, the life expectancy for people with CF has increased to over 30 years. CF is caused by mutations in the gene encoding the CFTR protein, which resides in the outer surface of cells lining the lung and intestine, where it regulates the movement of chloride. The most common mutation of the gene, $\Delta$F508, yields a protein that does not fold properly and is thus degraded before it reaches the cell surface. NIDDK-supported investigators have identified a class of small molecule by a high-throughput screening method that corrected the processing of $\Delta$F508 CFTR, thus increasing its transport to the cell surface. The investigators showed that this molecule caused a significant increase in CFTR function. These studies identified the bisaminomethylbithiazoles as the most promising class of compounds for further development. These compounds may yield a drug that could be useful in the treatment of CF caused by $\Delta$F508 CFTR mutation.

*Tuberous sclerosis complex (TSC)*

Tuberous sclerosis complex (TSC) is an autosomal dominant tumor disorder with an incidence of 1 in 6000 live births. This disorder can result in multiple tumors of the kidney, brain, heart and lung. TSC is caused by mutations in either of two genes, TSC1 and TSC2, which code for the proteins, hamartin and tuberin, respectively. These two proteins form a complex that inhibits a cellular kinase, mTOR. When either of these proteins is mutated, the mTOR signaling pathway is activated. NIDDK-supported researchers studied whether an mTOR inhibitor, CCI-779, or interferon-gamma could decrease the development of tumors in a mice model of TSC2. Interferon-gamma is a known modifier of TSC. Treatment of the mice with either of these agents resulted in a reduction in the number of kidney angiomyolipomas and increased survival. These results provide preclinical data suggesting that these drugs may be useful in treating patients with TSC.

**Rare Diseases Research Initiatives**

The NIDDK has joined the NINDS, NIMH, NCI, NIAMS, and the Tuberous Sclerosis Alliance in cosponsoring a Program Announcement entitled, "Understanding and Treating Tuberous Sclerosis Complex." The purpose of this initiative is to encourage research to understanding or treat Tuberous Sclerosis Complex (TSC). The genes that cause TSC, TSC1 and TSC2 are known, understanding of the pathways in which they act is increasing, and animal models now exist that mimic certain features of the disease. As a result, there is a remarkable opportunity to increase knowledge about the mechanisms that cause TSC and translate this knowledge into therapies for this often devastating disorder. Included within the scope of this Program Announcement are studies of the molecular genetic, developmental, and pathophysiological studies, preclinical therapy development, and clinical research.

The NIDDK has joined NICHD and NIDCD in cosponsoring three Program Announcements on "Innovative Therapies and Clinical Studies in Screenable Disorders." The purpose of these Announcements is to encourage translational research on potential therapeutic interventions for conditions that can be screened for in the newborn and "high priority" genetic conditions for which screening could be possible in the near future. Special emphasis is placed on research relating to some of the "high priority" conditions identified in the report, "Newborn Screening: Toward a Uniform Screening Panel and System," which the Maternal and Child Health Bureau sponsored and the American College of Medical Genetics (http://mchb.hrsa.gov/screening) developed. In these Program Announcements, a "high priority" condition is a condition for which the development of an effective therapy would make the condition amenable to newborn screening. Research designed to understand the natural history of these conditions will facilitate the identification of potential therapeutic targets, with an ultimate goal of developing more targeted and effective therapies.

The NIDDK funded a grant to support the development of the International Registry For Hereditary Calcium Stone Diseases. The Registry will include data from patients with Primary Hyperoxaluria and Dent Disease.

The NIDDK has joined NINDS and NIA in cosponsoring a Program Announcement entitled, "Targeting Diseases Caused by Protein Misfolding or Misprocessing." The purpose of this initiative is to invite applications to identify and optimize small molecule reagents of any kind that specifically ameliorate a protein folding or processing defect in inherited diseases. Because so many mutant proteins, once folded and exported to their normal location, retain some functional activity, "chemical chaperones" have potential to rescue mutant phenotypes in many diseases, including many which are rare. Agents which selectively upregulate or modulate the cell's endogenous chaperone and quality control activities may also prove useful. The initiative solicits applications for research efforts at all stages in the therapeutic continuum.

In March 2004, the NIDDK sought to encourage cystic fibrosis (CF) research by issuing an RFA, entitled "Cystic Fibrosis Research and Translational Core Centers," to support both basic and clinical studies. As a result of this RFA, two CF Research and Translation Core Centers were funded. The first Center will emphasize the translation of basic knowledge into applied therapeutics. The second will facilitate the discovery and evaluation of novel small-molecule therapies for CF.

**Rare Diseases-Specific Conferences**

On September 19 and 20, 2005, the Primary Sclerosing Cholangitis Research Workshop was held at the National Institutes of Health. This meeting was sponsored by the NIDDK, ORD and the Morgan Foundation. The aims of the meeting were to address current clinical issues and investigative efforts, identify research hurdles and to define and prioritize future research needs in order to advance diagnosis and develop effective treatments. Over 115 meeting participants attended with a national and internationally recognized faculty. Recognizing the need to create an atmosphere that promoted dialogue between clinicians and investigators, ample time for questions and discussions were included after each session. The final session of the meeting focused on the investigative needs and engaged the entire audience. A meeting summary manuscript by the organizers is in progress which will include a list of research needs identified by the audience that will be submitted for publication.

On August 24 and 26, 2005, the NIDDK and ORD supported the "Sixth Annual Symposium on Familial Amyloidotic Polyneuropathy and Other Transthyretin-Related Disorders," and the "Fifth International Workshop on Liver Transplantation in Familial Amyloidotic Polyneuropathy." A pre-meeting was organized on August 23 to discuss how to encourage crosscutting research that could move the field closer to new clinical applications that would reach across the amyloidoses. Of the 18 individuals invited to the pre-meeting, some also attended the concurrent conferences and others came in specifically for the pre-meeting. The group developed a list of research priorities for this area of research. A second meeting is being planned for 2006.

Two special issues of Urologic Research in November and December 2005 and two special sections in the American Journal of Nephrology (March-April and May-June 2005) contained papers presented at the NIDDK-supported meeting "7th International Workshop on Primary Hyperoxaluria." This included a significant paper about consensus diagnostics by Milliner,

entitled “The primary hyperoxalurias: an algorithm for diagnosis.” Am J Nephrol. 2005 Mar-Apr;25(2):154-60.

**Activities with Rare Diseases Patient Advocacy Groups**

On January 24 and 25, 2005, a Symposium was held on “Glomerular Disease,” where the patient advocacy group, NephCure, participated in the planning process. The purpose of the meeting was to discuss recent observations and potential opportunities for improving diagnosis and therapeutic interventions in the areas of focal segmental glomerulosclerosis (FSGS) and glomerulonephritis**.** Based on the outcome of this Symposium, the NIDDK is planning to develop an initiative for research in glomerular disease in 2006.

# NATIONAL INSTITUTE ON DRUG ABUSE (NIDA)

## Overview of Rare Diseases Research Activities

## Background

The National Institute on Drug Abuse (NIDA) provides national leadership on the prevention and treatment of drug addiction by conducting and supporting biomedical and behavioral research, health services research, research training, and health information dissemination. NIDA plans, conducts, fosters, and supports a comprehensive program of research and research training relating to the causes, prevention, treatment, patterns, and consequences of drug abuse and addiction. Research is performed in NIDA's own laboratories and through contracts and grants made to scientific institutions and individuals. Training relevant to fundamental sciences and clinical disciplines of drug addiction is performed via institutional and individual research training awards, and through collaborations with other research institutes and federal health agencies. NIDA collects and disseminates research findings and related educational materials for health professionals, educators, and the public. In addition, NIDA coordinates with institutions, professional associations, and agencies both domestically and abroad that specialize in the treatment and research of drug addiction. Specifically, NIDA coordinates with the Substance Abuse and Mental Health Services Administration (SAMHSA) on services research issues as well as on other programmatic issues.

## Size of Drug Addicted Population

In the United States (U.S.), opiates such as heroin and other narcotics are abused by more than 1 million persons, and stimulants such as cocaine and “crack cocaine” by more than 2 million (National Survey on Drug Use and Health, 2004). However, even the lowest estimates from any source put addiction levels for these substances at figures well above the 200,000 threshold generally used for defining orphan products. Incidence and prevalence figures for addiction to controlled substances are difficult to estimate, as they vary from type of drug, community, and supply availability (generally a function of supply interdiction/law enforcement). Some addiction indications affect less than 200,000 persons in the U.S. The total cost to society of drug abuse and addiction in the U.S. has been estimated to exceed $500 billion per year (including alcohol and tobacco).

## Drug Addiction as an Orphan Disease

Although drug addiction is a serious public health concern, historically, it has been treated as an orphan disease because the pharmaceutical industry rarely profits from marketing drugs for the treatment of addiction, and there is little incentive for pharmaceutical companies to pursue research and development of new treatment medications for this population. Although total numbers of persons afflicted may seem sufficient in the aggregate, unlike other disease states, many of these persons are not treatment-seeking upon diagnosis. Therefore, the actual patient population is less than the total number of persons afflicted. Additionally, many of these persons will be treated in publicly funded clinics where companies perceive reimbursement as modest or

inadequate and perhaps subject to artificial cost controls. Finally, much of the U.S. treatment system is based on non-pharmacological treatment modalities.

A further complication is that some treatment agents may themselves be abusable and will be strictly controlled. For example, methadone is classified as a Schedule II controlled substance for use in opiate maintenance therapy. Some 900 U.S. clinics licensed to dispense methadone serve approximately 190,000 persons per year, with a pharmaceutical market value of only about $40 million per year. When compared to other treatment indications, most manufacturers view the drug addiction treatment market as unattractive based on low projected return on investment. Each of these points is well documented in the Institute of Medicine Report on *the Development of Medications for the Treatment of Opiate and Cocaine Addiction, 1995,* and is well known to the pharmaceutical and market research industries. Therefore, while opiate and cocaine addiction do not strictly fit the definition of orphan products, in practice, they certainly are treated as such. An example was the development and approval of levomethadyl acetate hydrochloride (trade name ORLAAM), an alternative to methadone for the treatment of opiate addiction. Despite the facts that human data on 6,000 subjects from government-sponsored studies was available for ORLAAM and the government had a large supply of the compound available for anyone interested in obtaining a New Drug Application (NDA), no private sector entity was interested in completing the development of this compound. Ultimately, NIDA contracted with a company to complete the work. Similarly, the development of the narcotic antagonist naltrexone was largely a NIDA-funded effort. Therefore, these products should be viewed as entirely "orphan-like" insofar as their ability to attract private sector sponsors.

**History of NIDA Rare Diseases Research**

Currently, there are four medications for the treatment of opiate addiction that have received orphan product designation. These drugs are ORLAAM, naltrexone, buprenorphine, and buprenorphine combined with naloxone. NIDA was substantially involved in the development of these products. ORLAAM received NDA approval in 1993. Naltrexone, an opiate antagonist for use in detoxified patients, was approved in 1985. Currently, orphan exclusivity for ORLAAM and naltrexone has expired. Additionally, ORLAAM's distributor notified physicians that distribution of the product would be discontinued in 2004, due to poor sales in the U.S. and its withdrawal from European markets. ORLAAM's orphan product designation expired in 2000, and thus there is no legal requirement for a manufacturer to maintain the product in the U.S. market.

The opiate partial agonist buprenorphine and a combination of buprenorphine plus naloxone have also received orphan designation (see details below) and were approved for marketing in the U.S. on October 8, 2003. These products represent a major success, as the FDA designated them both as orphan products. Buprenorphine became the first product to receive an orphan designation based on economic rather than population based rationale (i.e., the product would not recoup its developmental expenses in 7 years of exclusivity in the U.S. market.)

## Recent Scientific Advances in Rare Diseases Research

The discovery of opiate receptors by NIDA-funded scientists in the 1970s opened a new era of neurobiological research that is ongoing. Scientists continue to map brain receptor system types and subtypes, continuously gaining understanding of their structure and function. This information could help design interventions (behavioral, chemical, and genetic) that could be used to treat brain disorders.

A generation of research has shown that drug addiction is a complex biomedical and behavioral disease that affects parts of the brain that underlie and mediate human emotions. Hence, drug addiction is a brain disease that can and often should be treated with medicine.

Medications reestablish normality to brain function and behavior so that the addicted patient has the *opportunity* for rehabilitation through counseling, psychotherapy, vocational training, and other therapeutic services. While the mechanisms of many central nervous system disorders are still to be elucidated, scientists working in the field of drug abuse have now identified and cloned the putative site of action in the brain for every major drug of abuse. In addition, recent application of microarray technology to characterize drug effects on gene expression has identified intracellular proteins that are altered by drugs of abuse after their initial receptor interactions. Thus, the potential to develop new treatments is enormous.

## Rare Diseases Research Initiatives

As described in the history section above, NIDA considers medications for the treatment of drug addiction to be de facto orphans. Thus, the development of medications for the treatment of drug addiction could be considered rare diseases research occurring within the context of an urgent public health need and a wholly inadequate private sector response. Therefore, NIDA's Medications Development Program effort can be considered as part of a rare diseases research initiative.

## Functions of the Division of Pharmacotherapies and Medical Consequences of Drug Abuse

In 1990, the Medications Development Division (MDD) was established in NIDA. In 1999, the MDD became the Division of Treatment Research and Development (DTR&D). In 2004, DTR&D became part of the Division of Pharmacotherapies and Medical Consequences of Drug Abuse (DPMCDA). The functions of MDD within the new division remained the same; namely, DPMCDA conducts studies necessary to identify, develop, and obtain FDA marketing approval for new medications for the treatment of drug addiction and other brain and behavior disorders; develops and administers a national program of basic and clinical pharmacological research designed to develop innovative biological and pharmacological treatment approaches; supports training in fundamental sciences and clinical disciplines related to the pharmacotherapeutic treatment of drug addiction; collaborates with (a) the pharmaceutical and chemical industry in the U.S. and other nations, and (b) other federal medications development programs; (c) academia, and works closely with the FDA to assure that research designed to show the clinical efficacy of new compounds is evaluated and approved expeditiously. In addition, the DPMCDA

operates within the larger context of a NIDA-wide Medications Development Program that incorporates basic research discoveries from both the intramural and extramural communities in the quest to develop new pharmacological treatments. This enables DPMCDA to access the latest research findings and test them in controlled clinical settings.

NIDA's current efforts are placing more emphasis on discovery and development of medications for treating cannabis and stimulant addiction, since there are no FDA-approved products for the treatment of these indications. Clinical trials for stimulant dependence primarily test marketed medications in collaboration with pharmaceutical companies. Efforts are also directed toward supporting, through grants and contracts, synthesis of novel compounds for screening and pharmacological testing.

Significant areas of research and development are summarized below:

**1.** *Opiate addiction treatment*

*Buprenorphine/Buprenorphine-Naloxone Combination***:** A major milestone and achievement for NIDA's Medications Development Program and for Reckitt Benckiser Pharmaceuticals, Inc. (NIDA's collaborator in a Cooperative Research and Development Agreement) was the October 8, 2002, FDA approval for the marketing of two new products for the treatment of opiate addiction. These two new products, known under the trade names Subutex and Suboxone, represent new tools in the arsenal of anti-addiction medications. Both have been designated as orphans, based on the expectation that these products will not recoup their developmental expense during their period of U.S. marketing exclusivity. Marketing of these products began in January 2003, but is subject to certain restrictions imposed by U.S. law and regulations. Nevertheless, these products may, under the conditions specified in law and regulations, be prescribed in a variety of settings, including physicians' offices. These medications offer additional treatment options to physicians and patients, and should expand treatment availability.

Subutex and Suboxone now join methadone as medications for the treatment of heroin and opiate addiction. Their availability represents the culmination of several years of research and development between NIDA and Reckitt Benckiser Pharmaceuticals, Incorporated. The unique pharmacology of Subutex (buprenorphine) and Suboxone (buprenorphine combined with naloxone) and the statutory changes enacted by the Drug Addiction Treatment Act of 2000, as contained in P.L. 107-273, "The Children's Health Act of 2000," authorizes appropriately trained physicians to prescribe these products in settings other than the existing, but limited, Opiate Treatment Programs (OTPs). In addition to increasing treatment availability across the U.S., these new medications provide another option for patients who either have no OTP programs available, or who cannot avail themselves of these programs. As of the date of this report, SAMHSA reports that 9,808 physicians have taken the training required by law to prescribe these two medications, and 6,767 have registered as providers.

*Depot Naltrexone***:** Naltrexone, a marketed long-acting, orally effective opioid antagonist, was approved in 1983 for blocking the pharmacological effects of exogenously administered opiates.

It is an adjunct to the maintenance of the opioid-free state in detoxified, formerly opioid-addicted individuals.

One of the major obstacles to the success of naltrexone has been patient compliance with therapy. Naltrexone must be taken at least three times per week and has no effect other than to block the effects of heroin. Because of this, many patients forget to take or choose to stop taking their medication. Therefore, the greatest success with naltrexone has been in a limited population of highly motivated, formerly opiate-addicted patients.

During 1999, NIDA completed, via a Small Business Innovative Research (SBIR) grant to Biotek, Inc., the production and preclinical testing of a batch of 120 doses of a sustained release form (depot) naltrexone. These doses produced a blood level of about 2 ng/ml, which remains relatively constant over a 30-day period when administered subcutaneously in humans. Depot naltrexone, in an inpatient clinical study, blocked the subjective response to 25 mg IV heroin challenges for at least 4 weeks in most subjects. This study was completed in 2000. A two-site outpatient double-blind study was subsequently carried out to test the product in a real-world setting. This outpatient study began in November 2000 and was completed in 2003. The results were reported at a scientific meeting in June 2004 and are pending publication. The results indicate a significant effect of the depot preparation to limit relapse to heroin in the trial.

Additionally, the NIDA Intramural Research Program conducted clinical trials to test another investigational sustained release formulation of naltrexone supplied by Alkermes, Inc. This study provided safety and duration of effect information on this potential treatment product. Alkermes is seeking approval initially for the depot product to treat alcohol abuse.

The oral form of naltrexone was approved in 1994. Alkermes reports that their dosage form of depot naltrexone reduces heavy drinking behavior in males (but not females). They have received an approvable letter from the FDA.

Thus, the feasibility of a sustained-release formulation of naltrexone for the treatment of opiate and alcohol addiction is moving rapidly from concept to clinic, towards potential regulatory approval and marketing. The product could be marketed by mid-2006.

**2.** *Cocaine addiction treatment*

Several small studies of potential cocaine addiction treatment agents have been completed and are in various stages of data analysis. Significant findings will be followed up in larger clinical trials as warranted. In addition, NIDA continues to work toward the discovery and development of new molecular entities, such as kappa opioid antagonists, for cocaine addiction treatment.

*Ondansetron***:** A 5HT3 antagonist that can block dopamine release and increase GABA tone and has shown efficacy in reducing cocaine use at a dose of 8 mg/day in a recently completed NIDA study. A follow-up Phase IIb study to explore and confirm the results of the Phase IIa study with two doses of Ondansetron at 8 mg and 16 mg and placebo is being considered.

*Modafinil*: A non-dopaminergic stimulant that has shown reduction in cocaine use in a pilot study (Dackis 2004). It is currently being studied in a multisite trial for confirmation of efficacy. Recruitment for this study is under way; two-thirds of the subjects have been recruited. Two NIDA grant projects are also pursuing a variation on this protocol independently.

*Atomoxetine*: A norepinephrine uptake inhibitor approved for ADHD, currently in Phase I trials for safety in cocaine-abusing subjects. A Phase II study in co-morbid cocaine subjects with ADHD is planned, pending the outcome of the safety study.

B*aclofen*: A GABA-B agonist that has been shown in a pilot trial to reduce cocaine use in heavy users. Data analysis of a multisite clinical trial is underway.

*Disulfiram***:** Antabuse, marketed as aversive therapy for treating alcoholism; shows promise in the treatment of cocaine addiction.

Three efficacy trials conducted with different populations of cocaine-addicted individuals suggest that disulfiram, in combination with each of three different therapeutic interventions (cognitive behavioral treatment, 12-step facilitation, and clinical management), might be effective in treating cocaine addiction. In cocaine-alcohol abusers, disulfiram treatment showed sustained effect on reduced cocaine and alcohol use 1 year after cessation of the therapy. Disulfiram treatment of cocaine abusing, opioid-addicted patients maintained on methadone resulted in significant decrease of the amount and frequency of cocaine use. A preliminary study showed that disulfiram also decreases cocaine use in cocaine-opioid addicts maintained on buprenorphine.

NIDA is currently sponsoring two large outpatient clinical trials with disulfiram as the treatment for cocaine addiction: (1) a study on 160 opioid-cocaine-addicted patients maintained on methadone, conducted at the University of Arkansas; and (2) a study on 150 opioid-cocaine-addicted patients maintained on buprenorphine, conducted at Yale University.

A study conducted at the University of Pennsylvania evaluating disulfiram and naltrexone alone and in combination treatment of 208 alcohol-cocaine addicted individuals was completed in October 2004. The combination of medications was more likely to be associated with 3 weeks of continuous abstinence than either medication alone or placebo.

Finally, NIDA has completed a Phase I clinical pharmacology/safety study examining the interactions between disulfiram and intra venous administered cocaine. Data analysis will begin soon. A large-scale Phase III multi-center trial testing disulfiram in cocaine addicted patients is in consideration.

*Vigabatrin***:** A GABA agonist that has been shown in animal models to decrease self-administration of cocaine and to block dopamine release following cocaine administration in PET imaging studies. A Phase II trial for cocaine dependence is planned as a proof-of-concept study of Vigabatrin against placebo to assess efficacy in reducing cocaine use. A 5 site trial is planned; 135 patients will be recruited.

*Talampanel*: An AMPA antagonist that blocks stress-induced reinstatement in preclinical studies. This mechanism may make it a candidate for Phase II testing in a relapse prevention paradigm. Prior to the Phase II study, a Phase I cocaine safety interaction study will be conducted.

*Dopamine Agonists***:** The activation of the dopaminergic reward system in the brain appears to be the principal neurochemical mechanism involved in the addiction to stimulants such as cocaine and amphetamine. Chronic abuse of these drugs results in dopamine deficiency in the brain, which has been hypothesized to lead to stimulants craving, depression, anhedonia, and dysphoria.

Most recently, studies in rodents, and to a lesser extent in monkeys, have differentiated the roles of D1 and D3 receptors with regard to cocaine. The D1 system may inhibit the effects of cocaine, while the D3 system may block conditioned cues. Compounds that affect both systems are under study.

*Kappa Opioid Antagonists***:** While the discovery of a selective kappa opioid antagonist for potential use in preventing relapse to opiates has been a goal of NIDA for several years, the recent discovery and evaluation of JDTic, a highly selective kappa antagonist synthesized by a NIDA grantee, has provided a rationale for effectiveness in preventing relapse to cocaine. Interestingly, JDTic has been shown to prevent stress-induced reinstatement of response for cocaine in a rat model of cocaine relapse. The compound is currently undergoing initial preclinical safety testing to determine its development potential as a medication.

*Glucocorticoid and Corticotropin Releasing Factor (CRF) Antagonists***:** Studies have shown that cocaine causes the release of stress hormones known as glucocorticoids in both rats and humans. Evidence from rat studies suggests that glucocorticoid antagonists and CRF antagonists reduce cocaine self-administration in a dose-related manner. NIDA will follow up on these basic research findings with additional studies aimed at developing a potential treatment for cocaine addiction. DPMCDA is attempting to obtain CRF antagonist compounds from pharmaceutical company sources.

*Cocaine "Receptor" Imaging Studies***:** In addition to the categories of compounds being tested as described above, a new imaging technology is being investigated for its value in predicting efficacy of potential cocaine treatment medications. Research in the field of structure-activity relationships has revealed highly selective and potent binding ligands for the dopamine transporter. NIDA intramural researchers have identified three "generations" of such compounds, with each succeeding generation being more selective and potent than the previous one. RTI-55, the first potent compound, was shown to be an effective *in vivo* labeling agent in animal studies and was subsequently examined in human imaging studies by SPECT. A second compound, RTI-121, was found to be more selective for the dopamine transporter but had a higher apparent lipid solubility and exhibited a lower specific-to-nonspecific binding *in vivo*. NIDA researchers are testing new compounds and are also utilizing some older compounds (e.g., WIN-35,428) in brain imaging studies. Procedures have been developed for estimating the

occupancy of transporter sites *in vivo*. Dopamine transporter imaging studies of cocaine abusers have been completed. This technology may make it possible to estimate the effectiveness of a potential treatment compound or regimen by correlating receptor occupancy (as shown in imaging studies) with actual clinical results. NIDA will continue to follow this line of research.

Additionally, NIDA is participating in an effort with NIMH, NINDS, and NIAAA to develop appropriate imaging ligands that will be essential to the study of many brain and CNS conditions, as well as the effects of various treatments.

*Immunology***:** DPMCDA presently supports an immunotherapy program for the treatment and prevention of drug abuse. This program has led to the clinical development of vaccines for cocaine and nicotine addiction. Although these vaccines are being developed as therapeutic vaccines, their potential as prophylactic vaccines could be explored once efficacy is established in patient populations. This program has also led to the preclinical development of monoclonal antibodies, which have been designed to rapidly reverse the effects of methamphetamine, MDMA, and phencyclidine overdose. If successful in humans, monoclonal antibody treatment will not only provide a rapid reversal of drug effects in an emergency room setting, but could also reduce or prevent the long-term medical problems (neurotoxicity and addiction) associated with stimulant drugs of abuse.

*Cocaine Vaccine*: There is presently no established pharmacotherapy for cocaine abuse, but active immunotherapy with a cocaine vaccine has potential as a therapeutic intervention. The cocaine vaccine TA-CD (owned by Celtic Pharma/Xenova) was developed through a NIDA-funded grant. Evaluation of safety, efficacy, and immunogenicity of the vaccine at Yale University in an open-label, 14-week dose-escalation study in 18-cocaine dependent subjects showed that the vaccine was well tolerated, that subjects reported attenuation of cocaine's euphoric effects, and that cocaine specific antibodies persisted at least 6 months (Martell BA, et al., 2005). Dr. Tom Kosten at Yale University presently has a NIDA-funded research grant for Phase II efficacy testing of the vaccine in 132 methadone-maintained cocaine abusing subjects.

*Treatment of PCP Abuse and Overdose*: NIDA has been funding a project, currently in its 12th year, to Dr. Michael Owens at the University of Arkansas to develop a new generation of monoclonal antibody-based medications for treating PCP overdose. These medications function as pharmacokinetic antagonists, and are designed to provide a rapid reversal of drug effects in an emergency room setting, as well as reduce or prevent the long-term medical problems associated with their use. In rats, a single dose of a mouse anti-PCP mAb reduced the locomotor effects and brain concentrations of PCP in overdose studies (Hardin JS, et al., 2002). The antibody has also proven effective in preventing the adverse health effects of PCP in rats in chronic dosing studies (Laurenzana EM, et al., 2003). A chimeric (mouse/human) form of the mAb has been reported (Lacy and Owens, in press).

NIDA is funding a Phase I study of an STTR Fast Track grant to Inflexion Therapeutics (in collaboration with Dr. Owens) for the large-scale agricultural production of the chimeric anti-PCP monoclonal antibody in tobacco plants, as well as the advanced preclinical development needed for Investigational New Drug filing (IND). If the IND is approved, a Phase II

collaboration with Dr. Tom Kosten, is planned to test the effects of the PCP antibody in PCP users in outpatient treatment. Thus, the initial efficacy testing will be done on outpatients before possible testing of PCP overdose in emergency room settings.

*Treatment of Methamphetamine Abuse and Overdose***:** NIDA is presently funding research by Dr. Michael Owens for the development of a new generation of monoclonal antibody-based medications for treating the abuse and overdose of methamphetamine, amphetamine, and ecstasy (MDMA). These medications would function as pharmacokinetic antagonists, and are designed to provide a rapid reversal of drug effects in an emergency room setting, as well as reduce or prevent the long-term medical problems associated with their use. The components of the project are focused on the production of highly selective and potent fully human anti-methamphetamine, anti-amphetamine, and anti-MDMA monoclonal antibodies; behavioral pharmacology studies to test the effectiveness of the medications in reducing the addictive properties of these drugs in animal models; pharmacokinetic and pharmacodynamic studies for evaluating the fundamental medical consequences of rapid input of these drugs into the central nervous system and the protective effects of these medications in overdose situations; and the large-scale production of these antibodies in tobacco plants. The team has been successful in producing fully human anti-methamphetamine and anti-amphetamine monoclonal antibodies that block self-administration (in rats) of methamphetamine and amphetamine, respectively, at specific dose levels (Byrnes-Blake KA, et al., 2003). They have also assessed the dose levels that are necessary to reverse drug effects in animal models, and have made advances in developing the technology for large-scale production of monoclonal antibodies in plants. This technical innovation makes the monoclonal antibody approach commercially feasible.

*3. Methamphetamine addiction treatment*

Methamphetamine is a potent psychomotor stimulant that has gone through episodic periods of widespread use and abuse in the U.S. Cocaine abuse and addiction surpassed use of methamphetamine in the 1970s and 1980s, but methamphetamine abuse and addiction has been re-appearing in many regions of the U.S. and is widespread in western U.S. cities such as San Francisco, San Diego, Los Angeles, Denver, and Phoenix. According to the 2005 National Survey on Drug Use and Health, approximately 12 million persons (aged 12 and older) had used methamphetamine at least once in their lifetimes, 1.4 million had used it in the past year, and 600,000 had used it in the past month.

There are no accepted treatment medications for methamphetamine addiction or abuse. As a result, NIDA has developed a Medications Discovery Program for methamphetamine and is funding a number of extramural and intramural studies to develop medications to treat methamphetamine abuse.

*Ondansetron***:** There is evidence that selective serotonin 5-$HT_3$ receptor antagonists attenuate behavioral responses to d-amphetamine and methamphetamine, suggesting that 5-$HT_3$ receptors modulate brain dopamine in animals. This action of 5-$HT_3$ receptor antagonists may therefore reduce the rewarding effects of abused substances. Ondansetron is a selective 5-$HT_3$ receptor antagonist that decreases stimulated dopamine release and has been shown to reduce the

development of behavioral tolerance and sensitization to cocaine following a period of acute and chronic withdrawal. Furthermore, suggestions that 5-$HT_3$ antagonists may also reduce discomfort or post-cessation anxiety following psychostimulant withdrawal prompted NIDA to test whether Ondansetron might have these effects following cocaine use cessation. NIDA, through a Methamphetamine Clinical Trials Group (MCTG) established with UCLA, conducted a Phase II double blind, placebo-controlled, dose-response trial with Ondansetron. The study, completed in 2003, showed no significant effects for any Ondansetron dose compared to placebo. No further development is planned for this indication for Ondansetron.

*Aripiprazole*: This is a typical neuroleptic drug that has been approved by the FDA to treat schizophrenia and bipolar mania. It is a functional antagonist at the dopamine $D_2$ receptor level in a hyperdopaminergic environment, a functional agonist at the same level in a hypodopaminergic environment, a serotonin antagonist with serotonin 5-$HT_{2A}$ receptors, and a partial agonist with serotonin 5-$HT_{1A}$ receptors. It has moderate affinity for $alpha_1$-adrenergic and histamine ($H_1$) receptors. Long-term methamphetamine abuse results in schizophrenia-like symptoms. By extension, aripiprazole may have potential as a methamphetamine abuse therapeutic. NIDA is currently conducting a Phase I safety interaction study with aripiprazole and methamphetamine at UCLA and NYU.

*Lobeline*: This medication is a derivative from Indian tobacco plants. Because it stimulates a subclass of nicotine receptors, it was tested in the clinic for its potential as a smoking cessation therapeutic. Additionally, lobeline redistributes dopamine in nerve terminals by preventing dopamine uptake into synaptic vesicles without inhibiting MAO-B. In contrast, methamphetamine enters synaptic vesicles and inhibits MAO-B. Studies in rats revealed that lobeline decreases methamphetamine self-administration without affecting the rats' ability to self-administer sugar water. These data suggest that lobeline may reduce acute rewarding effects of methamphetamine and corresponding abuse liability. NIDA is collaborating with a pharmaceutical company to conduct a Phase I double-blind, placebo-controlled, ascending-dose pharmacokinetic, safety, and tolerability study of lobeline, with plans to test it as a methamphetamine abuse therapeutic.

*Bupropion*: This medication has been approved to treat depression and promote smoking cessation. It is a dopamine uptake inhibitor that is well-tolerated and has a good safety record. NIDA is testing bupropion for its ability to alleviate the dysphoria seen in early abstinence and reduce methamphetamine craving and relapse to drug using. A Phase II, double-blind, placebo-controlled study with bupropion is now complete. Analysis of the primary outcome showed a trend for significance ($p=0.09$) for the total sample ($n=150$ patients). However, when the group was split according to baseline use into high and low/moderate users, bupropion was significantly efficacious in reducing methamphetamine use for the low/moderate users ($p=0.03$). No effect was seen for the high users. A follow-up Phase II study is under consideration to examine bupropion's efficacy in the low/moderate use population.

*Modafinil*: This is a non-dopaminergic stimulant used to treat narcolepsy through modulation of the glutamate/GABA system. Recently, it has been shown that Modafinil blunts cocaine euphoria under controlled conditions and improves clinical outcomes in cocaine-addicted

patients receiving standardized psychosocial treatment. Because methamphetamine is a stimulant like cocaine, NIDA is endeavoring to evaluate Modafinil's potential as a methamphetamine abuse therapeutic. NIDA is in the process of conducting a Phase I safety trial, as requested by FDA, prior to conducting a Phase II trial.

*Topiramate***:** This medication is a fructopyranose derivative approved for treating seizure disorders. In a clinical trial, it was found to be superior to placebo in improving drinking outcomes in alcohol-addicted individuals. Data from a pilot proof of concept study suggest that topiramate may be a useful medication for preventing relapse to cocaine use. Shared stimulant properties of cocaine and methamphetamine may mean that topiramate could also help prevent relapse to methamphetamine use. To that end, NIDA is providing grant support to conduct a Phase I safety interaction study between topiramate and methamphetamine. This study was completed with no safety concerns. A collaboration is planned with OrthoMcNeil pharmaceuticals to launch a Phase II outpatient study for topiramate for methamphetamine dependence.

**4.** *Medications development for cannabis-related disorders*

The treatment of cannabis-related disorders (CRDs) is an issue of great public health concern. Currently, marijuana is the most commonly used illicit drug in the U.S., recent estimates from SAMHSA reporting 14.6 million past-month users. In 2004, marijuana was used by 76.4 percent of current illicit drug users. An estimated 56.8 percent of current illicit drug users used only marijuana, and 19.7 percent used marijuana and another illicit drug. Among persons aged 12 or older, the overall rate of past-month marijuana use was about the same in 2004 (6.1 percent) as it was in 2003 (6.2 percent) and 2002 (6.2 percent). In 2004, 3.2 million persons were using marijuana on a daily or almost daily basis over a 12-month period, similar to the estimates in 2002 and 2003.

Sufficient research has been carried out to confirm that the use of cannabis can produce serious physical and psychological consequences. The consequences of cannabis use may be due to the acute effects of the drug or to the chronic exposure that may ultimately produce addiction. The use of a large amount in a short period of time may induce hallucinations, delirium, and other perceptual manifestations similar to a psychotic episode. Chronic users of cannabis may experience difficulty in stopping or controlling drug use, develop tolerance to its subjective and cardiovascular effects, and eventually present withdrawal symptoms after sudden discontinuation of use.

Unfortunately, there is currently no effective pharmacological treatment for CRDs and limited research focused on identifying and developing medications to treat these disorders. Several factors support the timeliness of an accelerated program to develop medications to treat CRDs. First, newly marketed medications are available whose mechanisms of action may have potential therapeutic effects on the clinical manifestations of CRDs. Second is the recent discovery of an endogenous cannabinoid system with specific receptors and endogenous ligands. Third, the availability of genetically engineered knockout mice that lack functional cannabinoid receptors make it possible to study genetic predispositions to the effects of cannabinoids. Fourth, the

subsequent development of reliable preclinical models to study the rewarding and addiction-producing effects of THC, combined with the preceding, enable understanding of basic therapeutic mechanisms. Finally, the discovery and development of new chemical entities--some of them already being investigated at the clinical level--target the cannabinoid system and have beneficial therapeutic potential. All of these factors are setting the stage for the development of medications to treat CRDs.

5. *New research projects for medications development for cannabis-related disorders*

Based on the needs described above, NIDA funded several new research grants through RFAs, the goal of which was the development of safe and effective medications for the treatment of CRDs. Pre-clinical and clinical studies are focusing on the treatment of marijuana, hashish, or other cannabis derivatives use disorders. Medications studied under this RFA are aiming to treat CRDs, such as abuse and addiction, intoxication, delirium, psychosis, and anxiety. They are also focusing on the specific symptoms of the disorder, such as withdrawal, craving or relapse, complications, (e.g., cognitive impairment), sleep disorders/disruption of normal rhythms, or the clinical surrogates of use, such as depression and other mood disorders.

The rationale for choosing the medication(s) to be investigated is based on a top-down approach, a bottom-up approach, or both approaches combined. The top-down approach tests marketed medications available for other indications that may also be promising candidates for the treatment of CRDs. The bottom-up approach involves the identification and testing of new chemical entities that, because of their chemical characteristics and mechanisms of action, are being developed specifically for CRDs.

NIDA is currently funding research to evaluate the safety and efficacy of cannabinoid antagonists, anticonvulsants (valproic acid), antidepressants (e.g., fluoxetine, bupropion, selegiline), and antipsychotic medications (e.g., clozapine, olanzapine) for the treatment of cannabis dependence.

*Consequences of Drug Addiction*

As a result of the reorganization that created DPMCDA, the program of research on medical consequences of drug abuse and co-occurring infections now resides within this division. As described below, these studies involve marketed medications and would not typically be performed by the public sector. Under this program, the supported studies may be categorized into four major programs of research that are in various stages of development and progress:

1. Metabolic and endocrine disorders of HIV/AIDS and drug abuse;
2. Pharmacokinetic/pharmacodynamic drug-drug interactions between medications used in the treatment of drug addiction (e.g., methadone, newly FDA-approved buprenorphine), Infections (e.g., HIV, HCV, TB), and mental disorders;
3. Medical and health consequences of chronic use/abuse of licit and illicit drugs of abuse and co-occurring infections (e.g., HIV, HCV, TB, STDs); and

4. Oro-maxillary complications (e.g., facial and dental injuries) associated with drug abuse and co-occurring infections.

New avenues of research are planned that fit within these major themes:

- Metabolic and endocrine disorders of HIV/AIDS among drug abusers;
- Directly observed therapy for HIV-infected drug abusers;
- Hepatitis C;
- Issues in the medical management of HIV/HCV co-infections among drug abusers;
- Mini-symposium on TB among drug abusers; and
- Role of hormones and nutrition in drug abusers co-infected with HIV and HCV.

Injection drug use and sexual contact among users is a highly correlated vector in the spread of HIV, hepatitis, and tuberculosis, creating a public health problem of enormous magnitude. Medical consequences may range from drug addiction-based brain disease to effects on almost every organ system, including the central and peripheral nervous, cardiovascular, endocrine/hormonal, pulmonary/respiratory, renal, hepatic/metabolic, reproductive, immune, and other systems. For example, stimulants such as cocaine and methamphetamine increase the heart rate while constricting the blood vessels; in susceptible individuals, these two actions together set the stage for cardiac arrhythmias and strokes. Cocaine use decreases blood flow to the brain, increases heart rate, and elevates blood components that promote clotting effects, which can lead to stroke or heart attack even in those not otherwise at risk for these serious cardiovascular events. NIDA-funded research also shows that chronic cocaine use is associated with left ventricular dysfunction and increased calcium deposits in the coronaries of HIV-infected African-Americans, and that its use may also facilitate the entry of HIV into brain cells, leading to cognitive and memory impairment. The club drug methylene-dioxy-methamphetamine (MDMA), also known as "ecstasy," which many users mistakenly believe to be safe, has caused on rare occasions malignant hyperthermia, permanent kidney damage, and death. MDMA also damages serotonin nerve fibers in the brain. Heroin can cause a life-threatening kidney condition called focal glomerulosclerosis and PCP (phencyclidine) decreases heart rate and blood pressure, triggers violent aggression, and may cause muscle contractions strong enough to break a bone. Marijuana, often perceived by many as innocuous, is also associated with consequences ranging from memory to cognitive and motor problems in youths and adults (Khalsa, et al., 2002).

Injecting drug use further promotes blood clots, severe skin infections, and blood-borne infections, including life-threatening endocarditis, viral hepatitis, and HIV/AIDS. Abuse of some drugs is associated with impulsive sexual activity that elevates individuals' risks for acquiring and transmitting HIV/AIDS and other STDs.

In a relatively new area of research at NIDA, data show that nutrition may play an important role in HIV disease progression. Preliminary research shows that drug abusers with inadequate nutrition, particularly those with sub-optimal levels of anti-oxidant micronutrients like selenium and zinc, are at high risk of mortality if they are co-infected with HIV/AIDS. Clinical trials are under way to determine if supplementing with selenium, zinc, and other anti-oxidant micronutrients would slow the progression of HIV/AIDS. This research would have worldwide

implications, such that people in underdeveloped countries who cannot afford expensive antiretroviral therapy would benefit from an inexpensive treatment modality to slow disease progression and improve quality of life.

Research also shows that HCV is another blood-borne pathogen easily transmitted through contaminated drug injection paraphernalia. Furthermore, both viruses--HCV and HIV--frequently co-exist because of common routes of transmission. Liver injury seems to occur in HIV/HCV co-infection through the induction of a novel signaling pathway that is cooperatively activated by specialized protein molecules known as HCV E2 and HIV gp120, providing a rationale for therapeutic interventions. NIDA continues to support a wide spectrum of research on epidemiology, natural history, underlying pathogenesis, prevention, and treatment of HIV/HCV co-infections among drug abusers.

**Drug-Drug Interactions**

Research shows that some illicit drugs and drug abuse medications can interact with medications used for treating diseases, resulting in possible adverse effects and loss of efficacy. For example, an interaction can occur between methadone and the protease-inhibiting drugs that are currently the most effective treatments for HIV infection. This interaction can make the treatment ineffective and increase the toxic side effects from one or both drugs. In some cases, the presence of a protease inhibitor increases the metabolism (processing) of methadone sufficiently to cause patients to go into withdrawal. The most current research shows that interaction between a protease inhibitor and the newly approved addiction treatment medication buprenorphine does not result in withdrawal, making it easy for clinicians to effectively treat drug addicts co-infected with HIV. Identifying such interactions and developing alternative regimens remain high NIDA priorities under a new program of Research on Drug-Drug Interactions within the Branch of Medical Consequences of Drug Abuse and Co-occurring Infections.

*Conclusion***:** Due to the lack of pharmaceutical industry interest in developing new medications to treat addiction to controlled substances, NIDA has been substantially involved in the development of nearly all such medications since the Institute's inception in 1974. NIDA works with several government agencies, pharmaceutical companies, and academic institutions to develop pharmacological treatments for the treatment of drug addiction. Historically, drug addiction has been treated as an orphan disease because the pharmaceutical industry rarely profits from marketing drugs for the treatment of drug addiction, and little or no incentive exists for pharmaceutical companies to pursue research and development of new treatment medications for this population (currently, there are four medications for the treatment of opiate addiction have received orphan product designation -- ORLAAM, naltrexone, buprenorphine, and buprenorphine combined with naloxone).

Since there are no FDA-approved products for the treatment of cannabis and stimulant dependence, NIDA's efforts are currently shifting toward a greater emphasis on discovery and development of medications for these disorders. Clinical trials for stimulant dependence have primarily tested marketed medications as part of attempts to collaborate with pharmaceutical

companies. Efforts are also directed toward supporting, through grants and contracts, synthesis of novel compounds for screening and pharmacological testing. NIDA is currently sponsoring several clinical trials for the treatment of cocaine, methamphetamine, and nicotine dependence, and NIDA continues to progress research and development in an area that has truly been treated as an orphan disease.

*References***:**

Byrnes-Blake KA, Laurenzana EM, Carroll FI, Abraham P, Gentry WB, and Landes RD, Pharmacodynamic mechanisms of monoclonal antibody-based antagonism of (+)-methamphetamine in rats. *Eur J Pharmacol* 2003, 461:119-128

Khalsa JH, Genser S, Francis H, Martin B, Clinical consequences of marijuana. *J Clin Pharmacol.* 2002 Nov;42(11 Suppl:7S-10S).

Laurenzana EM, Gunnell MG, Gentry WB, and Owens SM, Treatment of adverse effects of excessive phencyclidine exposure in rats with a minimal dose of monoclonal antibody. *J. Pharmacol. Exp. Ther.* 2003, 306:1092-1098.

Martell BA, Mitchell E, Poling J, Gonsai K, and Kosten, TR, Vaccine pharmacotherapy for the treatment of cocaine dependence. *Biol. Psychiatry.* 2005 July 15; 58(2):158-64.

Wessinger WD, Wenger GR, Proksch JW, Laurenzana EM and Owens SM, A single dose of monoclonal anti-phencyclidine IgG offers long-term reductions in phencyclidine behavioral effects in rats. *J. Pharmacol. Exp. Ther*. 2002, 302:119-126.

## NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS)

### Overview of Rare Diseases Research

NIEHS supports research into the fundamental mechanisms of how environmental exposures interact with the human body to produce disease and dysfunction. This research on molecular pathways and environmental interaction has also yielded insights into the basic mechanisms involved in the pathogenesis of rare diseases and conditions. Diseases for which this research has yielded important clues in 2005 include autoimmune diseases, lung diseases, and birth defects.

### Recent Scientific Advances in Rare Diseases Research

### Autoimmune Diseases

*Myositis*

Autoimmune muscle diseases (the myositis syndromes) are acquired incurable disorders defined by chronic muscle inflammation resulting in muscle weakness. To begin to understand possible gene-environment interactions in myositis, NIEHS clinical researchers assessed over 500 subjects for season of disease onset along with autoantibody and genetic associations. While there were no seasonal patterns of disease onset in myositis patients as a whole, significant seasonal associations were present in the subgroups defined by disease-specific autoantibodies. In Caucasian patients with antisynthetase autoantibodies, in whom a gene called HLA-DRB1*0301 was found to be a strong risk factor, myositis onset peaked in March-April. Among the antisynthetase-positive patients, the seasonal association was predominantly in those with a clinical form of myositis called polymyositis and in men. In contrast, patients without myositis-specific autoantibodies showed a peak of onset in June-July; this seasonal association was seen in women but not in men and was more significant in a form of the disease called dermatomyositis, which was found to be associated with HLA-A*68 alleles. Additional studies to define the responsible environmental exposures at these times are underway. These findings, in conjunction with other data, suggest that diverse environmental agents, acting upon different immunogenetic backgrounds, result in distinct immune responses and clinical phenotypes. These results emphasize the importance of studying more homogeneous groups, based on clinical features, immune responses, ethnicity, gender and genetics, when attempting to decipher disease pathogeneses.

### Lung Diseases

*Bronchiectasis*

A non-cancerous lung disease, bronchiectasis is a relatively rare condition in which the bronchial tubes become enlarged and distended, forming pockets where infection may occur. Bronchiectasis is caused by various types of infections which damage and weaken the bronchial walls and interfere with the action of the lung's cilia. Patients may be predisposed to get this

condition with various congenital or inherited deficiencies such as immunological deficiency or cystic fibrosis; environmental exposures might also play a role in some people who develop this disease. In a study of people exposed to arsenic through contaminated drinking water in West Bengal, India, evidence was gathered to investigate if arsenic affects risk of bronchiectasis. Thirty-eight people in the study reported chronic cough and underwent computed tomography scans. Brochiectasis severity was almost 4 times worse in 27 participants with skin lesions, a common sign of arsenic exposure, than in the 11 subjects without skin lesions. Overall, subjects with arsenic-related skin lesions were 10 times more likely to have bronchiectasis than those without lesions. These results help buttress a growing body of evidence implicating arsenic in risk of developing non-malignant lung disease.

*Idiopathic interstitial pneumonia*

Idiopathic interstitial pneumonia (IIP) is the collective name for a group of disorders that can lead to scarring and inflammation of the lung known as pulmonary fibrosis. Pulmonary fibrosis makes the delivery of oxygen to the body's tissues difficult and is often fatal. About one-half of patients die within the first 5 years of being diagnosed with idiopathic pulmonary fibrosis. New research shows that risk of IIP might be elevated by an interaction between a specific genetic background and cigarette smoking. Researchers enrolled and evaluated 111 families with a diagnosis of IIP in at least two affected relatives. The sample included 309 people affected with an IIP and 360 unaffected relatives. Each participant completed a detailed health and environmental exposure questionnaire, a chest x-ray, and a lung diffusion test, which determines how well oxygen passes from the air sacs of the lungs into the blood.

The researchers found that there is a genetic basis for this disease. In addition to the fact that 111 families had 2 or more relatives with this disease, the researchers also found similar age-at-diagnosis and significant risk among siblings. Older people, males, and those who smoked also showed a greater risk of developing familial interstitial pneumonia. After controlling for age and gender, having ever smoked cigarettes increased the likelihood of developing this disease 3.6 times. This study enhances our understanding of one form of pulmonary fibrosis, which could help lead to strategies for genetic testing, prevention, and treatment of this disease.

*Chronic beryllium disease*

Beryllium is a metal with a variety of industrial applications. Inhalation of beryllium dust or particles can lead to beryllium sensitization and chronic beryllium disease (CBD). Beryllium sensitization is a condition in which a person’s immune system becomes highly responsive to the presence of beryllium in the body. CBD is a debilitating and often fatal lung disease characterized by lung-tumor formation. The number of cases of CBD is small, but the Department of Energy expects more workers to be exposed to beryllium as efforts move forward to decommission and deactivate former nuclear weapons production facilities. About half of individuals with sensitization already have CBD at the time of their first clinical evaluations. However, the time-lag between the two conditions is unknown. NIEHS-supported researchers recently conducted a study in 55 workers with beryllium sensitization. They found that over about 4 years of follow-up, 17 of the subjects developed CBD. The remaining 38 workers

remained disease free after almost 5 years. Those workers that progressed to CBD were more likely to have worked as machinists than in other occupations, however, there were no differences in age, sex, race, smoking status, or beryllium exposure time between those who developed CBD and those that remained sensitized. Based on these findings, it will be important to continue to follow the subjects to determine if all sensitized workers eventually develop CBD and the extent to which the conditions of those with CBD worsen. Although the researchers could not identify when the beryllium sensitization initially occurred, the time period from first beryllium exposure to the development of CBD ranged from under 4 years to 45 years, indicating that people with beryllium sensitization have a life-long risk of developing CBD.

**Birth Defects**

*Fetal failure to close the ductus venosus*

In normal mammalian development, a fetal blood vessel, the ductus venosus, is open in order to shunt blood from the liver to the developing brain and heart where it is needed. Shortly before birth, this blood vessel is closed in order to allow for normal liver development. There are people, however, for whom the ductus venosus fails to completely close and the long-term consequence of this failure is glaucoma, difficulty breathing, and central nervous system toxicity.

Researchers have identified a specific protein receptor, the aryl hydrocarbon, or Ah receptor (AHR), as being critical in directing the body to close the ductus venosus. Capitalizing on the fact that levels of this receptor increase when exposed to the environmental contaminant, dioxin, researchers studied genetically engineered mice that had low levels of AHR. In control mice, the ductus venosus failed to close properly, leading to smaller than normal livers. However, in mice exposed to dioxin shortly before birth, the ductus venosus closed normally. These researchers have now begun investigations with clinicians to determine in children with unclosed ductus venosae have disruptions in the AHR pathway and if AHR inducers could be an effective treatment. Currently these people must undergo surgery to close this vessel.

**Conferences Accomplishments**

**Gene/Environment Interactions in Rare Diseases That Include Common Birth Defects -** Symposium sponsored by NIH Office of Rare Diseases, and NTP Center for the Evaluation of Risks to Human Reproduction; June 28, 2005, at St. Pete Beach FL:

Rare syndromes often feature specific types of birth defects, which are often major diagnostic clues as to the presence of a given disorder. Despite this specificity, not everybody with the same syndrome is equally or comparably affected, and not everyone with a specific birth defect manifests the same syndrome or is affected with all the features of a particular syndrome. This symposium attempted to explore how much of this variability is due to genetic factors and how much is due to environmental factors. The specific types of birth defects examined included cardiovascular defects, holoprosencephaly, clefts of the lip and/or palate, neural tube defects, and diaphragmatic hernias.

**Regulation of Ion Channels** – 2005 FASEB Summer Research Conference sponsored by NIH Office of Rare Diseases, NIEHS, NHLBI, Cellectricon, Inc., Icagen Inc., MERCK and Co., Inc., June 4 – 9, 2005 at Snowmass, CO:

This conference provided a review of the field of ion channel regulation and the influence of ion channels on human disease, including CNS degeneration, renal defects, and osteopetrosis.

**Conferences Plans**

**Omega-3 Fatty Acid Supplementation During Pregnancy and Infancy** – Workshop sponsored by NIEHS, ORD, OD, and NCCAM, March 8 – 9, 2006.

An upcoming workshop that will examine the biologic rationale for a role of omega-3 fatty acids in modulating the immune and inflammatory responses in early life and will help inform decisions about opportunities for prevention trials.

**Bench to Bedside Awards Sponsored by ORD and NIEHS**

- UVA Sensitivity in Smith-Lemli-Opitz syndrome: Possible Involvement of Cholesta -5, 7, 9(11)-trien3B-01.
- Analysis of Global Gene Expression Patterns and Mitochondrial DNA Damage in Lymphocytes of Friedreich's Ataxia Patients Undergoing Idebenone Treatment in Phase II Double-Blind Placebo Controlled Study.

## NATIONAL EYE INSTITUTE (NEI)

### Overview of Rare Disease Research Activities

The National Eye Institute was created on August 16, 1968, by Public Law 90-489 for the purpose of supporting and conducting research for improving the prevention, diagnosis, and treatment of diseases that affect the eye and vision. Eye diseases and blindness cost the Nation an estimated $38.4 billion per year. More than 12 million people in the United States suffer some significant impairment of vision. Over the years, vision researchers supported by the NEI have conducted many pioneering studies that have greatly advanced our understanding of eye diseases, including those classified as rare, and provided eye care professionals with new tools and methods to prevent or cure many sight-threatening conditions. In October of 2003, the NEI released its strategic plan for vision research National Plan for Eye and Vision Research. This plan is the seventh in the series that dates back to the publication of Vision Research Program Planning in 1975. The development and publication of the aforementioned plans address the visual health needs, including rare diseases of the eye and visual pathways, of the Nation.

### Recent Scientific Advances in Rare Disease Research

*Retinitis pigmentosa and related disorders*

Retinitis pigmentosa (RP) is a group of blinding hereditary retinal degenerative diseases that are characterized by a progressive loss of vision due to the degeneration of photoreceptor cells. RP patients frequently report night blindness and loss of mid-peripheral vision during adolescence, and are usually legally blind by the age of 40. Photoreceptor cells of the retina (the rods and cones) are responsible for the capture of light and the initiation of an electrical signal to the brain in the process of vision. The study of signaling in photoreceptor cells, termed the visual phototransduction cascade, has provided a detailed molecular description of this pathway.

*RPE65 and the visual cycle:* Vitamin A and its derivatives are critical components to vision. In photoreceptor cells, a vitamin A derivative, called 11-*cis*-retinaldehyde, combines with a protein called opsin to form rhodopsin. Rhodopsin is the key molecule that absorbs light and begins the cascade of molecular events that converts light to the chemical and electrical signals that our brains process to visualize our surroundings. When light hits rhodopsin, 11-*cis*-retinaldehyde is changed to another vitamin A derivative called all-*trans*-retinaldehyde. However, once rhodopsin is converted to this form, it can no longer absorb light. Through a process called the visual cycle, 11-*cis*-retinaldehyde is renewed so that it can again participate in the visual process. Studying the gene expression underlying the visual cycle, scientists discovered that a previously known protein called RPE65 is responsible for the chemical conversion of spent rhodopsin. Mutations in the RPE65 gene are known to cause a range of retinal degenerative diseases that vary widely in severity. For example, some mutations are associated with Leber's congenital amaurosis, a rare eye disease that causes blindness in infants, while others result in mild to moderate forms of retinitis pigmentosa. A more precise understanding of RPE65 will help clarify our knowledge of the visual cycle and the diverse diseases that emerge from alterations in this gene.

*Macular degeneration*

*Stargardt-like macular dystrophy (STGD3):* STGD3 is a rare, inherited form of juvenile macular degeneration that shares many clinical features with AMD, including the accumulation of deposits called lipofuscin in the retina prior to the degeneration of the photoreceptor cells. As of yet, there are no suitable animal models for AMD, and so the development of a rodent model for STGD3 may offer insights into both diseases. In 2001, alterations in a gene called ELOVL4 were found to cause STGD3. This year, vision researchers developed a transgenic mouse model with a mutant form of the ELOVL4 gene. The mutant ELOVL4 mice were found to possess some of the hallmarks of macular degeneration, including accumulation of lipofuscin in the retina, abnormal neural activity and localized degeneration of photoreceptor cells in the retina. The availability of the ELOVL4-mutant mouse will facilitate our understanding of the basic pathogenesis of macular degeneration and offer a model to evaluate therapeutic interventions.

*Autoimmune diseases*

*An improved understanding of uveitis:* Uveitis is the name given to a group of blinding, inflammatory eye diseases that are often autoimmune in nature. A collaborative team of researchers has found new evidence that the thymus gland plays a critical role in the development of uveitis. Immature lymphocytes created from bone marrow stem cells enter the thymus and develop into a wide variety of T cells (T stands for thymus) that have affinity for infectious agents that invade the body. The thymus also contains a catalog of tissue-specific self-antigens. Self-antigens are those cell elements that hold potential to initiate an autoimmune response. The developing T cells that have affinity for these self-antigens are eliminated in the thymus, thus creating tissue tolerance and preventing the potential for autoimmune diseases. In the current study, NEI scientists found that human thymus samples contained several eye-related self-antigens that have been previously associated with the development of uveitis in animal models. However, the expression of these tissue antigens varied remarkably among the individual samples. Previous studies have found evidence that susceptibility to autoimmune disease decreases when the level of expression of a self-antigen within the thymus is robust. These findings greatly clarify the mechanisms that determine the susceptibility to uveitis. The discovery that the thymus plays a role in the disease also opens a new avenue of investigation to expand our understanding of uveitis and to develop therapies that prevent the disease or limit vision loss.

*Corneal dystrophy*

*Gene discovery for corneal dystrophy:* Francois-Neetens mouchetée fleck corneal dystrophy (CFD) is a rare genetic corneal dystrophy characterized by numerous small white flecks scattered in the cornea. Vision researchers have identified mutations in a gene called phosphatidylinositol-3-phosphate 5-kinase, type III (PIP5K3) that cause CFD. PIP5K3 is part of a family of enzymes that help regulate the formation and intracellular location of lipid products. It is thought that mutations in the gene disrupt the transport of lipids within the membranes of corneal cells, resulting in the characteristic flecks that appear in the corneas of people with CFD. These flecks

are thought to be lipid deposits. Besides providing insight into the pathophysiology of CFD, this discovery provides a new avenue of exploration into both corneal biochemistry and physiology.

**Rare Diseases-Related Program Activities**

The National Advisory Eye Council and the NEI have established the following goals for rare disease research in the National Plan for Eye and Vision Research.

- $ Understand the pathogenesis of inherited retinal diseases.
- $ Continue to develop models and a coordinated system to share animal model data and resources in the vision community.
- $ Characterize the genes and proteins expressed in tissues of the ocular surface; determine the functional consequences of changes in expression and molecular interactions and determine the epigenetic, hormonal, neural, and environmental influences under both normal and pathological conditions.

## NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES (NIGMS)

### Overview of Rare Diseases Research Activities

The National Institute of General Medical Sciences (NIGMS) supports broad-based fundamental research that is targeted toward understanding the general biological mechanisms that operate in the functioning of all biological system rather than being relevant to any specific organ system or disease. Examples include basic studies on the structure and function of organelles and membranes at the cellular and molecular level; investigations into the replication, organization, and function of the genome in organisms ranging from bacteria to man; development of improved instrumentation and technology for the investigation of biological problems; studies on bio-related organic chemistry for the elucidation of biosynthetic pathways and the development of new synthetic strategies for molecules of biological interest; and investigations of pharmacologic mechanisms at the molecular level. In general, investigations related to specific diseases are supported only if the mechanisms under investigation are widely applicable across disease or organ system lines.

*Human genetic cell repository*

The NIGMS Human Genetic Cell Repository provides a valuable resource for investigators studying genetic disorders. The Repository, located at the Coriell Institute for Medical Research in Camden, New Jersey, collects, characterizes, maintains, and distributes cell lines and DNA samples from patients and families with a wide variety of genetic disorders and from normal persons whose tissues serve as controls. Nearly 10,000 unique cell lines, representing over 500 different inherited human disorders, and more than 4700 DNA samples are available to qualified investigators. The Repository stimulates research on rare diseases by providing access to these cell lines, both fibroblasts and transformed lymphoblasts, and DNA samples derived from these cell lines, that otherwise are not readily available. The cell lines requested most frequently in the last year were those from patients with rare diseases such as ataxia-telangiectasia, xeroderma pigmentosum, fragile X-linked mental retardation, spinal muscular atrophy, Huntington disease, cystic fibrosis, Seckel syndrome, Nijmegen breakage syndrome, Friedreich ataxia, and neurofibromatosis. Recent acquisitions to the collection include samples from patients with the following rare disorders: Angelman syndrome, congenital disorder of glycosylation 1G, isovaleric academia, Langer mesomelic dysplasia, leprechaunism, Gaucher disease, LCHAD, Sotos syndrome, spinocerebebellar ataxia 8, trifunctional protein deficiency, and Lennox-Gasteaux syndrome. These cell lines, as well as those previously acquired, are used for biochemical, cellular, and molecular studies to help elucidate the causes of genetic defects. The disease-related mutation has been characterized at the molecular level in nearly 1300 of the genetic cell lines. The molecular defect is known for most all of the newly acquired cell lines that are accepted into the Repository. The Repository also houses an expanding collection of chromosomally aberrant human cell lines; some of these have been evaluated with array comparative genomic hybridization (aCGH). It supplies DNA isolated from complete panels of well-characterized human-rodent somatic cell hybrids and from chromosome-specific somatic cell hybrid panels for nearly every human chromosome. Sets of cell lines (and DNAs derived from them) that represent the CEPH family collection and other extended families, the National

Human Genome Research Institute's DNA Polymorphism Discovery Resource and International HapMap Project, and other identified populations that represent the genetic diversity of humans are available from the Repository. These samples will help researchers map and identify genes that are involved in the etiology of complex genetic diseases.

**Recent Scientific Advances in Rare Diseases Research**

*Gene defective in Fanconi anemia is associated with BRCA1 function*

Fanconi anemia is an inherited disorder that presents, not just with a profound anemia due to bone marrow failure, but with a diverse array of congenital abnormalities, growth retardation, and a predisposition to the development of a variety of cancers. During chromosome replication, cells from Fanconi patients appear to be limited in their ability to repair the DNA damage caused by exposure to excessive sunlight, radiation, or carcinogenic chemicals. The resulting chromosomal instability produces a high rate of chromosome breakage that can lead to cell death or the development of cancer. Extensive research has identified defects in eleven separate genes that can each cause Fanconi anemia, but very little is known about the specific functions of any of these genes. Surprisingly, none of the proteins coded for by these genes has an identified role in DNA repair.

New work, resulting from a collaborative effort involving NIGMS supported investigators in the US and Germany, has now implicated a causal mutation at a gene locus, BRIP1, not previously associated with Fanconi anemia. The gene defect was discovered in five individuals from four families following whole genome scans using arrays of 50,000 single nucleotide polymorphisms (SNPs). The patients were selected from the International Fanconi Anemia Registry; three of the individuals were Inuit and two were Hispanic. All five were homozygous for the same C->T mutation that leads to a premature stop codon and the absence of detectable BRIP1 protein. The BRIP1 protein was previously known to function as a DNA unwinding protein (helicase) that, fittingly, is involved in a DNA repair pathway. The BRIP1 protein has been shown to interact directly with the BRCA1 protein, which plays a role in the repair of DNA double strand breaks and is associated with increased risk of breast cancer when it is defective. This work on the rare disease, Fanconi anemia solidifies the link of the disease with chromosome instability and helps to clarify the mechanism of BRCA1 in a common medical condition.

*Antley-Bixley syndrome with disordered steroidogenesis: POR gene variants*

The cranial-facial abnormalities that characterize Antley-Bixley syndrome have previously been attributed to mutations at the fibroblast growth factor receptor 2 (FGFR2) gene loci. Deficiencies in this gene, however, could not account for the ambiguous genitalia and altered steroid hormone metabolism that accompany the skeletal anomalies in some affected infants. NIGMS-funded researchers have now confirmed that infants who only show skeletal malformations do have defects in the FGFR gene, but children who show abnormal genitals and abnormal steroid metabolism in addition to the skeletal dysplasia have a defective gene for the enzyme, NADPH cytochrome P450 oxidoreductase (POR) rather than mutations at FGFR2. Beginning in utero, P450 enzymes in the fetal adrenal gland and in sex organs are critical for

formation of progesterone, testosterone and estradiol. Failure to form these steroid hormones in sufficient quantities will lead to abnormal development of sex organs. Poor reductase activity will prevent P450 mediated formation of progesterone and poor sex steroid formation. Identification of this abnormal P450 gene indicates that these infants require a treatment regimen that would involve a different team of doctors, i.e., pediatric endocrinologists, because they would require supplementation with steroid hormones .

## NATIONAL HEART, LUNG, AND BLOOD INSTITUTE (NHLBI)

### Overview of Rare Diseases Research Activities

The NHLBI provides leadership for a national program in the causes, diagnosis, treatment, and prevention of diseases of the heart, blood vessels, lungs, and blood; in sleep disorders; and in the uses of blood and the management of blood resources. Through research in its own intramural laboratories and through extramural research grants and contracts, it conducts and supports an integrated program that includes basic research, clinical trials, epidemiological studies, and demonstration and education projects.

Although much of the research supported by the NHLBI addresses common conditions such as hypertension, coronary heart disease, and chronic obstructive pulmonary disease, a significant amount of research is devoted to rare diseases in children and adults. NHLBI activities related to rare disease research in fiscal year (FY) 2005 are described below.

### Recent Scientific Advances in Rare Diseases Research

#### Heart and Vascular Diseases Programs

*Abetalipoproteinemia*

Abetalipoproteinemia is a disorder of lipid metabolism characterized by the absence of apoprotein B-containing lipoproteins from the plasma. Symptoms include diarrhea, severe fat malabsorption, and acanthocytosis, a rare condition in which the majority of red blood cells have multiple spiny cytoplasmic projections. Other symptoms, including blindness and neurologic defects, all appear to be secondary to defects in the transport of vitamins A and E in the blood. The NHLBI funds research grants to study the genetic, biochemical, and metabolic aspects of abetalipoproteinemia. Recent research suggests that the disease is the result of abnormal processing of apolipoprotein B (apoB) caused by an absence of microsomal triglyceride transfer protein (MTP). The major defining function of MTP is its ability to transfer lipids between small unilamellar vesicles *in vitro*. Recently, NHLBI-funded researchers developed new methods to measure the lipid transfer activity of MTP. They devised simple fluorescence assays to measure transfer of triacylglycerol (TAG), phospholipids (PLs), and cholesteryl esters (CEs) by MTP. Because the new assays are very easy to perform, the investigators recommend their use for routine MTP activity measurements. The new assays should facilitate studies of MTP and may help researchers to identify specific inhibitors for individual lipid transfer activities, characterize different domains involved in transfer, and isolate mutants that bind to, but cannot transfer lipids.

*Antiphospholipid syndrome (APS)*

APS symptoms include recurrent abnormal blood clotting, a history of fetal deaths, and autoimmune diseases such as thrombocytopenia (an abnormal decrease in the number of platelets in the blood). Individuals with APS have an increased risk of developing atherosclerosis. Many patients with the disorder also have systemic lupus erythematosus (SLE). APS is characterized

by the presence of circulating autoantibodies to certain phospholipids, chiefly cardiolipin, as well as to the SLE anticoagulant. The NHLBI supports research to determine whether genetic factors predispose individuals to develop APS autoantibodies, to design standardized immunoassays for reliable detection of individual antiphospholipid antibodies, and to investigate the role of APS autoantibodies in atherogenesis. Research shows that some APS autoantibodies bind to oxidized phospholipid (OxPL) components of the oxidized LDL lipoprotein (OxLDL). Recently, NHLBI-supported researchers investigated the binding of OxLDL to cells called macrophages, an interaction which is implicated in atherogenesis. The investigators used natural mouse autoantibodies against OxLDL to study the molecular basis of the interaction between OxLDL and macrophages. One of the autoantibodies, designated E06, was shown to inhibit the uptake of OxLDL by macrophages. Since E06 interacts with the phosphocholine (PC) headgroup of PC-containing OxPLs present in OxLDL, the results suggest that the PC group facilitates the binding of OxLDL to macrophages and that it might be a therapeutic target.

*Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD or ARVC)*

ARVD is a family of rare cardiomyopathies that result in abnormal heart rhythm and sudden cardiac death. Most forms are believed to be caused by the inheritance of autosomal dominant mutations in genes that remain largely unknown, but that clearly affect myocardial integrity. ARVD is characterized by marked, selective, right ventricular dilatation, myocardial cell death, and replacement of heart muscle with fat cells and fibrous tissue. Expression in gene carriers is variable, but in those who display symptoms, the disease is frequently fatal. Although relatively rare, ARVD has devastating consequences in otherwise healthy, young individuals and is thought to account for as many as 25 percent of sudden deaths in persons under the age of 65. The NHLBI supports the Multidisciplinary Study of Right Ventricular Dysplasia, an integrated network of three separate groups, to investigate genotype-phenotype relationships in familial forms of ARVD. The program uses clinical and genetic approaches to identify patients with ARVD. Recently, the Multidisciplinary Study of Right Ventricular Dysplasia initiated collaboration with the Japanese ARVC Registry to set up a parallel study. Investigators in Japan and in the U.S. will work closely to standardize study protocols and to perform joint analyses. Enrollment is expected to begin in Japan in spring 2006.

Several scientific advances in ARVD research have recently been reported. In FY 2005, researchers from the Multidisciplinary Study of Right Ventricular Dysplasia, in collaboration with investigators from Italy, showed that ARVD1 is caused by mutations in the TGFß 3gene. Also in FY 2005, researchers reported that mutations in the ARVD9 gene are found in familial ARVD. Their results show that approximately 25 percent of people screened for ARVD have the ARVD9 mutation. Now, family members participating in the Multidisciplinary Study of Right Ventricular Dysplasia can be screened for ARVD9 mutations and plans are being made to disseminate the genetic information back to the patients. Other recent accomplishments of the Multidisciplinary Study of Right Ventricular Dysplasia include quantification of echocardiographic abnormalities in individuals with ARVD, improved diagnosis of ARVD by magnetic resonance imaging using delayed enhancement to detect fibrosis, and quantitative comparison of right ventricular wall motion in ARVD patients and unaffected individuals.

*Bartter's syndrome*

Bartter's syndrome is characterized by salt imbalance and low blood pressure. Research on Bartter=s syndrome is currently being pursued as a part of the NHLBI Specialized Centers of Research on the Molecular Genetics of Hypertension. Researchers have discovered a mutation in a potassium channel that can lead to Bartter=s syndrome and have demonstrated that the channel is an important regulator of blood pressure and ion and fluid balance. Mutations in chloride channels have also been identified and implicated in the development of Bartter's syndrome. Researchers think that additional Bartter=s syndrome genes may exist. The hypotensive state caused by Bartter=s syndrome suggests that the mutated genes may protect against the development of high blood pressure.

*Beta-sitosterolemia*

Beta-sitosterolemia is a rare inborn error of metabolism characterized by increased absorption of dietary cholesterol and sterols from plants and shellfish. The distinguishing feature of the disorder, a 50- to 100-fold elevation in plasma plant sterol levels, reflects both an increase in absorption of sterols from the intestine and a decrease in excretion of sterols into bile. People with beta-sitosterolemia have a markedly increased risk of premature cardiovascular disease. Effective treatment is not available at present, although a number of drugs are under development. NHLBI-funded investigators are studying patients with beta-sitosterolemia and other mutations of sterol absorption, the basic mechanisms of sterol absorption, and related disorders of sterol metabolism. It is hoped that theses efforts will lead to pharmacological or dietary treatments for beta-sitosterolemia.

*Brugada's syndrome*

Brugada's syndrome, a rare inherited disorder characterized by cardiac electrophysiological abnormalities (right bundle branch block and ST elevation in the precordial leads), is associated with a high occurrence of sudden cardiac death. Mutations in the cardiac sodium channel gene, SCN5A, are associated with Brugada's syndrome as well as with other rare arrhythmic syndromes like Long QT syndrome subtype 3 (LQT3). The NHLBI supports research on Brugada's syndrome through two program project grants and a small portfolio of individual research project grants studying the molecular, clinical, and genetic bases of the condition. The NHLBI initiative, Exploratory and Developmental Research Grants for Investigations in Rare Diseases, currently supports a grant to elucidate the ionic basis of Brugada's syndrome.

Recent research advances in this area include four new reports on SCN5A, the gene associated with Brugada's syndrome. In the first report, NHLBI-funded investigators showed that mutations in SCN5A result in the expression of defective sodium channels that do not traffic to the cell membrane surface and therefore cannot be involved in generation of the cardiac action potential. They found that a sodium channel blocker could rescue the defect in isolated cells. In the second report, another group demonstrated that a mutation in an intron region (a non-coding region) of the SCN5A gene found in a single patient with Brugada's syndrome is associated with a loss of sodium channel function. The finding represents the first time that a mutation in a non-

coding region of SCN5A has been linked to an inherited arrhythmic condition and has important implications for screening for arrhythmia mutations. In the third report, investigators examined 39 SCN5A variants in healthy black, white, Asian, and Hispanic subjects. They found that 19 of the variants were found only in the black subjects. Three variants cited previously as Brugada's syndrome-causing mutations and one variant published previously as a possible LQT3-causing mutation were identified in the healthy cohort. The findings have implications for arrhythmogenic susceptibility and Brugada's/Long QT syndrome genetic testing. The fourth report showed that six out of eight common human SCN5A polymorphisms are associated with different electrophysiologic characteristics in isolated cellular expression systems. The findings have implications for the interpretation of previous studies of arrhythmia mutations.

*Cholesteryl ester storage disease*

Cholesteryl ester storage disease (CESD) is a rare syndrome characterized by an enlarged liver and spleen and abnormal liver enzymes. The disorder is known by a variety of names including lysosomal acid lipase deficiency, acid cholesteryl ester hydrolase deficiency, and Wolman disease. The severe infantile-onset Wolman disease and the milder late-onset cholesteryl ester storage disease (CESD) are seemingly caused by mutations in different parts of the acid lysosomal lipase gene. The mutations affect acid lysosomal lipase, an enzyme which removes lipids (cholesteryl esters and triglycerides) from lipoproteins. Acid lysosomal lipase deficiency causes a massive accumulation of lipids in tissues. The hypercholesterolemia that is common in individuals with CESD predisposes them to develop atherosclerosis. Researchers in the NHLBI's intramural program are conducting studies to determine the role of acid lysosomal lipase in the removal of lipids from the plasma and from tissues.

*Congenital heart defects*

Congenital heart defects encompass a constellation of abnormalities in the heart that occur during embryonic development. Abnormalities of the heart are the most common of birth defects, occurring in up to one percent of live births, and are an important cause of infant mortality, pediatric and adult morbidity, and shortened adult life expectancy. About one-third of affected infants and children require open heart surgery or interventional cardiac catheterization to repair or ameliorate their defects. Approximately the same proportion has associated extracardiac anomalies, such as chromosomal abnormalities and syndromes involving other organ systems.

The NHLBI has supported research in pediatric cardiovascular medicine since it first funded heart research grants in 1949. NHLBI-supported researchers have been instrumental in developing diagnostic fetal imaging techniques; surgical techniques, including various operations and refinements in cardiopulmonary bypass; and medical therapies now used to ensure healthy survival for most affected children. They also have made significant contributions to the epidemiology of congenital heart defects and to understanding the molecular and genetic basis of normal and abnormal heart development. In FY 2005, NHLBI-supported investigators found that microRNAs are part of the genetic control of heart development. They identified two microRNA genes that are regulated by heart development control genes, and showed that the

microRNAs in turn regulate genes involved in control of cardiac differentiation and proliferation. Also in FY 2005, another group of NHLBI-funded researchers showed that ablation of the recently discovered secondary heart field during early chick embryonic development causes cardiac defects including tetrology of Fallot and pulmonary atresia.

*DiGeorge syndrome*

DiGeorge syndrome, which occurs in about 1 in 4,000 live births, causes many abnormalities, including cardiac outflow tract anomalies, hypoplasia of the thymus and parathyroid glands, cleft palate, facial dysmorphogenesis, learning difficulties, and other neurodevelopmental deficits. It is usually sporadic, but may be inherited, and is caused by deletion of a segment of chromosome 22, which is known to contain numerous genes. The NHLBI supports both human and animal studies of DiGeorge syndrome through several individual research grants, program project grants, and a Specialized Center of Clinically Oriented Research (SCCOR) in Pediatric Heart Development and Disease. Much of the NHLBI-supported basic research on congenital heart defects also enhances understanding of DiGeorge syndrome because several of the most frequent cardiac malformations occur in conjunction with it. The recent identification of a gene associated with DiGeorge syndrome has enabled further research on how the gene is regulated and how it affects embryonic development through regulation of downstream genes. For example, in FY 2005, NHLBI-funded researchers used transgenic mice to conduct a systematic study of the spatial and temporal requirements for the gene during embryonic development. They discovered that gene expression in the lining of the pharynx is critical for normal development of a variety of tissues affected in DiGeorge syndrome, but that different tissues require the gene's activity at different times.

*Dysbetalipoproteinemia*

Dysbetalipoproteinemia, or type III hyperlipidemia, is a disorder with a strong heritable component characterized by the presence of beta-migrating very-low-density lipoprotein. It leads to the formation of characteristic yellow skin plaques (xanthomas) and predisposes to premature ischemic heart disease and peripheral vascular disease. The defect occurs in people with mutated forms of a protein, apoprotein E (apoE). A mutant form of apoE, apoE2, has been identified as the chief molecular defect. Animal models have been developed to facilitate basic research on dysbetalipoproteinemia. Mice expressing human apoE2 show increased plasma cholesterol and triglyceride levels. Similar increases in lipid levels are seen in humans with type III hyperlipidemia. Hyperlipidemia can be ameliorated in the mice by increasing expression of the low-density lipoprotein receptor by two-fold.

*Familial hypobetalipoproteinemia*

Familial hypobetalipoproteinemia (FHBL) is a disorder of lipid metabolism characterized by greatly reduced levels of apoprotein B (apoB)-containing lipoprotein cholesterol. The NHLBI funds research grants to study the genetic, biochemical, and metabolic aspects of the disease. At least three different types of FHBL exist, each resulting from a different genetic mutation. The three genetic forms of FHBL that have been reported: are (a) FHBL caused by mutations that

lead to the truncation of apoB, (b) FHBL linked to chromosome 3p21, and (c) FHBL not linked either to the mutations that cause apoB truncation or to the mutations on chromosome 3p21. Fatty livers (which have a five-fold increase in fat compared to normal livers) may be present in up to 80 percent of people with the form of FHBL that results from apoB truncation. In FY 2005, NHLBI-supported researchers compared affected individuals with mutations causing apoB truncation with those with mutations linked to chromosome 3p21. They found that, unlike individuals with mutations that cause apoB truncation, individuals with FHBL linked to chromosome 3p21 do not have fatty livers. They also found that the two genetic subtypes of FHBL differ in several other phenotypic features.

*Homozygous familial hypercholesterolemia*

Familial hypercholesterolemia (FH) is an inherited disorder characterized by elevated concentrations of low-density lipoproteins (LDL). The homozygous form of FH is rare (one in a million) but people who have it are very prone to premature coronary heart disease. Cholesterol derived from LDL, when deposited in arteries, leads to heart attacks and, when deposited in tendons and skin, causes yellow skin plaques (xanthomas). FH is caused by a mutation in a gene specifying the receptor for plasma LDL. LDL receptors facilitate the removal of LDL. When they are deficient or absent, the rate of LDL removal declines, causing the level of LDL in the plasma to rise. Although patients with FH have elevations in LDL cholesterol levels, plasma triglyceride levels are generally normal, suggesting that other mechanisms are responsible for clearing the majority of triglyceride-rich very-low-density lipoprotein (VLDL) and VLDL remnants. The NHLBI supports studies of the biochemistry, genetics, and potential treatment of FH; the regulation of LDL receptors; and blood cholesterol levels. In FY 2005, NHLBI-supported investigators conducted the first study of apolipoprotein B (apoB) metabolism in homozygous FH patients. They showed that the LDL receptor not only contributes significantly to the clearance of LDL from plasma but also plays a role in the clearance of larger apoB-containing lipoproteins.

*Klippel-Trenaunay-Weber syndrome (KTWS)*

KTWS is a very rare, vascular deformation disease of unknown incidence, involving capillary, lymphatic, and venous channels. It usually manifests as three symptoms: cutaneous port-wine capillary malformations, varicose veins, and enlargement of soft tissues and bone in one limb. KTWS symptoms are usually present at birth, with 75 percent of patients having symptoms before the age of ten. The NHLBI supports molecular research on characterizing the gene(s) responsible for KTWS.

*Lecithin cholesterol acyltransferase deficiency*

Lecithin cholesterol acyltransferase (LCAT) deficiency is a rare syndrome characterized by cloudy cornea, kidney failure, and extremely low levels of high-density lipoprotein (HDL). The disorder is inherited on chromosome 16 and is caused by a lack of the enzyme, LCAT, which aids in the formation of normal HDL. Researchers in the NHLBI intramural program are investigating molecular, cellular, and metabolic defects associated with human LCAT and their

role in LCAT deficiency. Patients with LCAT deficiency are being studied to clarify the natural history of the disorder and to develop better treatments. Intramural researchers are also performing studies in patients to determine the role of LCAT in HDL formation and in the development of atherosclerosis.

*Lipoprotein lipase deficiency*

Lipoprotein lipase deficiency (LPL) is a rare genetic lipid disorder characterized by extremely elevated triglyceride (fat). It is caused by a genetic defect that affects an enzyme involved in breaking down triglyceride and removing it from the blood. The specific gene defect, which is found on chromosome 8, is in the gene encoding the LPL enzyme. The excess triglycerides characteristic of the disorder are often deposited in the skin (eruptive xanthomas), back of the eye (lipemia retinalis), liver, and spleen and cause abdominal pain or pancreatitis in children. Researchers in the NHLBI intramural research program are conducting metabolic studies in patients with LPL deficiency to determine the role of the lipases in the metabolism of triglyceride lipoproteins and in the development of atherosclerosis and pancreatitis.

*Liddle=s syndrome*

Liddle=s syndrome is a disorder of severe hypertension, characterized by increased renal reabsorption of sodium resulting in hyperaldosteronism, or overproduction of the hormone aldosterone from the outer portion (cortex) of the adrenal gland. The hyperaldosteronism results in low potassium levels (hypokalemia), reduced acidity of the body (alkalosis), muscle weakness, excessive thirst (polydipsia), increased urination (polyuria), and hypertension. Research on Liddle=s syndrome is currently being pursued as part of the NHLBI Specialized Centers of Research on the Molecular Genetics of Hypertension.

*Long QT syndrome (LQTS)*

LQTS is characterized by a prolonged QT segment on an electrocardiograph, and is associated with fainting (i.e. syncope), ventricular arrhythmias, and, frequently, sudden cardiac death. About 70 percent of diagnosed cases are in women. LQTS comprises a family of related diseases that are often inherited and that are associated with alterations in cellular cardiac action potential repolarization caused by cardiac ion channel defects and derangements in the regulation of intracellular ion activities. The defects are directly associated with mutations in cardiac ion channel genes or indirectly with mutations in a gene encoding a protein that anchors ion channels to the cell membrane. In some forms of LQTS, affected individuals may inherit other abnormalities, such as deafness, and have varied clinical outcomes depending on their specific mutational patterns. Intense emotional states that affect the electrical stability of the heart may influence the likelihood of clinical events in LQTS patients. Researchers have identified mutations in eight specific genes that are associated with eight forms of LQTS (LQT1-LQT8). Three of them have been show to be ion channel gene mutations (KvLQT1, HERG, and SCN5A), which are associated with three forms of LQTS (LQT1, LQT2, and LQT3).

The NHLBI currently supports research on LQTS through two program project grants and several individual research project grants that address the molecular, clinical, and genetic bases of the condition. One award funds a study of trafficking signals that regulate potassium channel expression. Another project focuses on the associations between emotional states and clinical outcomes in LQTS patients. An additional investigator-initiated grant supports an international LQTS Registry that celebrated its 25th anniversary in 2005. The registry includes over 1,200 families with 2,949 affected individuals, 1,651 family members with borderline LQTS, and 2,967 unaffected family members. Investigators continue to conduct research to identify known genetic variants in registry members and to identify new variants and mutations. LQTS researchers also are assessing genotype-phenotype correlations and evaluating factors that trigger malignant ventricular arrhythmias. Recently, registry data was used to show that epinephrine infusion during electrocardiogram (ECG) testing helped investigators to predict whether patients had LQT1, LQT2, or LQT3 syndromes. The approach has potential for improving the clinical diagnosis of patients with LQTS, and especially of those with LQT1 syndrome.

Recent advances in LQTS research are described in five publications by NHLBI-funded scientists. One study describes two individuals with a severe variant of Timothy syndrome who also have a new form of LQTS (LQT8) that is associated with two mutations in a cardiac L-type calcium channel gene. A second study reports that one previously published and four newly described loss-of-function mutations in the ankyrin-B gene are associated with LQT4. Taken together, the findings support a new paradigm for human disease in which abnormal coordination of multiple, functionally related ion channels and transporters underlies the generation of the cardiac action potential. In a third study, researchers describe a patient with an SCN5A mutation associated with LQT3 who had a normal QT interval before antiarrhythmic drug administration but an increased QT duration after taking the drug. Further investigation showed that, in this patient, the mutant protein does not reach the cell surface and, therefore, cannot perform its function. Examination of the mutant sodium channel in isolated cells showed that the drug-induced increase in QT duration is caused by restoration of the channel's ability to reach the cell surface. The study supports the hypothesis that variable cell surface ion channel expression contributes to clinical variability in the LQT3 phenotype. The fourth study identified a region of the KCNQ1 potassium channel that is required for its normal surface expression. The fifth study, which analyzed five LQT-causing potassium channel genes, demonstrated the presence of compound mutations that are associated with expression of a severe phenotype in 20 of 252 affected individuals. The study suggests that the mutations are more common than expected.

*Marfan syndrome*

Marfan syndrome is an inherited connective tissue disorder associated with potentially severe cardiovascular complications such as aortic aneurysms and mitral valve prolapse, as well as non-cardiac complications such as dislocation of the eye lens. The disease occurs in about 1 per 10,000 persons and in all races. The NHLBI supports animal research on the assembly of microfibrils and their effects on cardiovascular development as well as a significant research portfolio on aortic aneurysm development and its treatment in the atherosclerotic population, which may have implications for treating aneurysms in people with Marfan syndrome.

*Niemann-Pick type C disease (NPC)*

There are several types of Niemann-Pick disease: types A (NPA), B (NPB), C (NPC), and D (NPD). NPC disease is a lipid storage disorder usually characterized by excessive accumulation of cholesterol in the liver, spleen, and other vital organs. Animal and basic studies show that the mutation that causes NPC interferes with lipid metabolism, cholesterol homeostasis, and intracellular cholesterol trafficking. The defects cause severe damage to the nervous system, bone marrow, and other tissues and organs in patients with NPC. Affected individuals have cardiovascular disease, enlargement of the liver and spleen (hepatosplenomegaly), and severe progressive neurological dysfunction. The gene deficiency in NP disease types A and B affects sphingomyelinase, an enzyme that breaks down the lipid sphingomyelin. The gene deficiency in NP disease types C and D affects the NPC-1 protein whose function remains obscure. Several NHLBI-supported grants fund research on the regulation of intracellular cholesterol movement as it relates to cholesterol accumulation in NPC disease. Recently, NHLBI-supported investigators obtained data supporting the hypothesis that certain agents that are incorporated into cell membranes increase the chemical activity of cholesterol by displacing it from its low activity association with phospholipids. The investigators described simple screens to help identify agents that increase or decrease the activity of membrane cholesterol.

*Smith-Lemli-Opitz syndrome (SLOS)*

SLOS is an inherited disorder caused by a defect in an enzyme active in the last step of cholesterol biosynthesis. As a result of the defect, synthesis of endogenous cholesterol is inadequate to meet biological demands for functions such as cell membrane production and bile acid synthesis. Newborns with SLOS have a distinctive facial dysmorphism; suffer from multiple congenital anomalies including cleft palate, congenital heart disease, genitourinary abnormalities, and malformed limbs; and exhibit digestive difficulties, severe developmental delays, and behavioral problems. Scientists now think that SLOS may be the cause of many previously unexplained cases of mental retardation. The NHLBI funds individual project grants for research on sterol balance and lipid metabolism in infants with SLOS, on the effectiveness of cholesterol-supplemented baby formula for ameliorating some of the behavioral and digestive symptoms of SLOS, and on the effectiveness of simvastatin therapy in lowering the plasma concentrations of toxic forms of abnormal cholesterol precursor compounds. In FY 2005, researchers published a study of cholesterol absorption in patients with SLOS. The results indicate that providing additional dietary cholesterol as egg yolk to SLO infants successfully raises plasma lipid levels in favorable ways, reflecting adequate if sub-normal absorption. Another 2005 publication describes a new mutation in a gene associated with SLOS.

*Supravalvular aortic stenosis (SVAS)*

SVAS is a vascular proliferative obstructive disease that affects the aorta and the coronary, carotid, and peripheral arteries. The incidence of SVAS is thought to be less than five percent of all congenital heart defects. SVAS is associated with a mutation in the gene for elastin, an extracellular matrix protein accounting for about 50 percent of the dry weight of the vascular wall. The NHLBI supports grants focused on SVAS. Research shows that SVAS is associated

with decreased elastin and altered arterial mechanics. In FY 2005, NHLBI-funded researchers reported on new findings using mice with a single deletion in the elastin gene ($ELN^{+/-}$), which are animal models for SVAS. Previous studies showed that the mice have hypertension and decreased arterial compliance and exhibit changes in arterial wall structure. Despite these differences, $ELN^{+/-}$ mice have a normal life span, suggesting that their arteries remodel and adapt to the decreased amount of elastin. In the study, the researchers tested the hypothesis that the arteries of $ELN^{+/-}$ mice remodel to adapt to decreased elastin levels. The results suggest that adaptive remodeling takes place during vascular development. The finding has implications for understanding human SVAS and indicates potential avenues of treatment.

*Tangier disease*

Tangier Disease is a rare syndrome characterized by a deficiency of high-density lipoprotein (HDL), mild hypertriglyceridemia, neurologic abnormalities, and massive cholesterol ester deposits in various tissues such as the tonsils. The disease is inherited and appears to be caused by excessive breakdown of HDL rather than by a fault in HDL synthesis. Tangier Disease patients have defective intracellular lipid trafficking that prevents removal of cholesterol from cells. The disease is caused by mutation in the gene for human ABCA1, a member of the ATP-binding cassette (ABC) transporter family located on chromosome 9. The NHLBI funds extramural research grants to investigate the cell biology and biochemistry of human ABCA1 and its role in Tangier Disease. NHLBI-supported investigators recently published a report on the relationship between ABCA1 expression and the breakdown of HDL.

**Lung Diseases Programs**

*Advanced sleep phase syndrome (ASPS)*

ASPS is a rare, genetically based sleep disorder characterized by an early evening onset of sleep, normal sleep duration, and spontaneous early awakening. The disorder leads to insomnia, excessive daytime sleepiness, and impairment of daily functioning and quality of life. The NHLBI supports basic research to elucidate the neural pathways through which the biological clock mechanism regulates sleep, clinical research to elucidate genetic risk factors, and applied research to determine the role of the biological clock in disturbed sleep and alertness of shift workers, school-age children, and drowsy drivers.

*Alpha-1 antitrypsin deficiency*

Alpha-1-antitrypsin (AAT) deficiency is an inherited deficiency of circulating AAT, a proteinase inhibitor that is manufactured primarily in the liver. The decrease in AAT is associated with emphysema, presumably due to inadequate protection of lung elastic fibers from enzymatic destruction by neutrophil elastase. Fifteen percent of the AAT-deficient population also develops liver disease. The NHLBI funds clinical and basic research on AAT deficiency, including studies of the molecular mechanisms that impair AAT secretion, methods of gene therapy delivery, and methods to increase the availability of defective, but partially active, AAT. NHLBI-supported investigators are defining abnormalities and degradation pathways associated

with AAT, characterizing the inflammation that leads to disease in various AAT deficiency states, developing alternative sources of AAT for augmentation therapy, and searching for small molecule therapeutic agents that enhance AAT secretion. A genetic study of families is looking for genes that may modify the nature and severity of the disease in different individuals. Two gene therapy clinical trials are testing whether the AAT protein is produced in skeletal muscle cells after injection of a viral vector construct that includes the AAT gene. In addition to research that specifically focuses on AAT, the NHLBI supports related studies addressing lung transplantation; the general causation of emphysema; the function, synthesis, secretion, and interaction of the enzymes that are inhibited by AAT; animal models of other enzyme inhibitor deficiencies; gene regulation; cellular signaling, injury, and repair; and protein processing.

*Asbestosis*

Asbestosis, an occupational lung disease, is characterized by interstitial pneumonitis and fibrosis resulting from exposure to inhaled asbestos fibers. In response to the deposition of asbestos fibers, macrophages and lymphocytes accumulate, type II alveolar epithelial cells and smooth muscle cells proliferate, fibrosis appears in the adjacent walls of respiratory airways, and the alveolar septa thicken. Asbestos fibers also can be associated with cell transformation and proliferation related to lung cancers. NHLBI-supported researchers are investigating the molecular and cellular events that trigger cellular proliferation in and regulate remodeling of lung tissue, which results in fibrotic lesions and perhaps, in malignant cell changes in response to asbestos.

*Bronchopulmonary dysplasia (BPD)*

BPD is a chronic lung disease characterized by disordered lung growth, changes in lung cell size and shape, and a reduction in the number of alveoli available for gas exchange. The NHLBI program in developmental lung biology supports basic and clinical research to increase understanding of BPD and identify treatment opportunities. The Collaborative Program for Research in BPD provides a well-characterized primate model of BPD for a multi-disciplinary exploration of the molecular mechanisms involved in the etiology of the disease. One of the participating centers of the NHLBI Specialized Centers of Research in the Pathobiology of Lung Development has identified nitric oxide (NO) as an important regulator of circulation in the lung during development. The NHLBI continues to support two clinical trials on the role of NO in preventing and treating chronic lung disease in premature infants. Both trials have reached their projected enrollment and have completed the analysis of the primary endpoints for the studies. Investigators plan to present results from the trials in 2006. Follow-up studies on neuro-developmental outcomes continue for both trials. The NHLBI also continues to collaborate with the National Institute of Child Health and Human Development Neonatal Research Network to fund a prospective, randomized study to test the individual factors of lower oxygen levels and volutrama reduction via nasal continuous positive airway pressure (nCPAP) oxygen delivery in very-low-birth-weight, premature infants. Enrollment for the study began in January 2005.

*Congenital central hypoventilation syndrome (CCHS)*

CCHS is a rare disorder characterized by normal breathing while awake, but shallow breathing during sleep (hypopnea) that is not effective in moving fresh air into the lungs. In severe cases, breathing is also ineffective in affected individuals who are awake. The NHLBI supports a basic research program to elucidate the anatomical and physiological factors responsible for generating neural rhythm and translating it into breathing. Research is focused on understanding how breathing is regulated and identifying the conditions under which reflexive generation of respiratory rhythm is suppressed. Identification of the neuronal pathways producing respiratory rhythm and pattern is a prerequisite for a full understanding of a variety of respiratory sleep disorders such as CCHS. Genetic and pathology studies of CCHS patients are now leading to identification of candidate genes and of specific areas of the brain stem involved in autonomic regulation including respiration.

*Congenital diaphragmatic hernia*

Congenital diaphragmatic hernia (CDH) is a developmental disorder that occurs once in every 2,400 births. Often CDH occurs in isolated fashion, i.e., it is not associated with any other life-threatening anomalies or chromosomal aberrations. Without surgical intervention, neonates die soon after birth because their lung tissue, compressed by their herniated viscera, is inadequately developed. In infants with CDH, hypoplasia of the lung and its vascular bed leads to pulmonary hypertension or persistent fetal circulation syndrome. NHLBI-funded researchers are investigating the causes and treatment of CDH.

*Cystic fibrosis*

Cystic fibrosis (CF) is a multi-system disease affecting a variety of epithelial tissues, characterized by defective transport of chloride and sodium across the cell membrane. More than 25,000 Americans have CF, which has an incidence of about 1 in 3,300 among Caucasians making it the nation's number one genetic cause of death for children and young adults. Lung disease is the major cause of morbidity and mortality in people with CF. Defects in a single gene, the CF transmembrane conductance regulator (CFTR) gene, give rise to the disorder. These defects lead to abnormal secretions, recurrent infection and inflammation, bronchiectasis (chronic dilatation of the bronchial tubes), and premature death. Increasing evidence suggests that defects in the CFTR gene do not function alone in determining disease outcome. The severity of pulmonary disease can vary greatly among individuals, even in those with identical CFTR mutations. Researchers hypothesize that the variation is due to the interaction of the defects in the CFTR gene with other genes that can affect the final disease presentation.

The NHLBI supports a program of basic, clinical, and behavioral research in CF focused on causes, pathophysiology, and treatment, specifically as related to pulmonary manifestations. In FY 2005, a grant in the area of cystic fibrosis and other pediatric lung diseases was awarded as part of an NHLBI-wide initiative establishing clinical proteomics programs to validate existing and new candidate protein markers for use in the diagnosis and management of diseases. The NHLBI also continues its partnership with the National Institute of Diabetes and Digestive and

Kidney Disorders, the Cystic Fibrosis Foundation (CFF), and several companies to sponsor "Early Antipseudomonal Therapy in Cystic Fibrosis", an investigator-initiated, multi-center, clinical trial. It is the largest clinical trial involving young (1 to 12 year old) children with CF ever to be conducted in the United States, and would be impractical without the Therapeutics Development Network (TDN) infrastructure, a unique resource for the CF research community, funded by the CFF and the National Center for Research Resources. The trial seeks to identify optimal methods for treating initial *Pseudomonas* infection to delay or prevent chronic infections that lead to irreversible lung destruction and eventual death. Recruitment is now underway at most of the 61 clinical sites. Also in FY 2005, the NHLBI funded several additional investigator-initiated grants for CF research. One grant the mechanisms underlying a recently identified defect in lipoxin production in CF patients as well as the therapeutic potential of lipoxin analogues. A pilot and feasibility grant is exploring a new, potential therapeutic approach for CF that involves replacing abnormal lung epithelium with normal functioning cells of the bone marrow. A final investigator-initiated grant explores the mechanisms by which ibuprofen exerts its anti-inflammatory effects in CF.

Several scientific advances related to CF were reported in FY 2005. For example, researchers found a deficiency of lipoxins, key regulators of neutrophilic inflammation, in the airways of children with CF. The defect is not observed in patients with other inflammatory conditions, including pneumonia, interstitial lung disease, and reactive airways disease. The research demonstrates, for the first time, that CF patients produce less lipoxin, increasing their susceptibility to neutrophil-mediated inflammatory damage and bacterial infection. The findings suggest that lipoxin analogs, or up-regulation of endogenous lipoxin production, may provide a new therapeutic approach for CF. Also in FY 2005, NHLBI-funded researchers showed that human bone marrow-derived adult stem cells can develop into airway epithelial cells, identical to the type lining the lungs and airways. Moreover, adding the correct version of the gene that is mutated in CF-to-CF mucosal stem cells (MSCs) restored the ability of the cells to transport chloride out of the cells, a function lacking in CF patients. The results suggest that CF patients' own bone marrow stem cells could be removed, genetically repaired, and transplanted into the lungs to improve lung function. Using a patient's own stem cells may overcome many of the immune complications that remain barriers to gene therapy. Finally, two additional NHLBI-funded studies are focusing on a new class of dual therapeutic agents (namely, proteasome inhibitors), which can enhance CFTR gene therapy. The identification of such compounds marks a new area for drug development, not only for CF, but for other gene therapy disease targets as well.

*Idiopathic pulmonary fibrosis*

IPF is a rare, chronic, lung disease in which functioning normal lung tissue is replaced by non-functional connective (scar) tissue, containing fibroblasts, myofibroblasts, and collagen. The causes of IPF are unknown, but the disease is commonly treated with corticosteroids, sometimes in combination with other immunosuppressant drugs, and less commonly with lung transplantation. Individuals with IPF develop abnormal, excessive scarring that can cause progressive shortness of breath, cough, and, in many cases pulmonary hypertension and right heart failure. Therapy is rarely effective and the disease progresses rapidly, resulting in death

over a relatively short time in most patients. NHLBI-supported extramural researchers are investigating the molecular and cellular events that trigger the alveolar injury seen in early stage IPF and initiate progression to the irreversible, fibrotic end stage of the disease. In addition, a clinical network to test drug therapy in patients with early and advanced IPF has been established.

In FY 2005, NHLBI-funded researchers reported that fibroblasts involved in tissue fibrosis, which were previously believed to originate locally in lung tissue, may originate in the bone marrow as progenitor fibroblasts and be attracted into lung tissue. The finding, which indicates that local lung tissue injury can elicit a systemic response, suggest new possibilities for treating IPF by suppressing fibrocytes in the bone marrow or intercepting them in the circulation. Other IPF-related research continues to focus on the transition of activated fibroblasts into myofibroblasts, which produce the growth factor TGFβ, a primary cause of tissue damage. In a recent trial, TGFß secreting myofibroblasts were targeted using immunosuppressant (interferon gamma) therapy. In another trial, 90 IPF patients were treated with pirfenidone, a drug with anti-inflammatory, antioxidant, and antifibrotic effects. The one-year, placebo-controlled trial did not demonstrate a difference in patient groups for the primary end point. In a third trial, 182 patients with IPF were treated for one year with standard therapy (prednisone and azathioprine) or with acetylcysteine (NAC) added to the other two drugs. Acetylcysteine is an antioxidant drug that can compensate for the reduced levels of glutathione present in IPF lung tissue. Results in the NAC treated patients compared with the prednizone and aziothioprine alone found some preservation of lung vital capacity and a higher lung carbon monoxide diffusing capacity. The favorable effects of NAC were attributed to its ability to ward off the toxicity of aziothiopine, known to deplete glutathione. Finally, an NHLBI-supported study showed that interstitial pneumonias, including IPF, can occur in families suggesting that genetic factors and environmental exposures have a role in the development of familial pulmonary fibrosis. An aggregation of fibrosis was found in families and in sibling pairs demonstrating transmission consistent with autosomal dominant inheritance of IPF as well as an interaction with environmental factors.

*Lymphangioleiomyomatosis (LAM)*

LAM is a rare lung disease that affects women, usually during their reproductive years. Symptoms develop as the result of proliferation of atypical, non-malignant, smooth muscle-like cells and the formation of cysts in the lungs. Diagnosis is usually made by lung biopsy. Common symptoms include shortness of breath, cough, and sometimes coughing up blood. Patients often develop spontaneous pneumothorax or chylous pleural effusion (collapse of the lung or collection of milky looking fluid around the lung). The clinical course of LAM is quite variable, but is usually slowly progressive, eventually resulting in death from respiratory failure. Although no treatment has been proven effective in halting or reversing LAM, lung transplantation is a valuable treatment for patients with end-stage lung disease. Some patients with Tuberous Sclerosis Complex (TSC), an inherited disease, develop lung lesions identical to those seen in LAM. In some cases, the clinical distinction between TSC and LAM is difficult to make. However, LAM-related lung dysfunction appears to be milder in individuals with TSC-LAM than in those who have LAM alone. The underlying genetic mechanisms that cause LAM

and TSC are controlled by abnormalities in the same genes, but TSC is inherited and LAM is a disease that occurs sporadically (does not appear to run in families). Mutations in the tuberous sclerosis complex gene, TSC2, can cause pulmonary LAM. The same types of mutations occur in the cells taken from LAM lesions in the lungs and cells taken from benign kidney tumors, known as angiomyolipomas. The cells in the lung and the kidney are thought to have a common genetic origin.

The NHLBI supports research on LAM in both its extramural and intramural programs. As part of the intramural program, the Institute has established a research laboratory at the NIH Clinical Center to study the causes and progression of LAM at the cellular, molecular, and clinical levels. The NHLBI extramural program supports a national LAM Patient Registry that is coordinated by the Cleveland Clinic Foundation. Although data collection from patients with LAM has ended, the Registry continues to help manage the collection, processing, and distribution of LAM tissue for current LAM projects and future research. After 2005, the Registry only will handle requests for LAM tissue. The NHLBI participates in the Trans-NIH TSC Coordinating Committee and assists in the management of the Rare Lung Diseases Consortium, which is developing a clinical trial of the use of rapamycin in patients with LAM. The Consortium is part of the Rare Diseases Clinical Research program supported by the NIH Office of Rare Diseases and the National Center for Research Resources.

A team of NHLBI-supported extramural researchers recently reported on antibody studies that have provided insight into the origin of the abnormal cells that cause LAM. Although LAM cells have many features in common with smooth muscle cells, the new studies show that the origin of LAM cells is different and that they should no longer be considered a variant of smooth muscle. A publication from another group focused on TSC2 gene function, which is associated with the abnormal proliferation, migration, and differentiation of LAM cells. The new study adds to the growing evidence of the neoplastic nature of human LAM cells and shows how TSC2 controls pathways responsible for cell migration and invasiveness. Malfunction of TSC2 in LAM permits the LAM cells to migrate and invade the lung. Understanding how TSC2 controls molecular signaling pathways for cell migration should enhance understanding of other rare and common lung diseases, including pulmonary arterial hypertension, asthma, and idiopathic pulmonary fibrosis.

Researchers in the NHLBI intramural research program have recently published four new studies related to LAM. One study showed that patients with LAM have low bone mineral density (BMD), which is related to loss of lung function and menopause. Lung transplantation resulted in a dramatic loss of BMD, suggesting that patients should be aggressively treated to maintain and improve BMD prior to transplantation. Patients with LAM responded to drugs such as the bisphosphonates, which are commonly used to treat osteoporosis and osteopenia. The second study, a retrospective review of patients seen at the NIH, showed that, in LAM patients matched for age and pulmonary function, there was no positive effect of progesterone treatment on pulmonary function. The third study showed that LAM cells with mutations in the TSC2 gene could be isolated from blood and other body fluids using a new cell isolation procedure. The procedure could be a useful method for relatively non-invasive diagnosis of LAM. The fourth

study showed that abdominal lymphangiomyomas in LAM patients vary in size during the day; this finding may help to differentiate them from other abdominal neoplasms such as sarcomas.

*Narcolepsy*

Narcolepsy is a disabling sleep disorder affecting over 100,000 people in the United States. It causes excessive daytime sleepiness and rapid onset of deep (REM) sleep. Other symptoms include abnormalities of dreaming sleep, such as dream-like hallucinations and transient periods of physical weakness or paralysis (cataplexy). Low cerebrospinal fluid levels of hypocretin, a neurochemical messenger linking sleep with the regulation of muscle tone and alertness, are highly specific to narcolepsy. The NHLBI supports basic and clinical research to identify brain abnormalities associated with narcolepsy that contribute to symptoms such as daytime sleepiness, sleep disturbance, and physical weakness.

*Persistent pulmonary hypertension of the newborn (PPHN)*

PPHN affects approximately 1 in 1,250 newborns. Due to inappropriate muscularization of fetal pulmonary vessels, the lung arteries of affected newborns fail to dilate after birth to allow for normal blood flow through the lung. Infants with PPHN are poorly oxygenated and require costly and prolonged medical care including: intubation of the airway, inhalation of oxygen, mechanical ventilation and, often, heart/lung bypass (extracorporeal membrane oxygenation). The NHLBI supports a spectrum of basic and clinical research grants concerned with achieving a mechanistic understanding of structural and functional defects of the pulmonary circulation in order to create new opportunities for correcting them. One of four NHLBI-funded Specialized Centers of Research on the Pathobiology of Lung Development is studying several aspects of the unique vascular response of the neonate to injurious stimuli to identify basic molecular mechanisms involved in the development of hypertensive pulmonary disorders such as PPHN.

Several recent advances have been made in research related to PPHN. Recent clinical studies point to a critical role for endogenous nitric oxide as a modulator of levels of vasoactive mediators whose net balance determines pulmonary vascular tone and reactivity. Investigators also have shown that several modes of delivering low dose nitric oxide (NO) to the newborn during the transition from fetal circulation to normal circulation are effective in preventing the development of PPHN in infants who apparently do not quite make the threshold level of needed nitric oxide. Studies on the function of the urea cycle, as well as genetic variants of the enzymes participating in the cycle, might lead to an alternative way to provide otherwise expensive NO therapy to a wider population of at risk infants. Thus, the new opportunities being explored for treating PPHN depend on a mechanistic understanding of the processes by which nitric oxide is generated in the course of normal development.

*Primary ciliary dyskinesia (PCD)*

PCD, also known as Kartagener's syndrome or immobile ciliary syndrome, is an inherited disease characterized by defects in the cilia lining the respiratory tract. Patients with PCD exhibit impaired ciliary function, reduced or absent mucous clearance, and susceptibility to

chronic, recurrent, respiratory infections, including sinusitis, bronchitis, pneumonia, and otitis media. The disease typically affects children ages 0 to 18 years, but the defect associated with it has a variable clinical effect on disease progression in adults as well. Many patients experience hearing loss and, in males, infertility is common. Another symptom, situs inversus (having organs on the opposite side from usual), occurs in approximately 50 percent of PCD patients. Clinical progression of the disease is variable with lung transplantation required in severe cases. For most patients, aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial superinfections are recommended. NHLBI-supported researchers are relating the molecular etiology of PCD to the ciliary phenotype. The researchers have established a PCD repository for DNA and phenotypic data. A large cohort of subjects with PCD is undergoing rigorous diagnostic evaluation and determination of the specific ultrastructural abnormalities in their cilia. The relationship of mutations in specific genes to ultrastructural defects and ciliary functions is being investigated. A pilot and feasibility grant awarded by the NHLBI in FY 2005 will use samples from the repository and employ high-throughput DNA sequencing technologies to conduct a comprehensive screen for mutations in candidate genes that cause ultrastructural changes and loss of ciliary function in patients with classic PCD. Subsequently, DNA samples from term neonates with respiratory distress will be tested for these mutations to explore the hypothesis that genetic ciliary defects, especially dynein abnormalities, are a leading cause of neonatal respiratory distress in term infants.

PCD represents a genetically heterogeneous hereditary disease. Three PCD genes, DNAI1, DNAH1, and DNAH5, have been identified. Recent findings provide evidence that the mutations responsible for ciliary defects in PCD cause altered localization of axonemal proteins within the cilia of respiratory cells. It is possible that an immunofluorescence-based method detecting changes in the axonemal proteins might provide a new technique to diagnose PCD.

*Primary pulmonary hypertension (PPH)*

PPH is a rare, progressive, and fatal disease that predominantly affects women, regardless of age or race. It causes deadly deterioration of the heart and lungs. The basic types of pulmonary arterial hypertension (PAH) are familial PAH (primary or genetic), PAH resulting from an unknown cause (idiopathic), and PAH that develops in patients as a result of another serious disorder such as scleroderma, chronic obstructive lung disease, cystic fibrosis, lupus, or as a result of the use of appetite suppressants (secondary). In FY 2005, the NHLBI supported a portfolio of more than 80 projects on PAH. They include research on the basic cell and molecular biology of PAH; research to identify the genes and gene mutations that predispose a person to develop PAH; and multi-disciplinary projects combining basic and patient-based research. One study is gathering genetic information on patients with PAH to investigate whether interactions between genes and environment might cause the disease. In addition, two small business innovation research (SBIR) Phase I grants were funded. One supports the initial development of a small molecule inhibitor of the receptor for urotensin-11 (U-11). U-11 is a potent vasoconstrictor, and the grant will analyze different analogs of an inhibitor for its receptor in hopes of developing a new drug to treat PAH. The other supports the development of a device to mimic small pulmonary arteries and serve as a model for studying and testing new drugs to treat PAH. The device will be lined with endothelial cells and have the capability to alter flow

rates as well as the gas and chemical content of fluid. Through its intramural program, the NHLBI also supports two ongoing clinical trials examining the underlying mechanisms of PAH in patients with sickle cell anemia.

Scientific advances in FY 2005 include results from two NHLBI-funded studies that combined the use of patient samples and animal models to demonstrate that gene therapy may be an effective treatment for PAH. The first study examined an inhibitor of angiopoietin-1 (Ang-1), a protein that stimulates smooth muscle cell proliferation and contraction and indirectly stimulates endothelial cell proliferation as well. The inhibitor blocked the development of PAH in a monocrotaline-induced rat model of PAH. The second study showed that blocking the activation of the receptor TIE-2 using a gene for a soluble form of the receptor decreased endothelial cell proliferation and vasoconstriction and inhibited PAH in hypoxic rats.

*Pulmonary alveolar proteinosis (PAP)*

PAP is a rare disorder in which accumulation of surfactant, a fluid secreted by the cells of the alveoli (the tiny air sacs in the lungs), results in respiratory insufficiency due to impaired gas exchange. The clinical course is variable, ranging from spontaneous resolution to respiratory failure, and can be complicated by serious secondary infections. Although the disorder occurs in three distinct clinical forms, primary (idiopathic), secondary, and congenital, over 90 percent of PAP cases are primary. The NHLBI supports a modest basic research program on the causes and pathogenetic mechanisms underlying PAP.

Monocyte chemotactic proteins 1-3 are important chemokines involved in regulating mononuclear cell migration and activation and growth factor secretion by epithelial, endothelial, and smooth muscle cells. A study published in FY 2005, showed that all three MCPs are increased in PAP lungs and serve to suppress the expression of their corresponding receptors on lymphocytes and alveolar macrophages. The increases in MCPs may thus decrease activation of macrophages and contribute to the accumulation of surfactant. Another recent study focuses on PAP diagnosis. Currently, the only definitive diagnostic test for PAP is open lung biopsy. However, previous studies showed that antibodies to granulocyte macrophage colony stimulating factor (GM-CSF) are increased in PAP, and that the antibody titer is correlated with disease pathogenesis. The new study describes an innovative, very sensitive, quantitative assay for GM-CSF antibodies that may become a useful clinical diagnostic test for PAP.

*Sarcoidosis*

Sarcoidosis is a disease involving organ systems throughout the body in which normal tissue is invaded by pockets of inflammatory cells called granuloma. Most sarcoidosis patients have granuloma in their lungs. The disease can occur in a mild form that disappears spontaneously or in a severe form that results in a life-long condition. Estimates of the number of Americans with sarcoidosis range from 13,000 to 134,000, and between 2,600 and 27,000 new cases appear each year. Up to 5 percent of individuals with pulmonary sarcoidosis die of causes directly related to the disease. The morbidity associated with sarcoidosis can be severe, resulting in significant loss

of function and decrease in quality of life. The causes of sarcoidosis are presently unknown, but disease development is thought to involve both a genetic predisposition and the immune system.

The NHLBI supports research on sarcoidosis in its extramural and intramural programs. The Institute funds laboratory-based research to investigate granuloma formation and to obtain a better understanding of initiating events, disease processes, and the contribution of susceptibility genes. It also supports A Case Control Etiologic Study of Sarcoidosis (ACCESS). ACCESS investigators have created a repository of DNA specimens collected from more than 700 sarcoidosis patients and paired controls. Another NHLBI-supported study of sarcoidosis, the Sarcoidosis Genetic Analysis Consortium (SAGA), ended in 2005. SAGA collected environmental, phenotypic, and genotypic data on 360 African American families with two or more affected siblings to identify sarcoidosis susceptibility genes and to determine how the genes and environmental risk factors act together to cause sarcoidosis. Data from SAGA are now being analyzed. In addition, intramural investigators are initiating a clinical trial that will evaluate atorvastatin as a treatment for pulmonary sarcoidosis. Atorvastatin may reduce inflammation, and reduce the requirement for prednisone.

In FY 2005, ACCESS researchers published several results. In one of them, investigators assessed lung involvement and the association of demographic and psychosocial factors with respiratory health in 736 persons with sarcoidosis. They found that 95 percent of patients had lung involvement, that slightly more than half reported shortness of breath, and that more advanced sarcoidosis was associated with a decrease in Forced Vital Capacity (FVC). Patients who had more airways obstruction and shortness of breath were more likely to be depressed and report a reduction in their quality of life. The investigators concluded that a "global" approach to sarcoidosis treatment, including careful assessment of shortness of breath, health related quality of life, lung function and radiographic changes, and any extrathoracic involvement, not only is important in management of individual patients, but also could be beneficial in assessing outcomes in future clinical trials. A second study published in FY 2005 focused on tissue biopsy for diagnosis of sarcoidosis, since diagnosis is most secure when supported by a tissue biopsy. Clinicians must decide which site offers the best chance of achieving a diagnostic biopsy with the least patient risk and discomfort. All 736 patients enrolled in ACCESS had a diagnostic organ biopsy (some had more than one site biopsied) within six months of recruitment. The biopsy sites were correlated with demographic data, chest radiographic stages, symptoms, pulmonary function, and associated organ involvement. The investigators concluded that biopsy diagnosis in sarcoidosis is almost always easily obtained. Many sites yield good results and the choice for biopsy is influenced by the presenting clinical constellation of organ involvement and the ease and safety of the biopsy procedure. In a third study, the ACCESS investigators collected data on industrial and occupational exposures in patient and control groups. They found that individuals exposed to industrial organic dust, especially Caucasian workers, are at greater risk of developing sarcoidosis. Individuals working for suppliers of building materials, hardware, and gardening materials and educators also were at an increased risk for sarcoidosis. Childcare work was negatively associated with sarcoidosis risk. Jobs with metal dust or metal fume exposures were negatively associated with sarcoidosis risk, especially in Caucasian workers. The researchers concluded that exposures in particular occupational settings may affect sarcoidosis risk.

**Blood Diseases and Resources Programs**

*Acquired aplastic anemia*

Acquired aplastic anemia is an unusual hematologic disease in which the bone marrow fails to produce red cells, white cells, and platelets resulting in severe anemia, low white blood cell counts, and low platelet counts. The NHLBI intramural research program conducts clinical and laboratory research on bone marrow failure syndromes, including aplastic anemia. Intramural researchers have conducted multiple laboratory experiments directed at the pathophysiology of aplastic anemia as well as clinical programs dedicated to the treatment of the disease by immunosuppression and stem cell transplantation. Recently, the intramural program completed two clinical trials testing treatments for mild and severe aplastic anemia and a study of molecular clonotyping of the T-cell response in patients with aplastic anemia. They also described mutations in the telomere repair complex genes in patients with apparent acquired aplastic anemia.

*Cooley's anemia*

Cooley's anemia (also called beta-thalassemia, thalassemia major or Mediterranean anemia) is a genetic blood disease that results in inadequate production of hemoglobin. Individuals affected with Cooley's anemia require frequent, lifelong blood transfusions to sustain life. Because the body has no natural means to eliminate iron, the iron contained in transfused red blood cells builds up over many years and eventually becomes toxic to tissues and organ systems. In addition, many children with Cooley's anemia have acquired other diseases such as hepatitis through years of transfusion exposure. Extramural research efforts of the NHLBI include identifying mutations in the globin gene cluster that lead to Cooley's anemia, determining the mechanism by which naturally occurring mutations significantly increase levels of fetal hemoglobin in adult red blood cells, developing therapeutic applications related to naturally occurring mutations, studying iron chelation, identifying clinically useful therapies and drugs for the disorder, and developing gene therapy strategies to reduce morbidity and mortality associated with Cooley's anemia.

The NHLBI continues to support research to enable the efficient identification and targeting of hematopoietic stem cells, to determine the effects of *ex vivo* manipulation of stem cells on the biologic properties of cells, and to improve vectors to facilitate the development of gene therapy for Cooley's anemia. The Institute also supports programs in therapy development as well as The NHLBI Thalassemia Clinical Research Network to test new therapies. The Network, which is composed of 5 clinical centers, was renewed in July 2005. Having completed four previous studies, the Network is now investigating the use of Deferoxamine with and without L1-Deferiprone to improve cardiac function in adults with Thalassemia major. The study is currently underway in the United States and additional international sites are being established. In addition, a plan is in place to create a Hemoglobinopathies Coordination Committee (HCC) to oversee and facilitate the collaboration of clinical studies between NHLBI sickle cell disease and thalassemia networks where appropriate.

Several new studies of Cooley's anemia recently have been published. For example, over the past 5 years the Thalassemia Clinical Research Network has studied the prevalence of low bone mass in patients with thalassemia. Recently, the Network reported on the prevalence of bone fractures among patients with thalassemia and the relationship of the fractures to low bone mass. Other recent work published by the Network includes reports from the L1-DFO Cardiac Clinical Trial on the use of innovative magnetic resonance imaging techniques to evaluate levels of iron stored in liver, heart, and brain. Recent papers from other investigators reported on advances in the measurement of iron in tissues using needle biopsy and on the development of new iron chelators to remove iron from tissues. Another current topic of interest in Cooley's anemia research is use of hydroxyurea and phenyl butyrate, which have promise for ameliorating symptoms in sickle cell disease patients, to treat thalassemia patients. A recent study showed that hydroxyurea has only a mild effect in raising hemoglobin levels in thalassemia patients and phenyl butyrate has no significant effect. Another recent paper published by NHLBI-funded researchers explored the changing demographics of Cooley's anemia patients in North America. Traditionally, most thalassemia patients in North America were of Mediterranean origin. As immigration from Southeast Asia increases, the population of thalassemia patients and the associated genotypes and phenotypes found in North America are changing. The paper focused on the importance of providing appropriate education, diagnosis, and management for a diverse group of patients. Other recent work focused on the development of vectors for gene therapy in sickle cell disease and thalassemia. Several new papers and reviews illuminated the field in preparation of future gene therapy trials. A final area of interest relates to pulmonary hypertension, which is quite prevalent among adults with sickle cell disease and thalassemia. Recent work in this area has focused on the role of nitric oxide (NO) in pulmonary vasodilation, the inhibition of pulmonary vasodilation by hemoglobin binding to NO, and the role of arginase in removing arginine, a substrate needed for production of NO.

*Creutzfeldt-Jakob disease (CJD)*

CJD is a slow degenerative disease of the central nervous system that is characterized by motor dysfunction, progressive dementia, and vacuolar degeneration of the brain. The disease is rare, but invariably fatal, and is associated with a transmissible agent. A protease-resistant protein or prion is the hallmark of all transmissible spongiform encephalopathies (TSE) including CJD. Therefore, the term prion diseases is applied to this group of neurodegenerative illnesses, which includes bovine spongiform encephalopathy (BSE) or Amad cow disease,@ scrapie in sheep, and chronic wasting disease in deer and elk. Prion diseases may cross the species barrier, the most notable example being the recent cases of variant CJD in humans caused by consumption of beef contaminated with BSE. Classical CJD occurs worldwide at a rate of 1-2 cases per million people per year.

A major goal of several NHLBI programs is to develop blood tests that would be suitable for screening the U.S. blood supply to detect TSE in asymptomatic individuals. The NHLBI and the National Institute of Neurological Disorders and Stroke are jointly supporting an extramural contract program to develop tests for TSE diseases. The lack of a rapid, sensitive, and specific test for TSE infectivity has slowed progress in the study and control of CJD and other prion

diseases. Because TSE agents are at very low concentrations in the blood of laboratory animals, investigators are developing methods to concentrate the agents so that they can be detected using currently available assays. A variety of different concentration approaches are being tried. The NHLBI also supports a grant to develop a fluorescence-based test to detect low levels of prion proteins.

*Fanconi anemia (FA)*

Fanconi Anemia (FA is an autosomal recessive bone marrow failure syndrome characterized by a decrease in blood cells and platelets, developmental defects, and cancer susceptibility. Many FA patients can be identified at birth because of congenital anomalies, although approximately 25 percent do not have birth defects. FA is a clinically heterogeneous disorder that currently can be divided into at least twelve complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M). Genes for 11 of the complementation groups have been cloned, and 10 independent genes have been characterized. The NHLBI supports studies designed to identify and clone the remaining FA genes. An ongoing NHLBI program project has taken a multi-disciplinary approach to identify causes of FA at the molecular and cellular level. The scientific areas represented in the program include molecular hematology, molecular genetics, mouse genetics, gene therapy, stem cell biology, and DNA repair. Continued efforts to develop protocols for the efficient identification and targeting of hematopoietic stem cells, to obtain information on how *ex vivo* manipulation of stem cells alters their biologic properties, and to improve vectors are expected to make significant contributions to enhancing the potential for a cure for FA. Recently the NHLBI funded a new study "Novel Molecular and Cellular Therapies in Fanconi Anemia" with a clinical component that is using gene therapy to treat FA patients.

*Hemophilia*

Hemophilia is a hereditary bleeding disorder caused by a deficiency in either blood coagulation factor VIII or factor IX. The approximately 20,000 individuals in the United States with hemophilia are dependent on lifelong treatment to control periodic bleeding episodes. The NHLBI supports a broad spectrum of activities on blood coagulation and its disorders. Hemophilia research includes viral and non-viral approaches for gene therapy, mechanisms of antibody inhibitor formation, modification of factors for improved therapeutics, safety of plasma derived products, and blood product-associated infections. In addition, basic genetic, molecular biology, and protein biochemistry studies of factors VIII and IX are supported to improve understanding of their mechanisms of action and their regulation. The NHBLI supports two program project grants related to hemophilia. One of the grants funds multiple approaches to develop gene-based therapies for hemophilia A and B. The other investigates immune responses to adeno-associated viral-mediated gene transfer of factor IX. Five new individual project grants were awarded in response to the RFA, Improved Therapies for Hemophilia and Hereditary Bleeding Disorders.

Previously, NHLBI-supported researchers reported that a novel intravascular delivery technique to achieve adeno-associated virus-mediated factor IX gene transfer produced therapeutic levels of factor IX in dogs with hemophilia. In FY 2005, the investigators published new findings

showing that factor IX levels have remained high in the animals for over 3 years, providing correction of the bleeding disorder. Another NHLBI-supported research team developed a recombinant factor VIII-O-phospho-l-serine complex and showed that the complex has reduced immunogenicity and retains *in vivo* activity in a mouse model of hemophilia. Other Institute-funded researchers used a gene transfer approach to create B cells expressing human factor VIII domains linked to immunoglobulin G carriers for antigen specific immune tolerance. Treatment of hemophilia A mice with these modified B cells was found to prevent or decrease the level of existing antibodies. Finally, researchers continue to conduct studies using mice that have factor IX gene mutations that mimic mutations known to occur in humans. The new animal models are expected to be valuable research tools.

*Hereditary hemorrhagic telangiectasia (HHT)*

Hereditary hemorrhagic telangiectasia (or Osler-Weber-Rendu disease) is a bleeding disorder caused by weakness of the vascular support structure. Its most common manifestations are red spots on the lips and bleeding from mucosal membranes such as in the nose. HHT also is characterized by focal swelling of blood vessels in different organs which may rupture and lead to bleeding. In an advanced stage, arterio-venous malformations often develop in the lung, brain, gut, and liver. Two gene defects have been identified in patients with HHT. One is in a gene associated with the protein endoglin and the other is in a gene related to activin receptor-like kinase. A correlation may exist between the gene defect and organ susceptibility to the disease. Recent studies using a mouse model of HHT show that inflammation induces significant structural changes in the microcirculation of the animals. The results suggest that, in addition to genetic factors, inflammation may contribute to HHT. The NHLBI supports a broad spectrum of research in hemostasis and thrombosis which should enhance understanding of HHT. The research focuses in part on understanding the biology of platelet activation, the mechanism of clotting, and the interaction of blood with the vascular surface.

*Lymphedema*

Lymphedema is caused by an accumulation of lymphatic fluid in interstitial tissue that causes swelling, most often in the arm(s) and/or leg(s), and occasionally in other parts of the body. Lymphedema can develop when lymphatic vessels are missing or impaired (primary or congenital), or when lymph vessels are damaged or lymph nodes removed (secondary). When the impairment becomes so great that the lymphatic fluid exceeds the lymphatic transport capacity, an abnormal amount of protein-rich fluid collects in the tissues of affected areas. Left untreated, this stagnant, protein-rich fluid not only causes tissue channels to increase in size and number, but also reduces oxygen availability in the transport system, interferes with wound healing, and can cause infection. The NHLBI supports studies to elucidate developmental, molecular, and cellular causes of lymphedema as well as research to develop better therapies for both primary and secondary lymphedema. The NHLBI funds several individual research projects investigating lymphedema. A new project funded in FY 2005 addresses the "Molecular Mechanisms of Lymphatic Muscle Contraction." In FY 2005, NHLBI-supported researchers reported that functional inactivation of a single allele of the gene Prox1 causes adult-onset obesity. The defect altered lymphatic vessels causing them to become leaky, suggesting that

certain types of lymphatic dysfunction contribute to adult onset obesity. Another recent study tested whether introducing the gene for VEGF-C, a protein that stimulates new lymphatic vessel growth, may offer a therapeutic approach for treating lymphedema. The results show that the effects of exogenous VEGF-C on normal lymphatic regeneration are transient and unsustainable and suggest that other lymphatic growth factors and modulators are necessary to develop and maintain physiologically viable lymphatic vessels.

*Paroxysmal nocturnal hemoglobinuria (PHN)*

PHN is a disease of the bone marrow in which acquired, somatic mutations in the PIG-A gene lead to expansion of a clone of cells unable to express glcosylphosphatidylinositol-anchored proteins on their surface. The disorder results in intravascular hemolysis, proclivity to venous thromboses, and bone marrow failure. The NHLBI intramural program conducts clinical and laboratory studies of bone marrow failure syndromes, including PHN.

*Sickle cell disease*

Sickle cell disease (SCD) is an inherited blood disorder that is most common among people whose ancestors come from Africa, the Middle East, the Mediterranean basin, and India. In the United States, approximately 50,000 individuals, primarily African Americans, have SCD (SS hemoglobin). One of every 650 African Americans (0.15 percent) is born with SCD, and about 8 percent are heterozygous for the sickle cell gene. SCD occurs when an infant inherits the gene for sickle hemoglobin from both parents (Hb SS = sickle cell anemia) or the gene for sickle hemoglobin from one parent and the gene for another abnormal hemoglobin from the other parent. SCD is caused by hemoglobin polymerization that in turn causes red blood cells to become hard, sticky, and shaped like sickles or crescents. When sickled cells go through small blood vessels, they tend to get stuck and block the flow of blood. This can cause anemia. It can also lead to the acute painful episodes that are the hallmark of the disease and can cause chronic damage to the brain, heart, lungs, kidneys, spleen, and liver. The median age at death for patients with SCD is 42 years for men and 48 years for women. Pulmonary complications account for a large proportion of deaths among adults with sickle cell anemia. According to the Cooperative Study of Sickle Cell Disease (CSSCD), in a prospective multi-center study of 3,764 patients, over 20 percent of adults had fatal pulmonary complications of sickle cell anemia. Acute and chronic pulmonary complications of sickle cell anemia, such as pulmonary hypertension, pulmonary fibrosis, and asthma are also common.

Finding improved treatments and ultimately a cure for SCD is a major commitment of the NHLBI. The NHLBI SCD program includes investigator-initiated and NHLBI-initiated components. The NHLBI extramural program supports a wide range of investigator-initiated SCD research, including basic research on pathophysiologic mechanisms, translational research involving early stage testing of new therapies in humans, late stage clinical trials (either as stand-alone projects or within multi-project clinical research networks), and a limited amount of patient outcomes research (e.g., quality of life research). Over the last five years the NHLBI, the NIH Clinical Center, and the National Institute of Diabetes and Digestive and Kidney Diseases have created a unified consortium of intramural investigators that is one of the largest SCD

translational research programs in the country. The largest of the intramural research protocols is addressing the prevalence, etiology, and treatment of secondary pulmonary hypertension, a leading cause of adult mortality in patients with SCD. The intramural clinical program is complemented by a basic science program focusing on pathophysiology and experimental therapeutics.

In FY 2005, the NHLBI continued to support several studies of SCD. Examples include the Multicenter Study of Hydroxyurea (MSH) Patients' Follow-up, which is assessing the effects of long-term hydroxyurea use in patients who participated in the adult hydroxyurea clinical trial that ended successfully in 1995; the BABY HUG (Infant Hydroxyurea Study), which is assessing the effectiveness of hydroxyurea in preventing onset of chronic organ damage in babies with SCD and; the Sibling Donor Cord Blood Banking and Transplantation Study to establish a cord blood bank for collecting sibling donor cord blood in families that currently have a child with sickle cell anemia or thalassemia. Investigators are evaluating the safety and effectiveness of matched sibling cord blood transplantation for treatment of children with SCD or thalassemia. Also in FY 2005, the NHLBI began the Multicenter Neurocognitive and Neuroimaging Study in Adult Sickle Cell Disease to assess baseline neurocognitive function and neuroimaging abnormalities in adults with SCD and to randomize the patients with subnormal neurocognitive scores to receive 6 months of transfusion versus standard of care, followed by reassessment of baseline neurocognitive function. In addition to the other activities, the NHLBI, with support from the National Center for Minority Health and Health Disparities, also awarded a grant supplement to the International Bone Marrow Transplant Registry to collect data on demographics and outcomes of patients with sickle cell anemia who received a blood or marrow transplant. The program is designed to promote a unified strategy for sharing data, since transplants for patients with sickle cell anemia are performed at many centers across the United States, with few performed at a single center.

NHLBI-funded researchers reported a number of scientific advances related to SCD in FY 2005. Investigators from the MSH study reported that brain natriuretic peptide levels in the blood are correlated with the presence of pulmonary hypertension. BABYHUG investigators presented two abstracts (on hydroxyurea pharmacokinetics and on the use of trans-cranial Doppler ultrasonography to measure cerebral blood flow in babies) at the 47$^{th}$ annual American Society of Hematology meeting. Investigators from the STOP II trial showed that discontinuation of transfusions given to prevent stroke in children with SCD resulted in a high rate of reversion to abnormal blood-flow velocities on Doppler studies, and to stroke events. As a result, in December 2004, the NHLBI issued a clinical alert to physicians who treat children with SCD advising that stopping transfusions cannot be recommended. It also urged physicians to discuss with patients and their families the stroke prevention benefits of continuing periodic transfusions and the associated risks.

Other FY 2005 advances include using a human computational genetics approach to explain the dependence of stroke risk (in individuals with SCD) on the presence of specific polymorphisms in as many as 20 different genes; establishing a relationship between polymorphisms in the genes klotho, bone morphogenic protein 6, and annexin A2 (that play a role in bone morphology, metabolism, and vascular disease) and osteonecrosis in patients with SCD; identifying 5-

hydroxymethyl-2-furfural as an anti-sickling drug that specifically binds to intracellular sickle hemoglobin and improves survival of transgenic sickle cell mouse models under low oxygen conditions; associating priapism with increased hemolysis and decreased circulating nitric oxide (NO) levels in SCD patients and ; documenting that the inactivity of the nitric oxide pathway in SCD is in part explained by increased blood levels of arginase, an enzyme that destroys arginine, which is need to produce NO.

*Systemic lupus erythematosus (SLE)*

SLE is an autoimmune disorder in which the body produces antibodies that harm its own cells and tissues. Typical symptoms include fatigue, arthritis, fever, skin rashes, and kidney problems. SLE affects more women than men. The risk of coronary heart disease in women with SLE is up to 50 times higher than in the general population. SLE patients have a higher incidence of blood clot formation (thrombosis) and spontaneous loss of pregnancy. Although its cause remains unknown and no cure is currently available, SLE symptoms can be controlled with appropriate treatment so that most patients can lead an active life. The NHLBI supports two major areas of research relevant to SLE. First, the NHLBI funds research on components of the blood that regulate bleeding and blood clotting (hemostasis and thrombosis) to understand the biology of platelet molecule function, mechanisms of blood clotting, and the interaction of blood components with blood vessel surfaces. Second, the NHLBI funds research on cardiovascular complications and risk factors that may help explain the elevated incidence of premature cardiovascular disease in women with SLE. In addition to these areas, the NHLBI supports a clinical protocol to treat SLE using allogeneic hematopoietic stem cell transplantation.

Antibodies to phospholipid-binding proteins, hallmarks of antiphospholipid syndrome (APS), are present in 50 percent of SLE patients versus only 1-5 percent of healthy individuals. In APS syndrome, the body produces aberrant antibodies, which bind to blood coagulation proteins, and which may lead to miscarriages, thrombosis, and stroke. The clinical manifestations and severity of the disease vary and could be due to genetic variations in the target antigens of the aberrant antibodies. Studies supported by the NHLBI on single nucleotide polymorphisms in proteins related to the protein C pathway in coagulation indicate that interacting genetic variations in the target proteins account, in part, for the variable thrombotic risk. The findings may help in the diagnosis of SLE patients at high risk for heart attacks and stroke.

*Thrombotic thrombocytopenic purpura (TTP)*

TTP is a potentially fatal disease characterized by low blood platelet levels and widespread platelet thrombi in arterioles and capillaries. The disease has a sudden onset and individuals with TTP often exhibit hemolysis, high fever, and neurological abnormalities. Management of patients with TTP is difficult due to the lack of specific diagnostic criteria and rapid progression of disease. The standard therapy for TTP is plasma exchange. Relapse after the acute phase is common. The clinical course differs significantly for patients with idiopathic TTP compared to patients with TTP provoked by predisposing conditions. A congenital or acquired deficiency of a plasma metalloprotease, ADAMTS 13, which cleaves large polymers of von Willebrand factor (vWF), has been linked to the disease. Despite advances in basic research on TTP, treatment

options are limited and mortality remains high. The NHLBI supports a broad spectrum of research in hemostasis and thrombosis that includes research on platelet biology, blood coagulation, thrombolysis, and the interaction of blood with vascular and artificial surfaces. Investigator-initiated grants studying TTP have led to the development of improved assays for ADAMTS 13. In addition, the Institute supports ongoing efforts to enable large scale production of recombinant ADAMTS 13.

**Rare Disease Research Initiatives**

**Ongoing Initiatives**

- Animal Models of Antigen-Specific Tolerance for Heart and Lung Transplantation
- Beryllium-Induced Diseases
- Bioengineering Research Partnerships
- Biology of Iron Overload and New Approaches to Therapy
- Blood and Marrow Transplant Clinical Research Network
- Cell-Based Therapies for Heart, Lung, Blood, and Sleep Disorders and Diseases
- Cellular and Molecular Mechanisms of Primary Pulmonary Hypertension
- Chronic Fatigue syndrome: Pathophysiology and Treatment
- Clinical Networks for the Treatment of Adult Respiratory Distress syndrome (ARDS)
- Comprehensive Sickle Cell Centers
- Coordination of Vascularization and Lung Development
- Cord Blood Transplantation Study
- Development of an Assay for Creutzfeldt-Jakob Disease
- Developmental Processes in Differential Expression of Globin Genes
- Diamond-Blackfan Anemia and Other Congenital Bone Marrow Failure syndromes: Underlying Molecular Mechanisms
- Exploratory Program in Systems Biology
- Functional Heterogeneity of the Peripheral, Pulmonary, and Lymphatic Vessels
- Genelink
- Genetic Aspects of Tuberculosis in the Lung
- Genetic Modifiers of Single Gene Defect Diseases
- Genetically Triggered Thoracic Aortic Aneurysms and Other Cardiovascular Conditions (GENTAC): National Registry
- Granulomatous Lung Inflammation in Sarcoidosis
- Hemophilia and Hereditary Bleeding Disorders: Improved Therapy

- Heritable Disorders of Connective Tissue
- Hutchinson-Gilford Progeria syndrome: Exploratory/Developmental (R21) Grants
- Idiopathic Pulmonary Fibrosis Clinical Research Network
- International Cooperative Biodiversity Groups (ICBG)
- Mechanisms of Fetal Hemoglobin Gene Silencing for Treatment of Sickle Cell Disease and Cooley's Anemia
- Mesenchymal Stem Cell Biology
- Molecular Mechanisms of Mucous Cell Metaplasia and Excess Mucous Secretion in Human Airway Diseases
- Multicenter Study of Hydroxyurea in Sickle Cell Disease: Patient Follow-Up Extension I
- Muscular Dystrophy: Pathogenesis and Therapies
- Myelodysplastic syndromes (MDS): Pathogenesis and Disease Progression
- Myeloproliferative Disorders (MPD): Pathogenesis and Disease Progression
- NHLBI Clinical Proteomics Programs
- NHLBI Lung Tissue Resource
- Novel Approaches to Enhance Animal Stem Cell Research
- Organ Transplantation: Clinical Trials
- Pathogenesis and Treatment of Lymphedema and Lymphatic Diseases
- Pathogenesis of SARS Lung Disease: *In vitro* Studies and Animal Models
- Pediatric Heart Disease Clinical Research Network
- Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG)
- Pediatric Mechanical Circulatory Support
- Plasticity of Human Stem Cells in the Nervous System
- Polymicrobial Diseases: Novel Approaches
- Programs for Genomic Applications (PGAs) for Heart, Lung, and Blood Research
- Programs of Excellence in Gene Therapy (PEGT) for Heart, Lung, and Blood Diseases
- Pulmonary Complications of Sickle Cell Disease
- Pulmonary Fibrosis: Molecular Targets and Interventions
- Rare Diseases: Exploratory and Developmental Research Grants
- Research on Microbial Biofilms
- Somatic Cell Therapy Processing Facilities
- Specialized Centers of Research (SCOR) in Fibrotic Lung Disease

- Specialized Centers of Research (SCOR) in Hematopoietic Stem Cell Biology
- Specialized Centers of Research (SCOR) in (a) Neurobiology of Sleep and Sleep Apnea and (b) Airway Biology and Pathogenesis of Cystic Fibrosis
- Specialized Centers of Research (SCOR) in the Pathobiology of Lung Development
- Stem Cell Plasticity in Hematopoietic and Non-Hematopoietic Tissue
- Thalassemia Clinical Research Network
- Transactivation of Fetal Hemoglobin Genes for Treatment of Sickle Cell Disease and Cooley's Anemia
- Transfusion Medicine/Hemostasis Clinical Research Network
- Treatment of HIV and Associated Complications in Hemophiliacs
- Tuberculosis Curriculum Coordinating Center
- Zebrafish Research Tools

**Initiatives Started in 2005**

*Chronic Fatigue syndrome: Pathophysiology and Treatment*

A renewal of a trans-NIH Program Announcement (PA), sponsored by the NHLBI and several other NIH Institutes and Offices, supports research on the pathophysiology and treatment of chronic fatigue syndrome (CFS) in diverse groups and across the life span. The objective of the initiative is to improve the diagnosis, treatment, and quality of life of all persons with CFS. Research on new hypotheses, heterogeneous population groups, research gaps, and common mediators influencing the actions among and between various bodily systems is encouraged. Topics of special interest to the NHLBI include: (1) the role of neuro-cardiovascular regulation in the loss of normal control of blood pressure, heart rate, and contractility in CFS patients and (2) factors and mechanisms involved in altered sleep state, circadian regulation, and other causes of impaired or ineffective sleep in CFS patients.

*Genetically Triggered Thoracic Aortic Aneurysms and Other Cardiovascular Conditions (GENTAC): National Registry*

A new Request for Proposals (RFP), initiated by the NHLBI establishes a national registry to facilitate research on best medical practices for the clinical management of genetic thoracic aortic aneurysms and other cardiovascular complications associated with connective tissue diseases such as Marfan syndrome. The registry will: (1) collect information on patients, care providers, hospitals, and clinical interventions; (2) collect blood and tissue specimens; and (3) maintain a repository of tissue and blood, family pedigrees, and data on extra-cardiac complications. The NHLBI will collaborate with the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the National Eye Institute, the National Institute of Dental and Craniofacial Research, and the National Human Genome Research Institute to standardize

reporting, by the registry, of patient characteristics, indications for surgical intervention and other treatments, and adverse events.

*Hemophilia and Hereditary Bleeding Disorders: Improved Therapy*

A new NHLBI-initiated Request for Applications (RFA) funds research on immune response and safety issues related to gene transfer or cell-based therapies for bleeding disorders. The objective of the research is to develop improved treatments and possibly cures for, hemophilia, von Willebrand disease, and other hereditary bleeding disorders. The initiative is co-funded by the National Hemophilia Foundation, an organization promoting education, research, and advocacy on behalf of people with bleeding disorders.

*Idiopathic Pulmonary Fibrosis Clinical Research Network*

A new NHLBI-initiated RFA establishes a network of clinical centers to conduct multiple treatment trials on patients with established idiopathic pulmonary fibrosis (IPF), a disease of inflammation that results in scarring of the lungs, and eventually interference with oxygen transport. The network will include six or seven clinical centers and a data-coordinating center. Each of the clinical centers is expected to enroll 40 to 50 patients per year for a 2-year interval of treatment and 2 years of follow-up. Trials may evaluate existing or new medications, combinations of medications, and defined management strategies. For patients who require an open lung biopsy for diagnosis, living lung tissue and blood will be stored for future studies on cellular genomic and immunopathogenic changes.

*Muscular Dystrophy: Pathogenesis and Therapies*

A new PA, sponsored in part by the NHLBI, supports studies of and develops therapies for muscular dystrophies (MD). Premature death due to MD is often caused by cardiac or respiratory failure. Though recent research has increased knowledge about genetic defects associated with many forms of MD, a corresponding improvement in the treatment of MD has not been achieved. Important research priorities include studies of gene and stem cell therapies, pharmacological approaches to treatment, and clarification of the role of inflammatory mechanisms.

*Myelodysplastic syndromes (MDS): Pathogenesis and Disease Progression*

A new RFA, initiated by the NHLBI, encourages research on the causes and progression of myelodysplastic syndromes (MDS) with the ultimate goal of discovering new therapeutic approaches. MDS are a collection of disorders in which the bone marrow does not produce enough blood cells. The diseases can develop due to exposure to certain chemicals or radiation, e.g., those used for the treatment of cancer. A rare, familial form may also develop in patients who have family members with MDS. The initiative supports basic stem cell biology experiments to increase understanding of the mechanisms of MDS and the use of gene expression analysis and identification of gene products or cellular molecular expression to increase understanding of the pathways of disease development and evolution.

*Myeloproliferative Disorders (MPD): Pathogenesis and Disease Progression*

A new RFA, initiated by the NHLBI and co-sponsored by the NCI, supports research on the genetic, biochemical, and molecular pathways that operate in the emergence and progression of myeloproliferative disorders (MPD), a group of conditions characterized by excessive proliferation and production of one or more of the bone marrow (myeloid) cells. Research focuses on analyzing genes that are expressed in MPD and identifying gene products that are associated with or responsible for disease development and progression to a malignant and fatal outcome. Such discoveries will be critical to the development of new therapies for MPD patients who are not suitable candidates for hematopoietic stem cell transplantation, the only curative therapy currently available.

*NHLBI Clinical Proteomics Programs*

The NHLBI is funding a new RFA to validate, on a systematic, comprehensive, large-scale basis, existing and new candidate protein markers that are appropriate for routine use in the diagnosis and management of heart, lung, blood, or sleep diseases and disorders. The initiative establishes an infrastructure for research teams to validate protein panels that may be used to predict disease susceptibility or to assist in differential diagnosis, disease staging, selection of individualized therapies, and monitoring of treatment responses. The validation process uses disease-associated biological samples and clinical data from ongoing and completed NHLBI clinical trials and epidemiologic studies. In addition, the programs provide education and skills development to ensure that scientists have the competencies and expertise needed to address the multifaceted challenges of clinical proteomics.

*Novel Approaches to Enhance Animal Stem Cell Research*

The NHLBI, along with several NIH Institutes, supports the renewal of a PA to facilitate the identification, isolation, and characterization of totipotent and multipotent stem cells from biomedical animal research models. The research generates reagents and develops techniques to characterize and separate stem cells from other cell types. The PA encourages the use of innovative approaches to the problems of making multipotent stem cells available from a variety of nonhuman sources and creating reagents that will identify multipotent stem cells across species and allow for their separation from differentiated cell types.

*Organ Transplantation: Clinical Trials*

The NHLBI funds a new trans-NIH RFA to enhance understanding of and ultimately reduce the immune-mediated morbidity and mortality associated with organ transplantation. A cooperative, multi-site consortium manages interventional, observational, and related mechanistic studies. The NHLBI supports the costs associated with heart and lung transplant patients. The objectives of the RFA are to: (1) evaluate new therapeutic regimens to overcome immunologic barriers to graft acceptance and/or long-term graft and patient survival; (2) evaluate approaches to the treatment or prevention of immune-mediated complications of transplantation; (3) investigate the underlying mechanisms of action of the pathologic processes, agents, or regimens under study;

and (4) develop diagnostic tests and/or surrogate biomarkers that will facilitate routine surveillance, early diagnosis, and ongoing monitoring of those processes that contribute to post-transplant morbidity and mortality.

*Pathogenesis of SARS Lung Disease: In vitro Studies and Animal Models*

A new NHLBI PA advances understanding of the pathogenesis of severe acute respiratory syndrome (SARS)) in the lung using *in vitro* techniques, existing animal models of related coronavirus infections, non-human primate models of SARS, and new rodent models. Research topics of interest include *in vitro* research on the role of collectins and other extracellular host factors; studies of endothelial and epithelial permeability; and investigations of the effects of SARS on fluid movements, growth, and differentiation of human lung cells (e.g., alveolar epithelial cells, fibroblasts). The primary focus is on human SARS coronaviruses, but research using engineered and related animal coronaviruses and animal cells pertinent to the pathogenesis of SARS may also be conducted.

*Programs of Excellence in Gene Therapy (PEGT) for Heart, Lung, and Blood Diseases*

A renewal in FY 2005 of an NHLBI-initiated RFA promotes the rapid translation of basic, preclinical studies of gene therapy for cardiovascular, pulmonary, and hematologic diseases into pilot studies in humans. During their second five-year operating period, the NHLBI Programs of Excellence in Gene Therapy (PEGT) are: (1) conducting preclinical projects to facilitate the translation of gene therapy into clinical studies; (2) conducting clinical Phase I/II studies to test the safety and efficacy of gene therapy procedures; (3) operating six national cores to provide no-cost resources and services to NHLBI-supported investigators conducting gene therapy research; and (4) training MD, MD/PhD, and PhD scientists in conducting gene therapy clinical trials.

*Pulmonary Complications of Sickle Cell Disease*

A new NHLBI-initiated RFA encourages collaborative research between investigators in hematology and pulmonary science that combines basic and clinical approaches to research on pulmonary complications of sickle cell disease (SCD). Acute sickle cell chest syndrome, the second most common acute clinical complication of SCD, is characterized by infiltrates in the lungs, and sometimes by fever, pneumonia, and thromboembolism of peripheral blood clots and/or fat emboli. The less common chronic form of sickle cell lung disease is characterized by pulmonary perfusion and diffusion defects, pulmonary hypertension, changes in the vessel walls such as intramural and perivascular connective tissue deposition, hyperplasia/hypertrophy of smooth muscle cells, and in some cases by intramural thrombosis. Further elucidation of the acute and chronic lung syndromes is required in order to develop more adequate therapies. Projects currently funded under this initiative include research on all the known pulmonary complications of sickle cell disease-vasculopathy due to hemoglobinopathy, asthma, pulmonary hypertension, and acute chest syndrome. A significant amount of human genetics work is also included.

*Thalassemia Clinical Research Network*

In FY 2005, the NHLBI renewed an RFA to continue operation of a cooperative network of five clinical centers and a data coordinating center conducting clinical trials to evaluate existing and future therapies for the treatment of thalassemia major (Cooley's anemia). The network enhances progress in moving effective therapies, e.g., fetal hemoglobin enhancing agents, gene therapy, or iron chelation, from the laboratory to the bedside through rapid and systematic collaborative testing in Phase II and Phase III clinical trials. A registry of thalassemia patients has also been developed and will be used to identify patients available for future trials.

**Initiatives Planned for the Future**

*Blood and Marrow Transplant Clinical Trials Network*

A renewal in FY 2006 of an NHLBI-initiated RFA, supported in collaboration with the National Cancer Institute (NCI), will continue for another five years the operation of a network to accelerate research on the management of hematopoietic stem cell transplantation. The objective of the program is to standardize existing treatments and evaluate new ones. The network, which will comprise 15 interactive clinical centers and a data-coordinating center, will provide a coordinated, flexible mechanism to accept ideas and build consensus from the transplant community. Network investigators will develop protocols; expeditiously perform multi-center Phase II and Phase III clinical trials; and provide information to physicians, scientists, and the public with the ultimate goal of improving stem cell transplantation therapy for diseases such as leukemia, sickle cell disease, thalassemia, and Fanconi anemia. As part of the renewal of the initiative, a collaborative arrangement will be established with the Pediatric Blood and Marrow Transplant Consortium to conduct trials in children.

*Clinical Hematology Research Career Development Program (K12)*

A new RFA, to be initiated by the NHLBI in FY 2006, will establish multidisciplinary career development programs in non-malignant clinical hematology research to equip new academic researchers with the knowledge and skills necessary to address complex problems in blood disease, transfusion medicine, and cellular therapies. Five K12 awards will be available for established clinical hematology investigators to recruit and mentor, during the five-year award period, four to six young physicians. Alternately, one slot at any one time may be dedicated to a non-physician health professional with doctoral preparation. Each scholar will receive two to three years of training in research design and methodology, clinical management of patients with rare hematologic diseases, and research opportunities in the diseases. Programs will include a core curriculum, didactics, and a short-term research project for each scholar. At the end of their training, candidates will be expected to submit a K23 grant application to allow them to continue their research as a faculty member at an academic institution.

*Diagnostic Laboratories for Diseases of Red Cells and White Cells*

In FY 2006, a new NHLBI-initiated PA will support laboratories developing new DNA chip analyses to diagnose, in red and white cells, heritable diseases that are difficult to diagnose using current methods. Studies in red cells may focus on disorders associated with hemoglobin mutations, surface antigen abnormalities, membrane defects, or enzymopathies. In white cells, analyses may focus on disorders associated with phagocytosis, adhesion, chemotaxis, opsonization, ingestion, degranulation, and oxidative metabolism. The creation of one or more gene chip diagnostic laboratories through the Small Business Innovation Research (SBIR) program would facilitate diagnosis of red cell and white cell diseases and thereby effect better treatment and ultimately better health care for individuals with these rare diseases.

*Liver Disease: New Technologies*

In FY 2006, the NHLBI will fund a new PA, initiated by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), to develop, through the small business research community, resources, research tools, instrumentation, biomarkers, devices, drugs, and innovative approaches for the diagnosis, monitoring, management, treatment, and prevention of chronic liver diseases. The PA summarizes the opportunities for transactional research appropriate for small businesses that are described in the February 2005 trans-NIH Action Plan for Liver Disease Research. The NHLBI is especially interested in developing effective treatments to reduce or eliminate the progression of Hepatitis C virus (HCV) to end-stage liver disease in hemophiliacs.

*Lung Response to Inhaled Highly Toxic Chemicals*

A new FY 2006 PA, initiated by the NHLBI and funded in collaboration with the National Institute of Environmental Health Sciences (NIEHS), will support research to investigate acute mucosal irritation in the upper and lower respiratory tract occurring after aerosol exposure to toxic chemicals. The goals of the research will be to develop methods for: (1) minimizing initial injury promptly, (2) retarding and ameliorating progressive mucosal irritation or inflammation, and (3) offering prophylaxis against pulmonary edema.

*Pediatric Heart Disease Network*

In FY 2006, the NHLBI will renew an RFA to operate, for a second five-year period, a network of interactive pediatric clinical research centers to promote the efficient evaluation of new treatments and management strategies that may benefit children with structural congenital heart disease, inflammatory heart disease, heart muscle disease, and arrhythmias. Therapeutic trials and studies will involve investigational drugs; drugs already approved but not currently used devices, interventional procedures, and surgical techniques. The network, which will comprise seven clinical centers and a data-coordinating center, will provide an effective, flexible framework in which to study adequate numbers of patients with rare diseases such as congenital cardiovascular malformations. Efficiencies will be achieved through standardizing procedures to recruit, characterize, monitor, and follow patients. Approximately 2,000 patients are expected to

participate in 6-12 different protocols over the 5-year project period. The network will also serve as a platform to train junior investigators in pediatric clinical research and as a vehicle for rapid and widespread dissemination of findings.

*Protein Interactions Governing Membrane Transport in Pulmonary Health and Disease*

A new, FY 2006, NHLBI-initiated PA will encourage research to delineate the global protein interactions governing membrane trafficking pathways operative in pulmonary disease and develop new therapeutic interventions. Completion of the human genome project and technological advances such as mass spectometry, genomics, and new protein purification methods, now make it possible to probe the molecular pathology of pulmonary diseases associated with misfolded proteins destined for delivery to the cell surface and subsequent secretion. Therapeutic approaches directed toward augmenting the processing and trafficking pathways offer promise for promoting selective stimulation of protein transport to the cell surface to mitigate or prevent lung disease.

*Proteomic Studies of Platelet Functions*

The NHLBI will issue a new RFA in FY 2006 to support the use of proteomic tools to identify platelet disorders. Although platelets play an important role in cardiovascular diseases and stroke, current tests cannot predict platelet function or guide clinical therapy. Furthermore, diagnosis of platelet disorders that increase the risk of bleeding remains difficult. Aggregation of platelets and secretion of platelet components have been studied for many years, but they are complex processes, and platelets may be activated by different agonists through multiple pathways. The RFA is intended to stimulate proteomic studies of platelets and to apply the technology to the study of platelets from a patient group that may have defective platelet function. The studies are expected to generate proteomic approaches and provide new markers of platelet function.

*RNA Interference (RNAi) Biology: Stability, Delivery, and Processing by Tissues*

A new FY 2006 RFA, which will be initiated by the NHLBI and supported by several other NIH Institutes, will increase understanding of the biology of RNAi. RNAi, an effective post-transcriptional strategy for silencing genes, potentially can be applied to develop new therapies for a wide range of heart, lung, and blood diseases. The initiative is expected to provide insight into the uptake and processing of RNAi by target tissues; assess its stability, half-life, and off-target effects; and determine optimal delivery methods for RNAi uptake.

*Sarcoidosis: Etiology of Multi-Organ Disease and Clinical Strategies*

In FY 2006, the NHLBI will initiate a new PA in collaboration with the trans-NIH Sarcoidosis Working Group. The PA will fund research to conduct innovative, multidisciplinary basic and clinical research on the etiology of sarcoidosis and on related host factors that might enhance susceptibility to sarcoidosis. The initiative focuses on symptomatic, multi-organ disease that

involves critical organs (e.g., lungs, heart, eyes, central/peripheral nervous system, kidneys, and abdominal viscera) and that creates serious illness and problems in disease management.

*Sickle Cell Disease Clinical Research Network*

A new RFA, which will be initiated by the NHLBI in FY 2006, will establish a clinical research network to conduct multiple Phase III randomized, controlled clinical trials to test the efficacy of new therapies to treat and prevent complications of sickle cell disease and, when appropriate, thalassemia. The interventions will be based on results from basic studies and Phase I and Phase II clinical trials conducted in programs such as the NHLBI Comprehensive Sickle Cell Centers Program. The network will comprise a data-coordinating center and up to 15 clinical centers that will enroll 50 or more patients per center per year to participate in multiple trials using common protocols. In addition, the network will create data sets that can be used to characterize patients and their clinical course, apply genomic and proteomic techniques for improved diagnostic and therapeutic approaches, expand clinical application of multimodal therapies, and examine patient-centered outcomes.

*Zebrafish Research Tools*

In FY 2006, the NHLBI, along with several other Institutes, will fund the renewal of a PA to develop new tools or genetic or genomic resources of high priority to the zebrafish community. The goal will be to use the new tools to advance the detection and characterization of genes, pathways, and phenotypes of interest in development and aging, organ formation, behavior, sensory processing, physiological processes, and disease processes. Areas of interest to the NHLBI in which zebrafish are likely to be a useful model include: (1) cellular and molecular functions of zebrafish genes that have the potential to model human cardiovascular, blood, and pulmonary, or sleep disorders; (2) the genetic bases of disorders of cardiovascular development and function; (3) the effect of mutations on subsequent organ development leading to such disorders as arrhythmia, cardiac hypertrophy, dilated cardiomyopathy, and heart failure; (4) developmental aspects of endothelial dysfunction as the basis for vascular disorders; (5) developmental defects in hematopoiesis and the relationship to disorders of the hematopoietic system; (6) the genetic basis of angiogenesis, and vasculogenesis; and (7) the genetic basis, regulation, and role of biological clock mechanisms in development and circadian behavior.

*Network for Cardiothoracic Surgical Investigations in Cardiovascular Medicine*

The NHLBI will issue a new RFA in FY 2007 to evaluate new surgical techniques, technologies, devices, and bioengineered products related to cardiovascular medicine through rigorous Phase I and II clinical trials. New surgical procedures and devices are often incorporated into clinical practice without objective evaluation of their relative benefit over established therapies. It is vitally important to ensure the validity and efficacy of such treatments as compared to less risky, less expensive, and less invasive treatment options. A cardiothoracic surgical network will help overcome some of the challenges of clinical research by providing standard measures and methods for investigations and outcomes, coordinating multidisciplinary teams, and providing resources for collection and interpretation of data. The network will be designed to allow

research teams led by cardiac surgeons to evaluate, in small-randomized trials, newer therapies and techniques, and will inform the development of larger Phase III clinical trials through separate funding.

*Specialized Centers of Clinically Oriented Research (SCCOR) in Pulmonary Vascular Disease*

In FY 2007, the NHLBI will initiate a new RFA to conduct multidisciplinary research on clinical questions related to the diagnosis, prevention, and treatment of pulmonary vascular disease. The program will address primary (idiopathic) and secondary pulmonary arterial hypertension, acute and chronic pulmonary thromboembolism, right ventricular dysfunction, and pulmonary vascular disorders of infants and children. Three to four centers will each carry out a minimum of three research projects directly related to a unifying theme. At least half of the projects in each center will be clinically oriented to assure that basic science findings are rapidly applied to clinical problems. Applicants may propose inclusion of a Clinical Research Skills Development Core to enhance the research skills of new clinical investigators.

**Rare-Disease Related Program Activities**

*Alpha-1 antitrypsin deficiency (AAT)*

The NHLBI is developing a COPD Awareness and Education Program to implement a nationwide campaign for education of the public and physicians regarding obstructive lung diseases, including AAT deficiency.

The Clinical Centers of the NHLBI-supported COPD Clinical Research Network held six COPD and Alpha-1 Education Days throughout the nation to educate physicians and the public about AAT lung disease and COPD. Participants received information about diagnosis and treatment of obstructive lung disease, including screening for AAT deficiency. The program was co-sponsored by the Alpha One Foundation.

*Arrhythmogenic right ventricular dysplasia (ARVD)*

In April 2005, NHLBI-funded researchers participated in the First International Symposium on ARVD, a "virtual symposium" held in conjunction with the International Society for Holter and Noninvasive Electrocardiography. The symposium included lectures, Webcasts, and radio interviews. The international audience of researchers was able to download files relating to recent scientific findings, discussions, and forums, and to ARVD education.

NHLBI-funded investigators produced diagnostic protocols as a resource for physicians contacting the ARVD registry. The protocols are intended to promote uniformity in the initial diagnostic testing of potential registry participants.

In FY 2005, investigators from the Multidisciplinary Study of Right Ventricular Dysplasia produced the fifth volume of "Lo Que Pasa: Newsletter of the Multidisciplinary Study of Right Ventricular Dysplasia."

*Bronchopulmonary dysplasia (BPD)*

The NHLBI sponsored the annual meeting of the Collaborative Program for Research in BPD, which was held in September 2005 in Bethesda, Maryland.

In October 2005, the NHLBI sponsored a meeting for investigators participating in an Institute initiative on the Coordination of Vascularization and Lung Development. The investigators discussed recent research progress. Their efforts should contribute to a more complete mechanistic profile of the development of BPD.

*Brugada's syndrome*

On September 14, 2005, the NHLBI sponsored a working group on "Modifiers of Arrhythmias: New Targets for Therapy and Prevention".

*Congenital Heart Disease*

The Weinstein Cardiovascular Development Conference is an annual meeting partially supported by NHLBI. The 2005 meeting was held on May 19-21, 2005, in Tucson, Arizona, and included reports given by NHLBI-supported investigators on congenital heart disease. The meeting was cosponsored by the NIH Office of Rare Diseases, the National Institute of Child Health and Human Development, the American Association of Anatomists, the March of Dimes, the American Heart Association, local organizations, and corporate sponsors.

The Pediatric Heart Network sponsors a Web site that provides information for parents and health care providers about completed, current, and future network studies and about how they can participate. http://www.pediatricheartnetwork.org/

*Cooley's anemia*

The NHLBI currently holds FDA investigational new drug approval for L1-Deferiprone for a clinical study of thalassemia. The Thalassemia Clinical Research Network has an active trial using this oral iron chelator.

The NHLBI sponsored a working group on "Facilitating Collaboration Amongst Investigators in Sickle Cell Disease and Thalassemia" in June 2005. The meeting included a panel of 18 scientific experts, along with representatives from the Sickle Cell Disease Association of America, the Cooley's anemia Foundation, the National Institute for Diabetes and Digestive and Kidney Diseases, and the NHLBI. Participants discussed scientific and management issues that are common to the two hemoglobinopathies. The major areas of interest are chronic transfusion therapy and management of iron overload; treatment of pulmonary arterial hypertension; development of new agents to increase fetal hemoglobin levels; creation of genotype/phenotype databases; development of clinical registries for the diseases; and roles of zinc and other nutrients in the formation of low bone mass.

The NHLBI co-sponsored, with the NIH Office of Rare Disease and the National Human Genome Research Institute, a working group entitled "An NIH Strategic Plan for the Development of Globin Gene Therapy for Treatment of Sickle Cell Disease and Beta-Thalassemia" in June 2005. The working group brought together leading investigators in the globin gene transfer field as well as patient advocates and FDA and industry representatives to discuss how the NHLBI can best facilitate translation of hemoglobin gene transfer into clinical trials for sickle cell disease and beta-thalassemia. Investigators indicated a need for assistance with lentiviral vector production and with the preparation of applications to regulatory agencies and advisory committees.

The Sickle Cell Disease Advisory Committee met in June and November of 2005. The committee advises the NHLBI on research activities related to hemoglobinopathies, specifically sickle cell disease and thalassemia. Members of the Cooley's anemia Foundation participated in the meeting.

In October 2005, members of the Cooley's anemia Foundation (CAF) met with the Director of the NHLBI Division of Blood Disease and Resources.

The Eighth Cooley's Anemia Symposium was held in March 2005 to illuminate the many unsolved but critically important issues in the understanding and treatment of thalassemia. The symposium was sponsored by the New York Academy of Sciences and the Cooley's anemia Foundation with support from the NHLBI, the National Institute of Diabetes and Digestive and Kidney Diseases, and the NIH Office of Rare Diseases. The Director of the NHLBI Division of Blood Diseases and Resources gave opening remarks to the assembly.

The Thalassemia Clinical Research Network held a Steering Committee meeting in October 2005. The meeting was attended by all of the Center and Satellite investigators including research and nurse coordinators. Several members from the New England Research Institute attended along with representatives from the NHLBI and the National Institute of Diabetes and Digestive and Kidney Diseases. Members of the Cooley's anemia Foundation also were invited to participate. Attendees discussed the active Cardiac Clinical Trial being conducted by the network as well as planned protocols.

In October 2005 a grantees meeting was held to present research co-sponsored by the NHLBI and the National Institute of Diabetes and Digestive and Kidney Diseases. The annual meeting, allows researchers to discuss results funded by an NIH initiative on imaging techniques for assessment of tissue iron.

The Cooley's Anemia Foundation sponsored a symposium entitled "Vectors and Visions: Curative Approaches to Thalassemia" in December 2005 during the American Society of Hematology meeting in Atlanta, Georgia. Attendees discussed the progress of transplantation and gene therapy for patients with thalassemia.

*Cystic fibrosis*

In September 2005, the NHLBI and the NIH Office of Rare Diseases co-sponsored a conference entitled "Host Response to Persistent Bacterial Load in Cystic Fibrosis." Attendees reviewed the state-of-knowledge in the following four areas: 1) the initiation and underlying mechanism of mucus adhesion to airway surfaces; 2) the interaction of bacteria with mucus; 3) host persistent inflammation/ tolerance to interluminal persistent bacterial load; and 4) the interaction of viruses with persistently bacterially stimulated airway epithelia.

*DiGeorge syndrome*

The Weinstein Cardiovascular Development Conference is an annual meeting partially supported by the NHLBI. The May 2005 meeting included reports by NHLBI-supported investigators on genetic mechanisms of cardiac malformations associated with DiGeorge syndrome. The meeting was cosponsored by the NIH Office of Rare Diseases, the National Institute of Child Health and Human Development, the American Association of Anatomists, the March of Dimes, the American Heart Association, local organizations, and corporate sponsors.

*Hemophilia*

The RFA, Improved Therapies for Hemophilia and Hereditary Bleeding Disorders, was co-sponsored by the National Hemophilia Foundation (NHF). This collaborative effort resulted in the NHLBI funding five new grants in FY 2005. The NHF plans to fund additional projects submitted in response to the RFA in 2006.

A recombinant porcine Factor VIII is being developed by Octagen Corporation, with SBIR support, as a treatment option for hemophilia A patients who develop antibody inhibitors that neutralize the activity of currently available types of replacement Factor VIII. In FY 2005, Octagen received a competing continuation of their Phase II SBIR grant to conduct clinical trials to support FDA licensure of the product.

*Long QT syndrome (LQTS)*

In September 2005, the NHLBI sponsored a Working Group on "Modifiers of Arrhythmias: New Targets for Therapy and Prevention."

*Lymphangioleiomyomatosis (LAM)*

The NHLBI and the LAM Foundation co-sponsored a conference entitled "Tribulations and Trials: The First Annual LAM/TSC Research Conference," in April 2005. The conference marked the 10$^{th}$ anniversary of the LAM Foundation. The meeting was held concurrently with the Rare Lung Diseases Consortium for the second year in a row and included joint sessions and patient sessions. As in previous years, the synergy achieved at this meeting across scientific disciplines and the interaction of researchers and patients proved very beneficial for advancing knowledge. The conference covered a wide range of topics including inflammation and fibrosis,

signaling pathways and basic biology of TSC1 and TSC2, translational research on LAM/TSC, autoimmunity and misfolding of proteins, TSC genes in the brain, and causes of epilepsy and behavioral phenotypes. Patient-oriented sessions with an emphasis on diagnosis, management, the status of trials of possible new treatments, and advocacy were an important aspect of the meeting.

*Lymphedema*

Staff from the NHLBI extramural program met frequently with representatives of the Lymphatic Research Foundation to discuss lymphatic research. Many of the discussions included the Trans-NIH Coordination Committee for Lymphatic Research.

The NHLBI has recruited an internationally recognized clinical scientist to initiate lymphatic disease studies within the NHLBI intramural program and across the intramural and extramural programs of the NIH. The investigator, who will conduct a research protocol at the NIH Clinical Center, also will develop clinical care guidelines about lymphatic diseases.

*Persistent Pulmonary Hypertension of the Newborn (PPHN)*

In October 2005, the NHLBI sponsored a meeting for investigators participating in an Institute initiative on the Coordination of Vascularization and Lung Development. The investigators discussed recent research progress in integrative development of the lung vasculature and lung structure.

*Primary pulmonary hypertension*

The NHLBI sponsored a working group entitled "Cellular and Molecular Mechanisms of Right Heart Failure", in October 2005. The working group brought together experts in both heart failure and lung disease for the purpose of furthering understanding of the mechanisms and the development of new treatments for right heart failure. Right heart failure is commonly associated with pulmonary hypertension and is often the cause of death in PPH patients. Several recommendations for future research directions were generated. For example, experts at the meeting identified a need for: 1) more studies of the right heart and of the differences, similarities, and interplay of the left and right heart; and 2) the development of new therapeutic strategies for right heart failure, including cell-based therapy, new drugs, and new combinations of existing drugs.

A genetic screening program for PAH has been approved. This NHLBI-supported program screens patients and their families for mutations of the BMPR2 gene that has been linked to PPH. The program is designed for early detection and to provide counseling to help families understand their risk of developing PPH.

The NHLBI continues to collaborate with the Pulmonary Hypertension Association to co-fund young clinical investigators pursuing career development (K08 and K23) awards in PAH.

*Pulmonary alveolar proteinosis (PAP)*

The NHLBI and the NIH Office of Rare Diseases supported the Third International Pulmonary Alveolar Proteinosis Scientific Conference in April 2005. The meeting brought international thought leaders in PAP research together with investigators of the National Center for Research Resources-supported Rare Lung Diseases Consortium to review ongoing research on pathogenesis, diagnosis, and therapy of PAP as well as the emerging role of GM-CSF in innate immunity. One important outcome of the meeting was the standardization, internationally, of methods used to detect anti-GM-CSF antibodies. A second was the creation of the PAP Foundation, a non-profit patient advocacy organization, the formation of which was due, in part, to a synergistic meeting format that included PAP patients, clinicians, scientists, and NIH staff.

*Sarcoidosis*

The NHLBI has revised the Sarcoidosis Fact Sheet. It is currently available on the NHLBI Web site.

In June 2005, the NHLBI co-sponsored the 2005 World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) Congress in Denver, Colorado. At the meeting, leading clinicians and medical investigators discussed research and new clinical strategies in the field. The 2005 WASOG meeting included sessions on the etiology of sarcoidosis, possible infectious agents and environmental factors related to the disease; immune mechanisms affected by sarcoidosis or antigens in related diseases; and management. Symptoms such as fatigue and issues related to disability were also discussed.

The NIH has established a trans-NIH Sarcoidosis Working Group. The Working Group is chaired by the Director of the NHLBI Division of Lung Diseases. The first meeting was held in November 2004. In addition to representatives from the NHLBI, the group includes representatives from the NIH Office of Research on Women's Health, the National Eye Institute, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the NIH Office of Rare Diseases, the National Human Genome Research Institute, the National Institute of Environmental Health Sciences, the National Institute of Allergy and Infectious Diseases, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Nursing Research, and the National Institute of Neurological Disorders and Stroke.

*Sickle cell disease*

The NHLBI co-sponsored, with the NIH Office of Rare Diseases and the National Human Genome Research Institute, a working group entitled "An NIH Strategic Plan for the Development of Globin Gene Therapy for Treatment of Sickle Cell Disease and Beta-Thalassemia" on June 28, 2005. The working group brought together leading investigators in the globin gene transfer field as well as patient advocates and FDA and industry representatives to discuss how the NHLBI can best facilitate translation of hemoglobin gene transfer into clinical trials for sickle cell disease and beta-thalassemia. Investigators indicated a need for assistance

with lentiviral vector production and with the preparation of applications to regulatory agencies and advisory committees.

In September 2005, the NHLBI sponsored a working group on "Barriers to Late-Stage Drug Development for Hemoglobinopathies." At the meeting, experts from the NIH, small and large drug companies, contract research organizations, and academia discussed and evaluated options for the best avenues to expedite drug development for hemoglobin disorders and other rare diseases.

The NHLBI sponsored a working group on "Facilitating Collaboration Amongst Investigators in Sickle Cell Disease and Thalassemia" in June 2005. The meeting included a panel of 18 scientific experts, along with representatives from the Sickle Cell Disease Association of America, the Cooley's Anemia Foundation, the National Institute for Diabetes and Digestive and Kidney Diseases, and the NHLBI. Participants discussed scientific and management issues that are common to these two hemoglobinopathies. The major areas of interest are chronic transfusion therapy and management of iron overload; treatment of pulmonary arterial hypertension; development of new agents to increase fetal hemoglobin levels; creation of genotype/phenotype databases; development of clinical registries for the diseases; and roles of zinc and other nutrients in the formation of low bone mass.

In August 2005, the NHLBI sponsored a working group on "Renal and Urologic Complications in Sickle Cell Disease." The working group assembled hematologists, renal specialists, and urologists to discuss the research needs and opportunities associated with the renal and urologic system problems in patients with sickle cell disease. The major recommendation was to use microalbuminuria and macroalbuminuria as surrogate markers for progression of renal disease in future clinical trials with drugs (such as angiotensin converting enzyme inhibitors) to prevent renal disease.

The NHLBI, with support from the NIH Office of Rare Diseases, recently sponsored a meeting to bring together transplant investigators to review data collected by the International Bone Marrow Transplant Registry and to develop a systematic plan to sustain an infrastructure for collaboration among U.S. centers treating and transplanting patients with sickle cell anemia.

In April 2005, NHLBI staff met with representatives of the Sickle Cell Disease Association of America (SCDAA) to discuss implementation of the Talent Sickle Cell Treatment Act. This event was sponsored by the U.S. Health Resources and Services Administration.

NHLBI representatives attended the annual meeting of the SCDAA in Baltimore in September 2005. The Director of the NHLBI Division of Blood Diseases and Resources gave a presentation on clinical research trends in hemoglobin disorders.

In April 2005, representatives from the NHLBI-funded Comprehensive Sickle Cell Center at the Children's Hospital of Philadelphia sponsored an education session on applying for NHLBI training grants at the National Sickle Cell Program meeting. At the same meeting,

representatives from the NHLBI-funded Comprehensive Sickle Cell Center at the Albert Einstein College of Medicine sponsored an education session on sickle cell pain.

In FY 2005, the NHLBI added a training supplement component to the Comprehensive Sickle Cell Centers program. Researchers at the high school through junior faculty levels are eligible for the supplements. Fifteen trainees at ten different centers were supported in FY 2005.

## NATIONAL HUMAN GENOME RESEARCH INSTITUTE (NHGRI)

### Overview of Rare Diseases Research Activities

The National Human Genome Research Institute (NHGRI) led the National Institutes of Health's (NIH) contribution to the International Human Genome Project (HGP). With the achievement of its final goal, the finished sequence of the human genome in April 2003, this project was successfully completed ahead of schedule and under budget, and has already begun to change the way we address rare diseases.

> On October 26, 2005, a different international consortium of dedicated scientists from six countries, again led by the NHGRI, announced the production of a very different map of the human genome, one that may prove even more powerful, because of its medical applications. This is the "HapMap," and once again, all of the data has been placed in the public domain. The Genome Project spelled out the letters of the DNA code that we all share. The HapMap provides detailed knowledge of the variation in the genome. The HapMap investigates those spelling differences in the human instruction book that predispose some people to diabetes, others to heart disease, and others to cancer. The HapMap reveals the way in which this genetic variation is organized into chromosomal neighborhoods. The HapMap provides insight not only into the causes of common disease, but provides an important genomic tool for the understanding of the genetics of rare disease.

### Inherited Disorders of the Immune System

The NHGRI Genetics and Molecular Biology Branch is conducting a research program to find the causes and develop better treatments for inherited disorders of the immune system. These include immunodeficiencies, in which gene defects impair the ability of the immune system to fight infections, and also disorders of immune cell regulation, in which autoimmunity may be seen. Current areas of investigation include severe combined immunodeficiency, hyper-IgE syndrome, certain inherited autoimmune diseases, including variants of autoimmune lymphoproliferative disease, and genetic determinants of susceptibility to HIV/AIDS.

*Severe combined immunodeficiency (SCID)*

Rapid New Test Developed for Inherited Immune Deficiency

Babies born with Severe Combined Immunodeficiency (SCID fail to develop a normal immune system. SCID babies can be infected by a wide range of viruses, bacteria, and fungi that are normally controlled by a healthy baby's immune system. Although a rare disease, SCID is known to the public from media accounts - and a made-for-TV movie starring John Travolta - about David, "the Bubble Boy," a Texas boy who spent his entire life in a germ-free environment, ultimately dying at age 12, in 1984, after a failed bone marrow transplant.

If undetected and untreated, SCID typically leads to death before the baby's first birthday. However, if SCID is diagnosed in time, there are now often effective treatments. One form of the disease can be treated with an injectable medication. All forms of the disorder can be cured

through the transplantation of bone marrow if a matching donor can be identified. Finally, SCID may be treated through human gene therapy, in which a normal copy of the defective gene is inserted into the patient's own blood-forming cells. The first gene therapy experiments in history were carried out at NIH in 1990 in two young Ohio girls with SCID. Today the girls are alive, continue to do well, and are involved in ongoing research at the NHGRI.

Researchers at the NHGRI have developed a new laboratory method that rapidly identifies babies born with inherited forms of SCID. The new genetic test, which still must be validated before widespread use, could someday be added to the panel of tests that already screens newborns for a variety of disorders. Developed in the NHGRI Division of Intramural Research (DIR), the new test can use the same dried blood samples already collected from newborns, and would provide the first accurate, high-throughput screen for immune deficiencies. Prior efforts to identify this disorder by counting white blood cells in newborns proved unreliable and expensive. Many babies are diagnosed with SCID so late that they develop fatal infections before their condition is recognized. It is, therefore, critical to identify affected children immediately after birth and since the babies lack overt clinical symptoms for some time, a molecular test is a good approach. No one knows exactly how many babies are born with SCID. Current estimates suggest that 1 in every 50,000 to 100,000 births may be affected
Chan K, Puck JM: Development of population-based newborn screening for severe combined immunodeficiency. Journal of Allergy and Clinical Immunology 115: 391-398, 2005.
http://www.jacionline.org/article/PIIS0091674904026351/abstract

*Hyper IgE syndrome (Job's syndrome)*

Hyper-IgE syndrome, also known as Job syndrome after the biblical character who was stricken with boils, is a rare primary immunodeficiency characterized by recurrent skin abscesses, recurrent pneumonia with development of lung cysts, and extreme elevations of serum IgE. The specific immune defect has not been discovered, and NHGRI researchers have undertaken genetic studies to map the disease.

*Autoimmune lymphoproliferative syndrome (ALPS)*

Autoimmune Lymphoproliferative syndrome (ALPS) is a rare syndrome in which patients have large lymph nodes and spleens, increased numbers of a rare type of lymphocyte called CD4-/CD8- T cells, or double-negative T cells, and defects in programmed cell death, or apoptosis of their lymphocytes. NHGRI researchers have found that most patients with ALPS have inherited mutations in the apoptosis mediator Fas. These patients' lymphocytes do not die when they should; instead, they accumulate and can attack the body's own tissues. Autoimmune diseases of the red blood cells, platelets and white blood cells are common in ALPS. NHGRI researchers have found some affected members of ALPS families who are more severely affected than others. In 2005, researchers have started to investigate genetic modifiers of disease severity in ALPS. They first investigated the major histocompatibility locus (MHC) because it is known to be involved in susceptibility to many autoimmune conditions. By performing HLA typing in members of families with many cases of ALPS due to Fas gene defects, we have found that specific alleles are protective, while others may be a risk factor for severe disease in ALPS. The

researchers have started a genome-wide approach to finding additional modifier genes. Such genes might turn out to be important determinants for more common sporadic autoimmune diseases besides ALPS.

**Developmental Disorders**

Anti-Cancer Drugs May Hold Promise for Premature Aging Disorder

There are currently no treatments for the rare genetic disorder Progeria. When they are born, children with Progeria appear normal. But, as they grow older, they experience growth retardation and show dramatically accelerated symptoms of aging such as, hair loss, skin wrinkling, and fat loss. Accelerated cardiovascular disease also ensues, typically causing death from heart attack or stroke by about the age of 12.

Building upon their success in identifying the gene that causes progeria, researchers at the NHGRI have discovered that drugs originally developed for cancer also can reverse the dramatic nuclear structure abnormalities that are the hallmark of cells from children with progeria. This is a serendipitous surprise, rather like finding out that the key to your house also works in the ignition of your car. The new work that provided this surprise involved using farnesyl transferase inhibitors (FTIs) to treat skin cells taken from progeria patients. If upcoming studies in a mouse model validate the results of the cell experiments and translate into improvements in the animals' conditions, a clinical trial of FTIs in children with progeria may begin as early as next year. This work also has important implications for understanding the aging processes that all people experience.

Capell BC, Erdos MR, Madigan JP, Flordalisi JJ, Varga R, Conneely KN, Gordon LB, Der CJ, Cox AD, Collins FS: Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. Proceedings of the National Academy of Sciences *(PNAS)* 102: 12879-12884, 2005. www.pnas.org/cgi/doi/10.1073/[nas.0506001102.

*Polydactyly syndromes*

A group of syndromes that include polydactyly with other malformations is the subject of a clinical-molecular study. This research study encompasses a range of phenotypes that include Pallister-Hall syndrome, the allelic disorder Greig cephalopolysyndactyly syndrome (GCPS), McKusick-Kaufman syndrome (MKS), and Bardet-Biedl syndrome (BBS). The clinical manifestations of these disorders include polydactyly, central nervous system malformations (with or without mental retardation and seizures), craniofacial malformations, and visceral malformations such as renal malformations or congenital heart defects. NHGRI Researchers study these disorders by a translational approach that begins in the clinic with careful clinical evaluation of the phenotypes by physical examination, imaging studies that include radiographs, ultrasound, MRI and CT scanning. They have shown that BBS and MKS can both be caused by mutations in the same gene. PHS and GCPS are caused by a wide spectrum of mutations in the GLI3 gene. The severity of the GCPS phenotype, specifically the mental retardation and learning disability, are correlated with these mutations. Patients with larger deletions have a

more severe form of the disease. Researchers are also performing a positional cloning analysis of the Greig cephalopolysyndactyly syndrome in the mouse using a sporadic mutant identified at a large breeding facility. This disorder has now been mapped to a 500 KB area of the genome and candidate genes are being sequenced in the area.

*Lowe oculocerebrorenal syndrome*

The oculocerebrorenal syndrome of Lowe is a rare X-linked metabolic disorder characterized by congenital cataracts, renal tubular dysfunction, and mental retardation. It is caused by mutations in the gene OCRL1 encoding a specific phosphatase. Research at NHGRI has resulted in the identification of the responsible gene, determination of its biochemical function, and the development of accurate enzymatic diagnosis for affected fetuses and individuals. The current focus of this research is to understand how a defect in this enzyme results in the various manifestations of the syndrome. Researchers are working both in cultured human cells and in animal models. They have demonstrated that the cell structural skeleton is disorganized in cultured cells from Lowe syndrome patients. They are investigating the role of intracellular calcium in bringing about this phenotype and have found abnormal calcium signaling in patient cells.

Researchers are also working to create a mouse model for Lowe syndrome, Mice deficient in the mouse version of the OCRL1 gene are normal. It is thought that the gene INPP5B which is closely related to OCRL1 may be compensating in these mice, so researchers are making a mouse deficient in both mouse Olrl1 and mouse Inpp5b but carrying the human INPP5B gene, in order to mimic the situation in humans more closely. Researchers have also collaborated with a group in Syracuse New York to demonstrate that certain patients with mutations in the OCRL1 gene do not have Lowe syndrome but have a milder disorder, Dent disease, that is limited to the kidney and has some but not all of the proximal tubular defects seen in Lowe syndrome.

*Hirschsprung disease*

Animals heterozygous for mutations in the SOX10 transcription factor exhibit multiple defects in neural crest development, including reduced numbers of melanocytes in the skin, an absence of myenteric ganglion in the colon, and deafness. A human congenital disorder, Hirschsprung disease, also exhibits rectocolic aganglionosis and can be associated with hypopigmentation caused by SOX10 mutations. Thus SOX10 mice, as well as other neural crest mutant mice, serve as mouse models for this disease. Investigation of the involvement of SOX10 in Hirschsprung disease and other neural crest related disorders is being explored. NHGRI intramural researchers have demonstrated that SOX10 directly controls the expression of the genes MITF and DCT. These researchers have established a system for adding genes back to neural crest stem cells in order to complement genetic defects. They have used this system to test hierarchial relationships between SOX10 and its target genes. They demonstrated that they can use this system to correct SOX10 defects in cultured cells, and have generated vectors to make this very efficient. They have shown that MITF is not sufficient to completely replace SOX10 in development. The researchers have also established a whole genome mutagenesis program to identify SOX10 genetic interaction factors. They have identified 7 heritable loci,

mapped five and cloned the mutation in four of the genes. These may become human modifier loci and interesting contributors to neural crest developmental pathways.

*Microphthalmia syndromes*

The project seeks to understand the clinical and molecular basis of syndromic microphthalmia. This disorder comprises anophthalmia or microphthalmia (small or absent eyes with blindness), mental retardation, and skeletal anomalies. NHGRI researchers have identified a large family affected by Lenz Microphthalmia and have mapped the gene to the short arm of the X chromosome. This result is surprising because another family with this disorder maps to the long arm of the X chromosome. This means that Lenz microphthalmia is probably an amalgam of two disorders. Researchers have used positional cloning to isolate the gene that is altered in the condition, which is called BCOR (BCL-6 co-repressor). In addition, researchers have discovered that mutations in this gene also cause the Oculo-facio-cardi-dental syndrome. They have determined that this disorder is actually an amalgamation of two distinct X-linked diseases and that one form of Lenz is caused by the same mutation as Oculofaciocardiodental syndrome and that both of these diseases are caused by mutations in the BCOR gene.

*Molecular genetics of Anabaptist diseases*

Old Order Amish and related Anabaptist sects (including Mennonites) are important for the study of genetic disease, as they represent a cultural and genetic population isolate. In addition, they are enthusiastic historians and have excellent printed genealogical records. NHGRI researchers have built the Amish Genealogy Database (AGDB) and several computational tools to analyze the database including PedHunter. These tools allow generation of pedigrees for genetic study in an accurate and rapid fashion. NHGRI researchers have also cloned the genes that are altered in Glycogen storage disease type 6, McKusick-Kaufman syndrome, Amish Nemaline Myopathy, and are currently working on Amish Microcephaly. They have identified the gene alteration in Amish Microcephaly, which codes for a protein that transports deoxynucleotides (DNA precursors) into the mitochondria. They have currently developed both mouse and zebrafish animal models for this disorder and are now characterizing the phenotypes of these organisms.

*Proteus syndrome*

Proteus syndrome is a rare, sporadic syndrome that causes progressive, patchy overgrowth, bony distortion or deformation, tumor predisposition, and mental retardation. NHGRI researchers are determining the natural history and etiology of Proteus syndrome. The natural history and the phenotypic range are being determined by clinical assessment and longitudinal follow-up of a cohort of patients. Very little is known about the natural history and the range of the phenotype of PS. The etiology of PS has been studied using various comparative molecular biology techniques including representational difference analysis, cDNA arrays, and other techniques. NHGRI researchers have clinically redefined the Proteus syndrome. This was done through evaluation of a series of 35 patients and an exhaustive survey of all cases reported in the

literature. This allowed them to establish new clinical diagnostic criteria for this disorder and delineate a novel disease entity, the hemihyperplasia-multiple lipomatosis syndrome.

*Alagille syndrome (AGS)*

Researchers at NHGRI have shown that mutations in the Jagged1 (JAG1) gene are responsible for Alagille syndrome (AGS) a developmental disorder affecting multiple organ systems, including liver, heart, eye, face, and vertebrae. Zebrafish is an excellent model for vertebrate development, and therefore researchers have initiated efforts to explore the role of jagged genes in zebrafish development and in developmental diseases like Alagille syndrome. As a part of this effort, they have isolated and characterized jagged homologous genes from zebrafish and studied their expression during early embryonic development. They have found that Jaggeds 1 and 3 are similar to each other and appear to have originated as a result of genomic duplication in zebrafish. Abnormal expression of RNA from the three JAG genes results in a decreased amount or neurons in certain pathways. Antisense nucleotide probes from these three genes are being used to explore their role in liver development. Combinations of Jagged and Notch (an important developmental gene) knockdowns alter zebrafish liver development in a manner compatible with an AGS model of disease.

*Congenital disorders of glycosylation (CDG)*

Congenital disorders of glycosylation are a diverse group of metabolic disorders presenting with a spectrum of clinical features ranging from severe neurologic manifestations and multisystemic involvement to hypoglycemia and severe gastrointestinal symptoms with normal development. There are now 17 types of CDG defined by distinct enzyme defects and genes all involved in the synthesis of N-linked oligosaccharides. The number of children and adults diagnosed with CDG in the United States is increasing rapidly with a wider variance in the phenotypes. It is clear that the clinical presentation and complications of these disorders are expanding. NHGRI intramural researchers are planning to include in these studies a new group of disorders called Congenital Muscular Dystrophies (CMD). The underlying metabolic bases of these disorders is the abnormal synthesis of O-linked oligosaccharide of the mannose type. There are similar clinical questions in both of these disorders that can be answered as more patient histories are accrued. The researchers' goal of the upcoming year will be to continue to identify and evaluate individual patients with CDG and CMD, to explore the clinical and biochemical features of untyped individuals and, through clinical research, continue to add to the compendium of clinical management strategies for physicians caring for these affected adults and children. Researchers have recently completed an invited review in GeneReviews, an online resource for physicians caring for individuals with rare genetic disorders.

The reference is Sparks SE, Krasnewich DM (August 2005) Congenital Disorders of Glycosylation Overview (or Congenital Disorder of Glycosylation Type Ia). In: GeneReviews at GeneTests: Medical Genetics Information Resource [database online]. Copyright, University of Washington, Seattle, 1997-2005. Available at http://www.genetests.org.

## Neurological Disorders

*Familial encephalopathy with neuronal inclusion bodies (FENIB)*

NHGRI researchers are exploring the clinical, laboratory, neuropsyche and imaging features of at-risk members of families with Familial Presenile Dementia with Neuroserpin Storage. Over the past year, the name of the disorder has been changed to Familial Encephalopathy with Neuronal Inclusion Bodies (FENIB). At the NIH Clinical Center, researchers have seen 25 individuals at risk for this disorder for full clinical evaluations. The clinical facet of this project continues to be a long-term exploration of the natural history of family members at risk. This will increase insight into the pathophysiology and clinical presentation of FENIB. These families provide not only rich clinical insight but the opportunity to understand the controversy surrounding presymptomatic testing in late onset neurodegenerative disorders. Further clinical delineation and assessment of counseling needs remain the clinical goals for this project. A parallel laboratory project includes the development of a mouse model for FENIB which will further help to elucidate the phenotype and genotypic variation.

*Huntington's disease*

The Prospective Huntington At Risk Observational Study (PHAROS) was initiated by the Huntington Study Group (HSG) in 1999 to characterize and measure the clinical onset of Huntington's disease (HD) in a diverse cohort of individuals who are at immediate risk (50:50) for having inherited the HD gene. Participants include healthy adults, age 30-55 years, who have not undergone testing for the HD gene and who wish to remain unaware of their HD gene status, yet are interested in contributing to knowledge about HD through research. Standardized clinical assessments are carried out at about 9-month intervals for up to 7 years of prospective observation by investigators who are also kept unaware of HD gene status in order to define early, HD-gene specific signs predictive of manifest disease. Nearly 500 research participants have already been enrolled in PHAROS at 36 sites in the U.S. and 6 in Canada. NHGRI-funded researchers enrolled the entire cohort of 1000 participants in 2001, and will request prospective follow up of all PHAROS participants to a planned conclusion in 2006. PHAROS will provide objective knowledge about the positive predictive value and reliability of early clinical signs of HD and their relationship to CAG and other genetic and environmental modifiers.

## Endocrine Disorders

*Multiple endocrine neoplasia type 1 (MEN1))*

Multiple endocrine neoplasia type 1 (MEN1) is characterized by multiple tumors of the parathyroid, anterior pituitary and GI endocrine tissues. NHGRI researchers have previously shown that mutations in the MEN1 gene are responsible for the MEN1 syndrome. The MEN1 encoded nuclear protein, Menin, binds the transcription factors JunD and NFkB, and can repress JunD and NFkB-induced transcription. By expressing wild type or mutant JunD in mouse fibroblast cell lines that do not express menin and JunD, NHGRI researchers found that interaction with menin is required for the growth suppressor function(s) of JunD. They have developed both conventional and conditional mouse knockout models, which yield phenotypes

that are remarkably similar to the human MEN1 disease, and have allowed delineation of the stages in tumor development. Researchers have developed tissue specific menin-inducible transgenic mouse models and are currently creating a drosophila model. Conditional knockout of menin in liver was well tolerated, a tissue not affected in MEN1 syndrome whereas similar loss in parathyroid or pancreatic islets resulted in tumors of the respective tissues. Expression changes associated with menin in cell lines and during tumorigenesis, are being studied, using a"ChIP on chip" approach, to understand the biology of menin. This is particularly relevant in light of the recent demonstration that menin is a critical component of a huge protein complex that includes MLL (mixed lineage leukemia), which plays key role in transcriptional regulation by methylation of the DNA associated protein Histone H3.

**Disorders of Vision**

*Rieger syndrome*

A continuing area of interest of researchers at NHGRI involves the homeodomain family of proteins, which play a fundamental role in a diverse set of functions that include body plan specification, pattern formation, and cell fate determination during metazoan development. Members of this family are characterized by a helix-turn-helix DNA-binding motif known as the homeodomain. Homeodomain proteins regulate various cellular processes by specifically binding to the transcriptional control region of a target gene. These proteins have been conserved across a diverse range of species, from yeast to human. A number of inherited human disorders are caused by mutations in homeodomain-containing proteins. One specific homeodomain protein, FOXC1, is implicated in Axenfeld-Rieger malformations. Patients with Axenfeld-Rieger malformations typically show a spectrum of ocular findings, including iris hypoplasia, a prominent Schwalbe line, iris adhesions, and goniodysgenesis. The most severe cases show elevated intraocular pressure, leading to the development of glaucoma. Studies are currently under way to examine the effect of mutations in the FOXH1 gene (another homeodomain protein) on the atomic structures of the resulting protein. The FOXH1 studies are being performed in collaboratively among several NHGRI intramural researchers, as part of a broader study aimed at understanding the function of this protein in development.

As an outgrowth of the studies on the homeodomain class of proteins, NHGRI researchers have developed and continue to maintain the Homeodomain Resource. This publicly available database provides a curated collection of information that includes full-length homeodomain-containing sequence data, experimentally derived structures, protein-protein interaction data, DNA-binding sites, and mutations leading to human genetic disorders. The Homeodomain Resource is freely available through the World Wide Web at http://research.nhgri.nih.gov/homeodomain/. Work is continuing in this area of homeodomain proteins to better understand these eye related mutations and their net effect on vision.

## Metabolic Disorders

*Methylmalonic acidemia and related disorders*

Methylmalonic acidemia is a genetically heterogeneous disorder of methylmalonate and cobalamin (vitamin B12) metabolism. Symptoms of MMA usually begin in the first few months of life, and include lethargy, failure to thrive, vomiting, dehydration, respiratory distress, hypotonia, and hepatomegaly. Acute episodes may include drowsiness, coma, and seizures, with subsequent developmental delays. An NHGRI research study encompasses the hereditary methylmalonic acidemias and cobalamin deficiency disorders. These metabolic disorders are genetically heterogeneous and collectively represent an important subset of the organic acidemias. Researchers have developed mouse and worm models of methylmalonic acidemia and have characterized both systems in the past year. The general goal of the research is to define the complications seen in the patients, replicate the findings in mice or other organisms and use the combined information to guide the development and testing of new therapies, such as gene and stem cell therapy.

The human subject research is focused on assessing the natural history of methylmalonic acidemia in the United States to further understand the treatment, outcome and complications in this group of disorders. The researchers have developed a patient database for outcomes research and have enrolled 40 participants in our clinical research studies since last year. They have studied the effects of solid organ transplantation on MMA, delineated a new neurologic syndrome in patients who have suffered from a disease-related stroke and described a range of eye findings seen in one subset of patients.

## Additional Activities

*Genetic and Rare Diseases Information Center (GARD)*

In order to respond to the public's need for information on genetic and rare disorders, NHGRI and the Office of Rare Diseases, NIH, maintains and supports the NHGRI/ORD Genetic and Rare Diseases Information Center. The Information Center focuses on meeting the information needs of the general public, including patients and their families, health care professionals, and biomedical researchers. The purposes of the Information Center are to: 1) serve as a central, national repository of information materials and resources on genetic and rare diseases, conditions, and disorders; 2) collect, produce, update, and disseminate information on the diagnosis, treatment, and prevention of genetic and rare disorders; and 3) coordinate with organizations and associations interested in genetic and rare disorders to explore networking capabilities, avoid duplication of effort, and identify information gaps.
See: http://rarediseases.info.nih.gov/html/resources/info_cntr.html

## NATIONAL INSTITUTE OF MENTAL HEALTH (NIMH)

### Overview of Rare Diseases Research Activities

The mission of the National Institute of Mental Health (NIMH) is to reduce the public health burden of mental and behavioral disorders through research on mind, brain and behavior. According to the NIMH-funded National Comorbidity Survey Replication (NCS-R), a landmark study released in May 2005 that documented the prevalence and severity of specific mental disorders, half of all lifetime cases of mental illness begin at age 14. In addition, the NCS-R demonstrated the severity of the mental health problem. Approximately 6 percent of the U.S. population is afflicted with a severely disabling mental disorder in a given year. Seriously, disabling mental illnesses such as schizophrenia, depression, bipolar disorder, anxiety disorders, and autism are the primary foci of research that NIMH supports and conducts. Additional research areas of importance to NIMH can be classified as rare diseases and include childhood-onset schizophrenia; pediatric bipolar disorder; pediatric HIV/AIDS; suicide; Gaucher disease; Williams syndrome; and Fragile X syndrome.

### Recent Scientific Advances in Rare Diseases Research

*Childhood onset schizophrenia*

Childhood-onset schizophrenia (COS) is a severe and unremitting disorder that is defined by the onset of psychosis by age 12. Patients with this rare illness have profound impairment in development and resemble adult patients with severe forms of schizophrenia. This year, researchers at NIMH have made several advances in the study of COS.

NIMH investigators demonstrated that healthy first-degree relatives of COS patients have subtle deficits in cognitive functions such as visual scanning, short-term memory, and executive functions (e.g. planning, problem solving, decision-making). These results support the hypothesis that COS is associated with genetic factors; similar data have been reported from studies on adult-onset schizophrenia

Schizophrenia is clearly associated with cognitive deficits, but the progression of these impairments over time is not clear. NIMH researchers evaluated children with COS to elucidate the relationship between the course of the disease and decline in IQ. The study showed that IQ declined around the time of illness onset. However, approximately 2 years after illness onset, IQ scores stabilized for up to 13+ years after onset of psychosis. This plateau in IQ occurred despite persisting psychotic symptoms and substantial loss of brain volume.

Schizophrenia has been associated with reduced activity in a class of neurons that communicate using the neurotransmitter gamma-aminobutyric acid (GABA). Previous studies have also suggested that the GAD1 gene, which encodes a GABA-producing enzyme, might be a candidate gene involved in the development of schizophrenia. In a recent study, NIMH researchers discovered an association between variations in the GAD1 gene and families of children with

COS. These results, in addition to data from similar studies on adult-onset schizophrenia, suggest that the GAD1 gene may be a common risk factor for schizophrenia.

*Pediatric bipolar disorder*

Recent research has indicated that there are pronounced differences among children affected with pediatric bipolar disorder (PBPD). One subset, termed "narrow phenotype" PBPD, in which patients exhibit cycling of moods including manic episodes, more closely clinically resemble adult bipolar disorder (BPD). This resemblance suggests that narrow phenotype PBPD and adult BPD may share some common underlying mechanisms. Consequently, cognitive deficits similar to those of adults with BPD might be especially likely to occur in patients with narrow phenotype PBPD. NIMH researchers tested cognitive function in patients with PBPD and a comparison group of healthy volunteers using a series of memory tests. Consistent with findings in adults with BPD, patients with PBPD performed more poorly than controls on measures of verbal learning and memory and delayed facial recognition memory. These findings suggest that deficits in verbal learning and memory, as well as some aspects of visual memory, characterize patients with narrow phenotype PBPD.

Little is known about neuropsychological and social-cognitive function in patients with PBPD. Identification of specific deficits that characterize PBPD would facilitate advances in diagnosis, treatment, and research on its causation. The purpose of a recent study by NIMH researchers was to test the hypothesis that youths with bipolar disorder would perform more poorly than matched healthy subjects on measures of social cognition, motor inhibition, and response flexibility. Social impairment in patients with narrow phenotype PBPD as opposed to healthy controls was measured using neuropsychological tests for awareness of appropriate language in interpersonal situations and for identification of emotional facial expressions. Patients with PBPD performed poorly on these tasks as compared to controls. They also performed more poorly on tasks involving response flexibility. However, they did not differ from healthy controls on tests of motor inhibition. These results are similar to findings for adult BPD and provide more evidence for a continuity between PBPD and adult BPD.

The NIMH studies mentioned above as well as other behavioral studies show that children with narrow phenotype PBPD have deficits in emotion regulation and cognitive tasks that have been associated with specific brain circuits. NIMH researchers, using magnetic resonance imaging, examined these brain regions in children with PBPD in comparison to healthy children. The results of the study showed that children with PBPD had alterations in areas of the brain consistent with previous findings on brain correlates of emotion regulation and cognition.

In adults with BPD, it has been shown that comorbidity with anxiety disorders is common and impacts symptom severity, response to treatment and suicide rates. There has been limited research on comorbidity with anxiety disorders in children with PBPD. NIMH researchers explored the association of comorbid anxiety with PBPD and found that children with PBPD demonstrate high rates of comorbid anxiety. The children with narrow phenotype PBPD exhibited greater functional impairment with an earlier age of onset of symptoms and more

hospitalizations. The narrow phenotype PBPD plus comorbid anxiety may represent a particularly severe variation of PBPD.

*Gaucher disease*

Gaucher disease (GD) is a rare, inherited metabolic disorder in which deficiency of the enzyme glucocerebrosidase results in the accumulation of harmful quantities of certain lipids throughout the body, particularly within the bone marrow, spleen and liver. There are three general forms of the disease, characterized by the extent of neurological complications. The Type 1 form does not involve neurological complications, whereas Type 2 and Type 3 do, to varying degrees, with Type 3 the more severe form. Little is known about the mechanism leading from lipid accumulation to disease, particularly in Types 2 and 3. Previous work from NIMH researchers has shown the cellular mechanism by which, in animal models, lipid accumulation can enhance neuronal cell death. Current NIMH studies have investigated whether similar cellular mechanisms are altered in human brain tissue obtained post-mortem from Gaucher disease patients. Results demonstrated similar alterations in Type 2 GD, but not in Type 1 nor Type 3, offering the potential of new therapeutic approaches for disease management in Type 2 GD.

There is wide variation in characteristics of patients with Types 2 and 3 GD, although they share the same genetic profile for the disease. The mechanism by which this defined genetic mutation leads to the expression of the disease is unknown. NIMH researchers investigated the contribution of genetic modifiers to this phenomenon by analyzing the range of characteristics encountered in a group of GD patients with the same genetic mutation. They found that these patients had some commonalities, such as age of diagnosis and slowed eye scanning movements, but varied greatly in terms of systemic complications, nervous system involvement, and language and developmental deficits.

An association between GD and Parkinson disease has been demonstrated by the concurrence of GD and parkinsonism (dysfunction in brain nerve cells that leads to shaking, stiffness and slow movement) in rare patients and the identification of mutations in the enzyme glucocerebrosidase, a deficiency of which results in GD, in patients with sporadic Parkinson disease. Using a different and complementary approach, NIMH investigators described 10 unrelated families of subjects with GD who then developed parkinsonism. These observations indicate that mutant glucocerebrosidase, even in carriers, may be a risk factor for the development of parkinsonism.

*Williams syndrome*

Williams syndrome (WS), caused by a deletion of approximately 21 genes on chromosome 7, is characterized by dysmorphic features, mental retardation or learning difficulties, and striking neurocognitive and social-behavior abnormalities. Individuals with WS eagerly and impulsively engage in social interactions; however, they experience non-social anxiety and worry constantly. Scientists have long suspected that a specific area of the brain, the amygdala, may be involved in this striking pattern of behavior. Scientists at NIMH used functional neuroimaging to investigate the response of the amygdala to social stimuli in WS subjects and healthy controls. The results showed that the amygdala functions abnormally in WS, with reduced response to highly socially

relevant danger signals than in controls; this result is consistent with the lack of fear to social situations found in people with WS. Whole brain scans revealed that the orbitofrontal cortex, which modulates the amygdala by assigning emotional values to a situation, was not activated in subjects with WS in contrast to healthy volunteers. These results suggest a genetically controlled neural circuitry for regulating human social behavior and that genetic changes due to WS affect this circuitry in a specific way.

Deficits in spatial navigation and long-term memory, major cognitive domains dependent on function of an area of the brain known as the hippocampus, have been described in WS. NIMH researchers used neuroimaging techniques to explore hippocampal function and structure in WS. Results showed profound reduction in indicators of hippocampal function, including reduced activity and differential response to visual stimuli in patients with WS. Hippocampal size was the same as in healthy volunteers but subtle alterations in shape were present. These data demonstrate abnormalities in the hippocampal formation in WS, and suggest that hippocampal dysfunction may contribute to neurocognitive abnormalities in WS.

To further investigate the abnormal brain structure and cognitive deficits in WS, NIMH researchers performed a detailed analysis of the surface of the cerebral cortex constructed from high-resolution magnetic resonance imaging scans acquired from participants with WS and from healthy controls. They found significant differences in surface architecture within specific regions of the cerebral cortex in the brains of participants with WS. These alterations correspond closely to measures of reduced gray matter volume in the same areas, providing evidence that the gray matter volume loss and abnormal cerebral cortex surface geometry may be related. In conjunction with previous work indicating functional alterations in these areas of the brains of WS patients, these findings further define brain alterations that underlie some of the cognitive deficits found in WS.

*Fragile X syndrome*

Fragile X is a genetic disorder characterized by cognitive impairment ranging from learning disabilities to severe mental retardation, behavioral dysfunction and subtle physical abnormalities. In patients with Fragile X, a specific protein, the fragile X mental retardation protein (FMRP), has been found to be defective. Research has indicated that FMRP plays an important role in the synthesis of a number of other proteins in the cerebral cortex. NIMH researchers used a mouse model of Fragile X in which a genetic deletion prevented FMRP synthesis. Comparisons between these mice and normal mice showed that mice lacking FMRP demonstrate a substantial decrease in cerebral protein synthesis in all brain regions around the time of young adulthood. This result suggests that normal changes in the brain that occur during young adulthood may be altered in Fragile X patients.

*Suicide*

In August 2005, NIMH-supported researchers reported in the *Journal of the American Medical Association* the results of a clinical trial using cognitive behavioral therapy (CBT). The study involved 120 adults who had recently attempted suicide. Participants were randomly assigned to

either receive CBT or not, though all participants were encouraged to receive usual care from clinicians in the community. Over the 18-month follow-up period, only 24percent of those in the CBT group made repeat suicide attempts, compared to 42percent of the usual care group. Although the groups did not differ significantly in suicidal thoughts, those who received cognitive therapy scored better on measures of depression severity and hopelessness, which the researchers suggest "may be more highly associated with a reduced risk of repeat suicide attempts."

**2005 Rare Disease-Specific Request for Applications**

*Suicide*

NIMH continues to address the many unanswered questions around the Food and Drug Administration (FDA) advisories regarding the possible risk of suicidality with selected serotonin reuptake inhibitors (SSRI) treatments for pediatric populations. The FDA's further efforts to gather adverse event data for adults taking antidepressant medication has made this a pressing issue for both pediatric and adult populations. During 2005, NIMH released a Request for Applications (RFA) entitled Antidepressant Treatment and Suicidality. With an anticipated award date of July 1, 2006, the Institute has set aside $1.5 million to support grants that address the multiple issues around this putative problem.

**Significant Ongoing Rare Diseases Research Initiatives**

*Suicide*

NIMH supports a number of ongoing projects on suicide prevention across multiple populations that address multiple risk and protective factors. In FY 05 NIMH facilitated collaboration across four developing centers on suicide prevention [cofunded by the National Institute on Alcohol Abuse and Alcoholism (NIAAA), the National Institute on Drug Abuse (NIDA)] and a Veterans Administration center. These collaborations involved the identification of overlapping or related center goals such as suicide history and risk measurement, treatment development for comorbid patient populations, addressing safety and ethical issues in registries of attempters, and treatment trial designs. The American Foundation for Suicide Prevention has offered to provide funding of pilot data resulting from these collaborative center efforts.

*Pediatric bipolar disorder*

In 2005, NIMH funded a 4-year competing continuation of the Treatment of Early Onset Mania, a multi-site clinical trial designed to test the safety and effectiveness of pharmacological interventions for children with bipolar disorder. The study, conducted at five clinical sites with coordination by Washington University, St. Louis, involves more than 500 children, ages 8-14, suffering from bipolar I disorder, with manic or mixed phase, randomized to treatment with lithium, valproate, or risperidone, alone or in combination, according to their clinical history.

NIMH partnered with the National Institute of Child Health and Human Development (NICHD) to launch a new clinical trial to test the efficacy and safety of treatment with lithium in pediatric bipolar disorder in response to the Best Pharmaceutical for Children Act of 2002. The study will enroll more than 100 children, ages 7-17, with bipolar I disorder over the next 3 years to conduct a series of studies aimed at examining the pharmacokinetics, efficacy and safety of treatment with lithium carbonate in children.

*Pediatric HIV/AIDS*

Following a meeting supported by the Office of Rare Diseases (ORD) in 2001, a study investigating the prevalence of psychiatric symptomatology--particularly focusing on attention deficit disorders in youth perinatally infected with or exposed to HIV-1--was designed. NIMH partnered with NICHD and the National Institute of Allergy and Infectious Diseases (NIAID) to fund this study, conducted in cooperation with the Pediatric AIDS Clinical Trial Group (PACTG). The PACTG is the largest clinical research program in the United States focused on the care and treatment of HIV-infected children.

The study protocol has been approved with ongoing enrollment of patients in multiple centers throughout the continental United States and Puerto Rico. An NIMH-funded sub-study will evaluate 800 children perinatally infected with or exposed to HIV-1 and their caregivers to identify, quantify, and characterize the psychiatric consequences of living with HIV from birth. The goal of this study is to develop an understanding of both the physiological and environmental mediating and moderating factors that lead to mental health abnormalities and to ultimately develop interventions to prevent or lessen the psychiatric consequences of living with HIV in children and adolescents. The study will also examine the impact of type and duration of antiretroviral treatment in the infected children as well as disease specific factors such as viral load and immunologic status. . The data obtained on the prevalence, severity, and etiology of mental health abnormalities in children affected by HIV will be of great importance in developing countries as antiretroviral therapy becomes more available and children, adolescents, and adults live longer and healthier lives.

*Childhood onset schizophrenia*

NIMH funds several ongoing studies investigating the cognitive and neurophysiological characteristics of childhood onset schizophrenia (onset before age 13) and early onset schizophrenia (onset before age 18). For example, in FY 2005 NIMH funded a project that tests the hypothesis that specific circuits within the brain are dysfunctional in schizophrenia. The study will evaluate and follow two groups of adolescents with liability to schizophrenia (patients with early symptoms before the onset of the disorder and siblings of patients with COS). They will investigate the relationships between distinctive clinical features, neuropsychological deficits and functional outcomes using functional Magnetic Resonance Imaging. Additionally, NIMH continues to fund studies of treatment for childhood onset schizophrenia, two of which are nearing completion.

**Rare Disease-Specific Conferences, Symposia, and Meetings**

*Suicide*

Following the recommendations of the September 2004 ORD-supported workshop, "Pragmatic Considerations of Culture in Preventing Suicide," NIMH collaborated with the Indian Health Service (IHS), Health Canada, the Canadian Institutes of Health Research, and SAMHSA to plan a meeting on indigenous suicide prevention. Meeting co-sponsors include the ORD, NIDA, NIAAA, Office of Behavioral and Social Sciences Research (OBSSR), and Office of Research on Women's Health (ORWH). The meeting of approximately 150 indigenous community representatives and researchers from the United States, Canada, and U.S. Territories will take place February 7-9, 2006, in Albuquerque. The purpose is to identify community-relevant research questions and sustainable research approaches to suicide prevention, as well as to facilitate networking among indigenous researchers.

In November 2005, NIMH and ORD co-sponsored a meeting that addressed the topic of antidepressant treatment and its putative suicidality side effects. Participants represented the FDA, VA, IHS, and the American Foundation for Suicide Prevention, as well as researchers with methodological expertise in observational studies of mental health services; decision making theory; real-time assessments; suicide risk assessment; biostatistics; and pharmacogenetics, pharmacodynamics and pharmacokinetics. Participants discussed potential research questions and best approaches towards addressing the many unknown issues surrounding this topic. Representatives from the pharmaceutical industry were also invited. A summary of this meeting has been drafted and is being reviewed for posting on the NIMH Web site.

## NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE (NINDS)

### Overview of Rare Disease Research Activities

The mission of the National Institute of Neurological Disorders and Stroke (NINDS) is to reduce the burden of neurological disease—a burden borne by every age group and every segment of society worldwide. The brain, spinal cord, and nerves are vulnerable to hundreds of disorders, most of which are rare. Even more common diseases such as stroke, epilepsy, and Parkinson's disease include rare subtypes. The NINDS supports research to uncover the causes of, and develop treatments for, individual rare disorders, while also promoting cross-cutting research on topics such as stem cells, gene therapy, and neuroimaging that will impact multiple rare neurological disorders.

The NINDS supports basic, clinical, and translational research on rare diseases through both its extramural and intramural programs. The NINDS also collaborates with the NIH Office of Rare Diseases (ORD) and rare disease patient voluntary organizations to stimulate specific research areas via workshops, grant solicitations, and strategic planning efforts. The Institute's primary support of research is through unsolicited, investigator-initiated grant awards, as investigators often have the greatest insight into the critical questions facing a particular field of research. Of the new grants funded by the NINDS in FY 2005, many focused on rare diseases. Examples include Charcot-Marie-Tooth disorder, spinal muscular atrophy, Huntington's disease, Niemann-Pick disease, Tourette syndrome, amyotrophic lateral sclerosis, Batten disease, Down syndrome, neurofibromatosis, Angelman syndrome, muscular dystrophy, hydrocephalus, Rett syndrome, spinocerebellar ataxia, and the mucopolysaccharidoses.

### Examples of Recent Scientific Advances in Rare Diseases Research

*Down syndrome*

Down syndrome is a chromosomal disorder caused by an error in cell division that results in the presence of an additional third chromosome 21 or "trisomy 21." It causes mild to moderate mental retardation and associated medical problems in one out of 800 live births, in all races and economic groups. One of the underlying cellular defects thought to play a role in Down syndrome is mitochondrial dysfunction, where the mitochondria, the cell's power stations, fail to meet the high-energy demand of nerve cells. NINDS intramural scientists recently discovered a connection between a gene implicated in Down syndrome and mitochondrial dysfunction. When levels of expression of this gene are altered in fruit flies, a variety of changes occur in the mitochondria, and cellular energy levels drop. Since neurons require a great deal of energy to function properly, these mitochondrial defects could contribute to the cognitive problems associated with Down syndrome. Furthermore, this research suggests that this gene may be important in other neurological disorders, such as Alzheimer's disease, where mitochondrial dysfunction is thought to play a role.

*Fragile X*

Fragile X syndrome is a genetic disorder that causes mental retardation and is associated with a variety of other psychiatric and neurological symptoms. Building on the discovery of the gene defect responsible for the syndrome, scientists developed fruit flies with a similar gene defect. These flies have cognitive, behavioral, and neuronal abnormalities that are reminiscent of the human disorder. Studying these flies led to a theory that implicates changes in a particular neurotransmitter system in fragile X. That theory has now led to the finding that the drug lithium or drugs called metabotropic glutamate antagonists that affect this neurotransmitter system, can reverse the short-term memory deficits, behavioral problems, and structural changes in the brains of these flies. These findings suggest that similar modulation of this neurotransmitter system should be explored as a therapeutic approach in individuals with Down syndrome.

*Muscular dystrophies*

The muscular dystrophies are a group of genetic diseases characterized by progressive weakness and degeneration of the skeletal muscles that control movement. There are many forms of muscular dystrophy which vary in their mode of inheritance, age of onset, severity, and pattern of muscles affected. A number of therapies for muscular dystrophy are showing promise in animal and cell-based models, including gene therapy. One of the biggest challenges in developing gene therapy for muscular dystrophy is delivering beneficial genes widely to muscle cells throughout the body. Now, researchers have shown in rodents that a virus called adeno-associated virus 8 (AAV8) can effectively deliver a gene to skeletal muscles and to the heart. In a hamster model of limb-girdle muscular dystrophy, delivery of the delta-sarcoglycan gene using the AAV8 virus corrected the degeneration and other problems normally seen in this disease. Similarly, after delivery of the gene agrin, the body weight of mice that mimic a severe form of congenital muscular dystrophy improved by 80 percent at 6 weeks of age, and the mice lived about four times as long as untreated mice. A next step in this research is to study AAV8 gene delivery in dogs with Duchenne-like muscular dystrophy to learn if the treatment is effective in larger animals.

*Neurofibromatosis*

Neurofibromatosis type 1 (NF1) is among the most common genetic disorders that affect the nervous system. In addition to producing tumors and other structural deformities, NF1 is associated with a spectrum of disabilities in learning and attention. Following the discovery of the gene defects that cause NF1, researchers generated a mouse model of the disease. By studying these mice, investigators identified a biochemical pathway affected by the gene, that basic studies had shown is critical for learning. A new study has found that the drug lovastatin, which inhibits this biochemical pathway, can reverse the learning disabilities in NF1 mice. Lovastatin is a common and well-tolerated drug used to control cholesterol. The study suggests that Lovastatin or possibly other inhibitors of this biochemical pathway may prove useful in treatment of the cognitive impairments associated with NF1 in humans.

*Spinocerebellar ataxia*

Spinocerebellar ataxia (SCA1) is a genetic disease that causes progressive loss of motor coordination and balance due to degeneration of specific cells in regions of the brain that help coordinate movement. This disease is one of at least eight inherited disorders caused by an abnormal repetition of part of the genetic code. These "repeats" in the gene lead to abnormal repeats of one of the protein building blocks, glutamine, in the protein produced from the affected gene. Previous studies had shown that these glutamine repeats have a toxic effect on cells, however it was not clear how the repeats cause different patterns of cell loss in each of the glutamine repeat diseases. Findings from studies of SCA1 mutant flies and mice showed that the protein affected by SCA1, ataxin-1, is not broken down and cleared normally when the abnormal glutamines are present, and the buildup of protein has a toxic effect on one type of nerve cells. A new study now uncovers a mechanism for how this protein accumulation leads to nerve cell death. The researchers showed that selective neuronal degeneration is mediated through interactions between Atx-1 and another regulatory molecule in the cells. Interestingly, build up of both normal ataxin-1 protein or ataxin-1 with the expanded glutamine repeat was capable of mediating nerve cell death through this interaction. This suggests that the neurodegeneration seen in SCA1, and in other glutamine repeat disorders, may be caused by having too much of a normal protein, not just by a protein that acts in an aberrant fashion.

*Tourette syndrome*

Tourette syndrome is a neurological disorder characterized by repetitive, stereotyped, involuntary movements and vocalizations called tics. Several decades of research suggest that there is a substantial genetic contribution to Tourette syndrome, but no responsible gene defects had been identified. Researchers have now identified defects in a gene, called SLITRK1, that can cause Tourette syndrome. Basic developmental studies have previously investigated SLITRK1 because of its role in guiding growing nerve fibers in the developing brain. Although this particular gene may account for a small percentage of Tourette syndrome cases, the finding is a starting point to study the underlying changes in the brain that lead to the disease and to develop better therapies.

*Transmissible spongiform encephalopathies*

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of rare degenerative brain disorders characterized by tiny holes that give the brain a "spongy" appearance, when brain tissue is viewed under a microscope. Symptoms of TSEs vary, but they commonly include personality changes, psychiatric problems such as depression, lack of coordination, and/or an unsteady gait. Symptoms may progress to the point where individuals have severe mental impairment. The public health importance TSEs has increased dramatically in recent years. First, evidence arose that an animal form of TSE, bovine spongiform encephalopathy (mad cow disease) can be transmitted to people. More recently, there are indications that TSEs may be transmissible through transfer of blood products from people with TSEs. Better detection of TSEs is thus a high public health priority, but the unusual nature of the infectious agent of TSEs, the prion proteins, precludes use of the usual methods to diagnose

infectious disease and detect blood contamination. By understanding how prions propagate in TSEs, researchers have developed a method to amplify the low level of abnormal prions in blood of experimental rodents more than 10 million-fold and detect the prions with conventional protein detection methods. Work is underway to apply this method to other animals and to humans. In addition to improving the safety of the blood supply and surveillance of cattle, therapies are more likely to work the earlier TSEs can be diagnosed.

**Recent and Planned Research Activities**

The NINDS released the following grant solicitations in FY 2005 to help encourage research on rare diseases. Some of these were issued in collaboration with other Institutes and patient voluntary organizations, as indicated below.

- Administrative Supplements for Senator Paul D. Wellstone Muscular Dystrophy Research Fellowships at Wellstone MD Cooperative Research Centers (with NIAMS, NICHD)
- Administrative Supplements for Muscular Dystrophy Workshops and Research Conferences (with NIAMS, NICHD, ORD)
- Mentored Clinical Investigator Career Development Awards in Muscle Disease Research (with NIAMS, NICHD, ODS)
- Muscular Dystrophy: Pathogenesis and Therapies (with NIAMS, NICHD, NHBLI)
- Ruth L. Kirschstein National Research Service Awards for Postdoctoral Fellowships in Muscle Disease Research (with NIAMS, NICHD, ODS)
- Shared Neurobiology of Fragile X syndrome and Autism (with NICHD, NIMH, Canadian Institutes of Health Research, Ireland Health Research Board, FRAXA Research Foundation, Cure Autism Now, National Alliance for Autism Research, Autism Speaks)
- Basic and Clinical Research on Rett syndrome and MeCP2 (with NIMH, NICHD, International Rett syndrome Association, Rett syndrome Research Foundation)
- Understanding and Treating Tuberous Sclerosis Complex (with NIDDK, NIMH, NIAMS, NCI)

FY 2006 solicitations include:

- Exploratory/Developmental Program for Translational Research in Muscular Dystrophy (R21) (with NIAMS)
- Translational Research in Muscular Dystrophy (U01) (with NIAMS)

The NINDS awarded a new grant in FY 2005 to support a phase II clinical trial on diflunisal in patients with familial amyloidotic polyneuropathy (FAP). FAP is an inherited disorder in which an abnormal liver protein causes widespread damage throughout the body, resulting in sensory and motor problems, neuropathy, heart disease, gastrointestinal disorders, and ultimately, death. The FDA has designated diflunisal an orphan drug and has provided partial funding for this clinical trial.

New NINDS intramural clinical studies begun in FY 2005 include phase II studies to evaluate the safety and effectiveness of phenylbutyrate for treating Huntington's disease, an experiment drug that promotes protein folding and stability (AT1001) for Fabry disease, and idebenone (an antioxidant) for Friedreich's ataxia. NINDS intramural researchers also began a clinical study to examine the brain activity associated with tics in Tourette patients.

**Significant Ongoing Rare Disease Activities**

The NINDS, together with NIAMS and NICHD, has been actively implementing the provisions of the Muscular Dystrophy Community Assistance, Research and Education Amendments of 2001 (MD-CARE Act, Public Law 107-84). The three Institutes, together with other government agencies with an interest in muscular dystrophy and public members, some of whom represent patient advocacy groups, are represented on the interagency Muscular Dystrophy Coordinating Committee (MDCC). In accordance with the MD-CARE Act, the MDCC developed an NIH Research and Education Plan for Muscular Dystrophy, and more recently developed a more comprehensive Action Plan for the Muscular Dystrophies, with over 70 research objectives to help achieve the effective detection, diagnosis, treatment, and prevention of all types of muscular dystrophy. In addition, NINDS, NICHD, and NIAMS jointly fund a network of six Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers, which bring together expertise, infrastructure and resources focused on major questions about muscular dystrophy and provide resources that can be used by the national muscle biology and neuromuscular communities. The NIH has recently announced the availability of supplements to the Centers to improve training and to support scientific conferences on focused topics. Among other efforts, grant solicitations focus on encouraging research to improve our understanding of the underlying pathogenesis of the muscular dystrophies and on enhancing translational research to develop therapies for these diseases.

The SMA Project is making encouraging progress towards its ambitious goal of having a drug for spinal muscular atrophy (SMA) ready for clinical trials by the end of 2007. The steering committee, with expertise in drug development from industry, the FDA, academia and the NIH, has developed a detailed drug development plan. The project has identified compounds that have sufficient effectiveness in SMA model systems and appropriate chemical characteristics to serve as leads for developing a drug. The project has also established the tools and facilities for systematically modifying these leads, testing the newly generated drug candidates in cell and animal models, handling the substantial informatics requirements, and coordinating the overall drug development scheme. The drug development process is now underway, with more than 300 new compounds already synthesized.

The NINDS, NHBLI, NIEHS, NCI, NEI, NIGMS, NHGRI, NIA, NIAID, NICHD, NCRR, and ORD formed the Trans-NIH Ataxia-Telangiectasia Working Group in FY 2005 to coordinate and focus NIH efforts to study and identify research needs for ataxia telangiectasia (A-T). The working group is in the process of developing a research plan that will serve the entire A-T scientific community.

**Workshops, Symposia, and Meetings**

In FY 2005, the NINDS led or participated in the following workshops relevant to rare diseases:

- CNS Manifestations of Tuberous Sclerosis Complex
- Second Scientific Workshop on Neurodegeneration with Brain Iron Accumulation
- Tenth International Congress on Neuronal Ceroid Lipofuscinosis
- 2005 CAG Triplet Repeat Disorders Gordon Conference (SCA, Myotonic dystrophy)
- Research Planning Workshop on Spasmodic Dysphonia
- Hydrocephalus: Myths, New Facts, Clear Directions
- Vascular Cognitive Impairment: Harmonization Criteria
- Research Planning Workshop on Spasmodic Dysphonia
- Neurobiology of Disease in Children Conferences (Tourette)
- Drug Screening For Ataxia-Telangiectasia
- Towards the Development of Pediatric Stroke Trials
- Challenges and Opportunities in Clinical Trials for SMA
- At the Crossroads: Common Pathways in Fragile X and Autism
- First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Disease
- 2005 NNFF Consortium for NF1, NF2 and Schwannomatosis

For FY 2006, NINDS is organizing several meetings on rare diseases, including neurosarcoidosis, episodic ataxias syndromes, ataxia-telangiectasia, glycosphingolipid-associated disorders, dystonia, skeletal muscle diseases, and multiple system atrophy.

## NATIONAL INSTITUTE OF NURSING RESEARCH (NINR)

### Overview of Rare Diseases Research Activities

NINR supports clinical and basic research to establish a scientific basis for the care of individuals across the life span: from management of patients during illness and recovery to the reduction of risks for disease and disability, the promotion of healthy lifestyles, promoting quality of life in those with chronic illness, and care for individuals at the end of life. NINR's rare diseases research investigators, using an interdisciplinary approach, examine strategies to control, manage, and prevent complications.

### Recent Scientific Advances in Rare Disease Research

*Childhood acute lymphoblastic leukemia*

A novel study to describe the patterns of pain, sleep, fatigue, and well-being of children with acute lymphoblastic leukemia (ALL) and their parents across the course of chemotherapy treatment is underway. Children with ALL and their parents will be followed from the induction phase to the maintenance phase of chemotherapy treatments to monitor symptoms. Results may contribute to the development of strategies to manage symptoms of ALL treatment for both patients and parents.

*Anorexia nervosa*

Anorexia nervosa (AN) involves distorted body image and fear of weight gain. While psychotherapeutic approaches for AN exist, research indicates the need for enhancing recovery programs. A randomized clinical trial tests the effectiveness of a cognitive-behavioral social identity intervention program to promote recovery from AN by allowing participants to develop new and separate positive self-cognitions.

*Cystic fibrosis*

Cystic fibrosis (CF) carrier testing has been recommended as an option for high-risk individuals, pregnant women, and couples planning pregnancy by the 1997 NIH Consensus Statement on Cystic Fibrosis. To learn more about the interactions between patient and contextual factors during decision-making about genetic knowledge, an investigation describes how these variables contribute to decision making about cystic fibrosis carrier testing by pregnant individuals in a primary care setting. Results may provide additional knowledge of decision making in health care settings.

*Duchenne muscular dystrophy*

Duchenne Muscular Dystrophy (DMD) is a degenerative, genetically inherited disease, which primarily affects voluntary muscles, the heart and breathing muscles. Onset occurs in early childhood allowing for interventions that may affect both the quality of life and longevity.

A study investigates spirituality in children (8-12 years old) with DMD through interviews and descriptions of drawings.

*Ovarian cancer*

A range of investigations of individuals with ovarian cancer are exploring dimensions of health behavior decision making, symptom management, and quality of life. One investigation explores health behavior decision making in a population of women after learning they are at high risk for hereditary breast and ovarian cancer based on results of genetic testing. Another investigation explores symptom representations and symptom interference with life activities, and quality of life in a sample of women with recurrent ovarian cancer. A randomized clinical trial tests the effectiveness of a standardized nursing intervention protocol (SNIP) on quality of life outcomes for adult women newly diagnosed with ovarian cancer, along with cost of care, symptom distress and other domains.

*Pediatric AIDS*

Investigations are evaluating several research topics of perinatally acquired HIV, HIV prevention in school-aged children, and health risk behavior in youth. One investigation evaluates HIV self-care practices of youth with perinatally acquired HIV disease (YPAHD) and how family caregivers manage aspects of transitioning self-care as YPAHD get older. Another investigation is testing a school-based prevention program that adapts a prevention curriculum to the needs of impulsive decision makers (N=5000 students) and is examining characteristics of the instructional environment. A third investigation evaluates the predictive value of contextual/risk factors, protective resources, and heath behaviors of school-aged children for health-risk behaviors in early adolescence (N=2200 children in grades 4-6). A fourth investigation is examining sexual health practices of homeless adolescents.

*Scleroderma*

Persons with scleroderma experience hardening of skin and connective tissue, as well as decreased quality of life. An investigation is developing a self-paced education and self-management intervention designed for persons with scleroderma delivered by the Internet. The intervention will be used in a clinical trial to improve self-management and quality of life for persons with scleroderma.

*Sickle cell disease*

A complication of sickle cell disease is pain during episodes of vaso-occlusion. Researchers explore the factors that contribute to the lack of pain relief to morphine in children with sickle cell disease (e.g. subtherapeutic morphine concentrations, individual genetic variability). Results may contribute to the development of additional pain management strategies for children with sickle cell disease.

*Tuberculosis*

An investigation explores the effectiveness of a cultural intervention for adherence to Latent Tuberculosis Infection (LTBI) therapy in Latino immigrants, a population with a high rate of tuberculosis cases. The cultural intervention incorporates Latino cultural values, and language conventions, with other LTBI education materials.

**Rare Disease Research Workshop**

The National Institute of Nursing Research (NINR), the Office of Rare Diseases (ORD), the Office of AIDS Research (OAR) and the Office of Behavioral and Social Science Research (OBSSR) at the National Institutes of Health (NIH) convened the workshop, "Cultural Dynamics in HIV/AIDS Biobehavioral Research Among Young People" on September 15-16, 2005 in Bethesda, Maryland. This workshop was organized to explore how the incorporation of an understanding of cultural dynamics can lead to the development of culturally appropriate interventions to prevent transmission of HIV among young people, and an improved understanding of the impact of individual and communal beliefs and values upon the quality of life of youth already infected by HIV. The workshop concluded that since change is constant among cultures and among individuals, strategies for addressing the needs of young people affected by HIV require frequent modification. However, there is a maturing body of research to support development of interventions for affected young people.

## NATIONAL CENTER FOR RESEARCH RESOURCES (NCRR)

### Overview of Rare Diseases Research Activities

The National Center for Research Resources (NCRR) provides laboratory scientists and clinical researchers with environments and tools that they can use to prevent, detect, and treat a wide range of diseases. This support enables discoveries that begin at the molecular and cellular level, move to pre-clinical and animal-based studies, and then are translated to clinical research, resulting in cures and treatments for both common and rare diseases. NCRR connects researchers with patients and communities across the nation to bring the power of shared resources and research to improve human health.

Through its support of multidisciplinary research resources, NCRR is uniquely positioned to provide resource support in partnership with other Institutes or Centers to address emerging clinical and basic research needs, such as for the study of rare diseases. Expansion of NCRR's efforts in new biotechnologies and instrumentation, development of animal models, and clinical research resources will foster interdisciplinary collaborations and advance NIH's efforts to study rare diseases.

### Recent Sciencitific Advances in Rare Diseases Research

*Bardet-Biedl syndrome*

Bardet-Biedl syndrome (BBS) is a group of rare disorders inherited as autosomal recessive genetic traits. Abnormal genes from both parents are required to cause the disease; people with only one abnormal gene in the gene pair are called carriers, and do not exhibit the disease. Major features of these disorders may include mental and growth retardation, obesity, delayed sexual development or underdeveloped reproductive organs, poor visual acuity and blindness, cardiomyopathy, kidney failure, and/or abnormal or extra fingers and/or toes. BBS expression varies both within and between families and diagnosis is often difficult. Researchers at Ponce School of Medicine in Puerto Rico, with support from the Research Centers in Minority Institutions Program, have studied 14 families with 34 affected individuals and 321 relatives (including 68 carriers). All families showed linkage to a location on chromosome 11, and researchers have characterized the genes in this area. Continued research may lead to a genetic test for BBS.

*Bronchopulmonary dysplasia*

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that predominately develops in premature infants. The disease is characterized by inflammation and abnormal growth/development of the airways following any lung condition that requires prolonged oxygen and/or mechanical ventilation therapy. It is most commonly a complication of respiratory distress syndrome but can occur due to oxygen toxicity, mechanical lung trauma, infections or pneumonia. At the NCRR-supported Southwest National Primate Research Center (NPRC), researchers have developed an immature primate model for neonatal BPD that approximates the

human situation in terms of lung development and long-term ventilator support, and has clinical, biochemical, and histopathologic features comparable to those described in extremely immature human infants with BPD. This model is being used to develop strategies in the diagnosis and treatment of this disease.

*Fanconi anemia*

Fanconi anemia (FA) is a genetically complex disease of congenital anomalies and genomic instability progressing to bone marrow failure and greatly elevated risk of cancer. FA affects 1 in 300,000 children and is usually fatal within 5 years of the onset of anemia due to bone marrow failure or cancers, including myeloblastic leukemia and squamous cell carcinomas. In order to develop an animal model of FA, investigators at the University of Oregon have sought to determine if the FA genes in zebrafish are conserved and act similarly to the human genes. Structural and genomic analysis of the genes revealed extensive conservation between zebrafish and humans. These results, along with supporting functional analysis studies, will facilitate developing a zebrafish model of FA, understanding FA genes in development and cancer, discovering new genes in the FA pathway, and developing a suitable assay for a small molecule screen for therapeutic compounds.

*Hermansky-Pudlak syndrome*

Hermansky-Pudlak syndrome (HPS) is a multi-system disorder characterized by unusual susceptibility or predisposition to bleeding resulting from a platelet storage pool deficiency, and, in some cases, pulmonary fibrosis or granulomatous colitis. It is also the major cause of albinism in Puerto Rico. HPS is caused by defects in many genes, seven of which have been identified in humans (HPS1-7). HPS gene products are frequently undetectable in cells and tissues, limiting therapeutic targets for this disease. Both basic and clinical researchers at the Research Centers in Minority Institutions (RCMI)-funded Genetics of Blood Disorders Research Program at the University of Puerto Rico Medical Sciences Campus have developed methods to detect HSP gene products and have identified mutations in one of the genes associated with this syndrome, HSP-1, in human lymphoblast cell lines. In addition, they have identified potential response elements for glucocorticoid hormones, cAMP, and phorbol esters that increase the expression of the mutated HSP gene. Future research will focus on using this information to learn more about the causes and treatment of this disease.

*Lyme disease*

Lyme disease is a tick-borne disease that can leave victims with a life-long burden of disability, if it is not diagnosed and treated early. Infection of humans results after being bitten by ticks that are infected with the spirochete bacterium *Borrelia urgdorferi*. Typical symptoms include fever, headache, fatigue, and a characteristic skin rash called erythema migrans. Infection can spread to joints, the heart, and the nervous system. Since the mid-nineties, an increased number of co-infections have been reported in individuals with Lyme disease, which has resulted in an increase in the duration and severity of this infection. Researchers at the University of Kansas Medical Center=s Center of Biomedical Research Excellence (COBRE) are using a mouse model to

investigate co-infection with spirochetal agent of Lyme disease, *Borrelia burgdorferi*, and the blood parasite, *Babesia microti*. Preliminary results indicate that *B. microti* induces initial T-helper type 1 responses, with significant down-regulation of IL-10, which in turn increases inflammation of joints and creates an adequate milieu for *B. burgdorferi* persistence. Furthermore, co-infected animals receiving recombinant IL-10 showed significantly less severe Lyme arthritis as compared with non-treated controls. These results indicate a predominant role played by IL-10 during infection with *B. microti*, and suggest a potential target for reducing the severity of this disease.

Research at the Tulane NPRC has led to the development of an FDA- and USDA- approved test for Lyme disease, the C6 test. This test reduces confusion in diagnosing the illness by providing more accurate results.

*Lysosomal storage disorders*

Lysosomal storage disorders are a group of more than 30 inherited disorders resulting from the body's inability to produce certain enzyme activities necessary to breakdown and recycle large molecules. This enzyme deficiency results in the accumulation (storage) of large molecules that cause cell damage evident at birth or with aging. The diseases can affect multiple organ systems, often including the brain, resulting in mental retardation and a short life span. A research team at the University of Pennsylvania, School of Veterinary Medicine, has identified cats and dogs with lysosomal storage disorders, including mucopolysaccharidoses, alpha-mannosidosis, Krabbe disease, I-cell disease, and Neiman Pick C disease. These models have been useful in the ongoing evaluation of therapies, including enzyme replacement, bone marrow transplantation, and gene therapy using retrovirus and adeno-associated virus vectors. Gene therapy studies have been successful in preventing many of the clinical manifestations of lysosomal storage disease in these animals. The models provide systems in which to study the treatment of human genetic diseases by new approaches, particularly stem cell therapies.

*Measles*

Measles is an infectious viral disease and is primarily a respiratory infection with a typical skin rash. Current vaccines for measles utilize the live, attenuated virus and while very effective, this vaccine is not appropriate for children under 6 months of age. Researchers at the California NPRC are investigating a DNA vaccine to protect infants from measles. In experiments using infant rhesus monkeys, vaccinated animals were protected when challenged with pathogenic measles virus at 5 months of age. This vaccination strategy models a practical approach to protecting infants against measles in the first year of life before the standard measles vaccination can be safely given. Two other programs at the California NPRC examine genetic markers of attenuation of the measles vaccine and the potential to establish models of long-term immunologic memory to measles vaccine in the rhesus monkey. The recombinant vaccines used in infected infant rhesus furthers knowledge on protection against measles infection.

**Significant Ongoing Rare Diseases Research Initiatives**

*The Rare Diseases Clinical Research Network (RDCRN)*

RDCRN, a collaboration between NCRR and the NIH Office of Rare Diseases with support from several other NIH institutes, includes 10 Rare Diseases Clinical Research Consortia (RDCRC) and a Data and Technology Coordinating Center (DTCC). Each RDCRC is focused on a subset of rare diseases with the mission to perform, in partnership with patient advocacy groups, longitudinal studies, clinical studies and trials, train new rare diseases clinical investigators, and educate patients and physicians. Each Consortium has several protocols in the process of review or implementation, including both longitudinal and intervention studies. The DTCC has enabled and trained site staff on a Web-based remote data entry system that incorporates RDCRN approved standards and Systematized Nomenclature of Medicine Clinical Terms (SNOMED CT) terms. In addition, they have developed and implemented an electronic adverse event reporting system that includes a standard reporting structure and automatic alerts and notifications. Other innovations include a sample tracking system for samples within studies, remote direct laboratory transfer, integrated vocabulary and laboratory standards, statistical support, Web site development and maintenance, and database querying tools. The RDCRN vocabulary unit continues its national collaborations with incorporation of unique concepts and terms necessary for rare diseases in national vocabularies, including SNOMED CT, Health Level Seven, and the National Library of Medicine ULMS.

The RDCRN Web site (http://www.rarediseasesnetwork.org) is a source of information for the public, physicians, patients, and investigators about rare diseases. The Web site includes information about current protocols at each of the participating sites and informative links. The DTCC has implemented a unique Web-based contact registry for patients who wish to learn about clinical studies. This registry is expected to expand to include telephone and paper based registration in the future. The RDCRN Coalition of Patient Advocacy Groups (CPAG), which includes all of the patient support groups affiliated with each of the consortia, has been active in supporting each other in their outreach efforts to patients afflicted with rare diseases, their families and the public.

Each Consortium has training programs focused on the creation of a cadre of clinical investigators interested in rare diseases.

*National Gene Vector Laboratories*

Established by NCRR in 1993, the National Gene Vector Laboratories (NGVLs) have two primary goals. The first is to provide gene vectors to eligible investigators for use in clinical gene transfer protocols. The second goal is to support the development of toxicology data for the applicant's Investigational New Drug (IND) submission to the Food and Drug Administration (FDA). NCRR supports the infrastructure of three NGVL vector production centers and two NGVL toxicology centers while NHLBI, NINDS, NIEHS, NIAID, NIDDK, NCI, NIAMS, NICHD, NIDCR and the Office of Rare Diseases provide supplemental funding to the NGVLs for specific vector production and toxicology studies that address their missions. The NGVLs

provide retrovirus, adenovirus, adeno-associated virus (AAV), DNA plasmid, herpes virus and lentivirus vectors at no charge to clinical researchers who are studying common disorders and rare diseases such as muscular dystrophy, alpha-1-antitrypsin deficiency, Fanconi's anemia, cystic fibrosis, and leukocyte adherence deficiency.

**Industry-Initiated Rare Disease Research at General Clinical Research Centers (GCRCs)**

The NCRR-supported GCRCs host much of the research focused on rare diseases, as there are few large clinics available specifically for these unusual disorders. While most of these studies are investigator initiated, there are industry sponsored phase 1, 2, and 3 trials of new interventions that utilize the GCRCs. Since the number of affected individuals is small, the expected market is also small, limiting the interests of many pharmaceutical companies. Recognizing the need to advance the development of new agents for these diseases, the Orphan Drug Act provides incentives for companies to develop and license agents for rare diseases. The NCRR, also recognizing the need and benefit from the support of new agents for rare disease, modified the guidelines for GCRCs for industry initiated studies and trials. Normally, companies that wish to utilize the resources of the GCRCs in the performance of their studies must pay for those resources. For industry-supported rare disease studies and trials, the GCRC may approve use of GCRC resources without charge. Several rare disease studies are already benefiting from this change, including cystic fibrosis, lysosomal storage diseases, and urea cycle disorders.

**Activities with Nonprofit Organizations**

In partnership with the Cystic Fibrosis Foundation (CFF), NCRR supports a novel approach to develop new therapeutics for cystic fibrosis, a rare genetic disease. The CFF Therapeutics Development Network (TDN) unites investigators focused on cystic fibrosis research to perform clinical trials of promising agents for treatment and cure of this disorder. NCRR-supported General Clinical Research Centers (GCRCs), which provide personnel, resources, and space for the conduct of clinical research, are utilized by many investigators in this network. In addition, NCRR supports a coordinating center, which provides informatics support for the management, conduct, and analysis of the studies. This biomedical informatics component includes a secure, interactive Web environment for network communication and data entry, as well as a biostatistical unit. These resources facilitate transfer of new discoveries from bench to bedside.

The National Disease Research Interchange (NDRI) founded in 1980, is a not-for-profit organization in Philadelphia. NCRR provides support for approximately one-third of their activities via a cooperative agreement. NDRI personnel obtain commitments from academic pathologists to provide human tissues for basic research and statements of need. These two lists, with very specific clinical details (but without patient identifiers), are kept in NDRI databases. When a tissue becomes available, a researcher is contacted by NDRI staff and asked if he/she can accept it. Upon positive reply, the pathologist is notified, prepares the tissue according to the researcher's protocol and sends it, anonymized, to the researcher. The researcher pays a relatively small fee. Through this cooperative agreement, NDRI facilitates laboratory research on a broad variety of rare and common diseases by providing tissues obtained from individuals

afflicted with such disorders to laboratory researchers engaged in studying those specific diseases. The agreement is currently co-funded by NCRR, the Office of Rare Diseases, and five other NIH Institutes and Centers.

## NATIONAL CENTER FOR COMPLEMENTARY AND ALTERNATIVE MEDICINE (NCCAM)

### Overview of Rare Diseases Research Activities

Congress endowed NCCAM with a broad mandate to conduct and support complementary and alternative medicine (CAM) research, provide for research training, disseminate information, and facilitate the integration of CAM and conventional health care. In fulfilling this mandate, NCCAM is supporting a broad portfolio of basic and clinical research, including investigations of a number of rare diseases. Many of these studies seek to test the safety and efficacy of CAM therapies and elucidate the mechanisms of action underlying CAM practices.

### Ongoing and Planned Rare Diseases Research

### Cancers

*Ovarian cancer*

Building on earlier research findings suggesting that acupuncture can stimulate the production of white blood cells, investigators at an NCCAM-funded developmental research center are studying whether acupuncture can boost low white blood cell counts commonly associated with chemotherapy for ovarian cancer, which in turn can leave patients susceptible to life-threatening infections. While there are existing conventional treatments for this condition, known as neutropenia, the conventional treatments are generally expensive and carry side effects of their own. In addition to assessing the effectiveness of acupuncture in counteracting neutropenia in ovarian cancer patients, investigators are also monitoring its effect on their quality of life.

*Sarcoma*

NCCAM investigators are conducting a randomized, controlled study to determine whether electroacupuncture is effective in treating chemotherapy-induced nausea and vomiting in patients with pediatric sarcomas, such as Ewing's sarcoma. In addition to exploring whether these particular side effects of chemotherapy can be eliminated or reduced, researchers are seeking to determine whether acupuncture can reduce the psychological tension associated with chemotherapy and reverse the resulting effects of stress on the neuroendocrine and immune systems.

### Other Rare Diseases

*Amyotrophic lateral sclerosis*

A group of scientists at an NCCAM-funded research center are studying a number of related CAM antioxidant therapies to determine whether they might have a role in treating amyotrophic lateral sclerosis (ALS). In particular, these investigators are focusing on antioxidants such as zinc, uric acid, and alpha-lipoic acid, using cell cultures and animal models to learn more about

their underlying mechanisms of action, dose-response effects, and relevant molecular and biological targets. Based on information gleaned from these studies, researchers will be able to make informed decisions about whether these CAM antioxidant therapies should be tested in humans – and ultimately whether there might be a combination of therapeutic actions that will slow the progressive death of motor neurons that defines ALS.

*Cystic fibrosis*

About 30,000 people in the United States have cystic fibrosis (CF), an inherited disease of the mucus and sweat glands that affects the lungs and other organs, and generally leads to premature death from respiratory failure. As mucus builds up in the lungs of cystic fibrosis patients, it creates ideal conditions for bacteria to thrive, resulting in repeated and damaging lung infections. In several different research projects, NCCAM-funded investigators are evaluating CAM treatments to prevent or decrease the severity of these infections. At one site, for example, NCCAM-funded researchers are exploring whether ginseng might be an effective alternative or supplemental treatment for infection with Pseudomonas aeruginosa, the most common cause of morbidity and mortality in patients with cystic fibrosis. Another NCCAM-funded investigator is studying the use of probiotics to eradicate Pseudomonas aeruginosa from the throats of children with cystic fibrosis, thereby avoiding the bacteria's infection of the lungs.

Other NCCAM-supported investigators are working to determine whether botanicals such as feverfew and the plant pigment quercertin (found in onions, apples, and berries) can prevent or lessen the disproportionate inflammatory responses typically observed in the lungs of cystic fibrosis patients, and ultimately provide them more years of productive life.

*Kidney disease*

NCCAM-supported researchers are investigating the possibility of using a Traditional Chinese Medicine preparation made from the mushroom Cordyceps sinensis to treat the kidney disorder IgA Nephropathy. This kidney disease stems from an accumulation of deposits of the protein immunoglobulin A (IgA) in the glomeruli of the kidney, preventing proper filtering of the blood and often leading to kidney failure. Conventional treatments for this disorder include corticosteroids, but these can have harmful side effects, leading investigators to explore other options.

*Mucolipidosis*

Lysosomal storage diseases, such as mucolipidosis type IV, Tay-Sachs, and Niemann-Pick, are inherited metabolic disorders marked by the accumulation of excess lipids and carbohydrates in the lysosomes of cells, resulting in abnormal swelling and organ damage in affected individuals. Therapeutic strategies for lysosomal diseases are very limited, but NCCAM-funded investigators are studying the effects of dietary compounds and natural products on the functioning of lysosomes and their ability to remove excess lipids and carbohydrates from the cells of patients suffering from mucolipidosis type IV and other related diseases.

*Liver disease*

S-adenosylmethionine (SAMe) is a naturally occurring compound that is also available as a dietary supplement. Investigators studying liver function have established that SAMe plays an essential role in regulating hepatocyte cell growth and death. In particular, while a reduction in hepatic SAMe levels generally facilitates liver regeneration, prolonged low levels of SAMe can lead to liver injury, non-alcoholic steatohepatitis (also known as non-alcoholic fatty liver disease) and possibly even liver cancer. Researchers supported by NCCAM are building on these findings by continuing to explore the role of SAMe in liver biology and pathology and its potential value as a therapeutic agent.

In addition, NCCAM and the National Institute of Diabetes and Digestive and Kidney Diseases issued a Request for Applications in FY 2005 for Phase I/II clinical trials of silymarin, an extract from milk thistle plant seeds. The aim of these trials is to determine the efficacy and optimal dose of silymarin in treating non-alcoholic steatohepatitis and chronic hepatitis C, either in conjunction with – or as an alternative to – standard therapies.

## NATIONAL INSTITUTES OF HEALTH CLINICAL CENTER (CC)

### Overview of Rare Diseases Research Activities

The NIH Clinical Center serves as the nation's premier research hospital for conducting clinical research to improve the health of human kind. It also serves as a national resource for clinical research by developing diagnostic and therapeutic interventions, enhancing systems to ensure the safe, efficient, and ethical conduct of clinical research, training clinical researchers, and leading the response to the nation's public health needs. As the nation's clinical research center, the NIH Clinical Center is dedicated to improving human health by providing an outstanding environment that facilitates development of diagnostic and therapeutic interventions; training of clinical researchers; and development of processes to ensure the safe, efficient, and ethical conduct of clinical research.

The Clinical Center achieves this mission through a culture that fosters collaboration, innovation, diversity, and the highest ethical standards. About a thousand clinical research studies are under way at the Clinical Center, most of them sponsored by the Institutes and Centers at NIH. These different institutes, centers, and offices study diseases such as rare cancers, rare heart diseases, or rare eye and dental problems, and rare nerve diseases, to name just a few. This research on rare diseases is subsumed under the individual institute, center or office chapters elsewhere in this report in this report.

Natural history studies, often in patients with rare diseases, make up about half of the studies. Understanding the basis for rare diseases often leads to new approaches to common problems. Most of the other clinical research studies are the early (Phase 1 and 2) trials that are the first applications of basic, bench-side research into new treatments and therapies in people. These studies can also be accessed by the public in a database of studies at http://clinicalstudies.info.nih.gov/ for details.

The Clinical Center not only provides support for the various institutes and centers at NIH that conduct intramural research projects at the Clinical center facility in Bethesda, Maryland; the Clinical Center also has its own research projects, with staff members serving as Principal Investigators. While most studies focus on more basic or broader issues than rare diseases *per se*, two Clinical Center investigators currently are conducting significant work on rare diseases. Mark Gladwin, M.D., Critical Care Medicine Department, leads a robust program exploring the prevalence, etiology, and treatment of secondary pulmonary hypertension. This clinical research program is complemented by a basic science program focusing on the pathophysiology and experimental therapeutics of Sickle Cell disease. Margaret Rick, M.D., Department of Laboratory Medicine continues both clinical and laboratory investigation on von Willebrand factor.

*Translational research program in sickle cell disease*

*Scope of sickle cell disease*: n the United States, there are 50,000 individuals with sickle cell disease (SS hemoglobin). One of every 650 African Americans (0.15percent) is born with sickle cell disease, and about 8 percent are heterozygous for the sickle cell gene. According to the

United States Census Bureau, there were approximately 35,509,000 African Americans in the year 2000 which would indicate that there are approximately 50,000 patients with sickle cell anemia in this country.

*Mortality and pulmonary disease*: The median age at death for patients with sickle cell disease is 42 years for men and 48 years for women (Platt, 1994). Pulmonary complications account for a large proportion of deaths among adults with sickle cell anemia. According to the Cooperative Study of Sickle Cell Disease (CSSCD), in a prospective multicenter study of 3,764 patients, over 20 percent of adults likely had fatal pulmonary complications of sickle cell anemia (Platt, 1994). Acute and chronic pulmonary complications of sickle cell anemia, such as pulmonary hypertension, pulmonary fibrosis, and asthma, are common but often under-appreciated by health-care providers.

*Scope of current intramural program*: Over the last four years, investigators have created a unified consortium of CC, NIDDK, and NHLBI intramural investigators and created one of the largest sickle cell disease translational research programs in the country and one of the largest minority research programs in the intramural NIH. The program has enrolled over 200 patients with sickle cell anemia and over 100 control subjects (48 African Americans) into six studies. The largest study explores the prevalence, etiology, and treatment of secondary pulmonary hypertension, a leading cause of adult mortality in patients with sickle cell disease. This clinical program is complemented by a basic science program, which is extremely well published in areas of pathophysiology and experimental therapeutics.

*Accomplishment*

Researchers are working on the characterization of the role of nitric oxide in the pathogenesis and treatment of sickle cell disease; have undertaken studies in both transgenic murine models and in humans with sickle cell disease; have identified several potential novel therapeutic approaches based on laboratory investigations; and have continued on characterizing the emerging syndrome of hemolytic anemia-associated secondary pulmonary hypertension.

Characterizing the emerging syndrome of hemolytic anemia-associated secondary pulmonary hypertension:

*Future Goals*

Pulmonary hypertension screening study: Determine prevalence, etiology, and diagnostic accuracy of echocardiogram including prospectively determine the prognosis of pulmonary hypertension and identifying hemoxygenase/VCAM-1 candidate gene polymorphisms.

## NATIONAL LIBRARY OF MEDICINE (NLM)

### Overview of Rare Disease Research Activities

The National Library of Medicine provides information resources useful to rare disease research and to those seeking information about conditions that affect them or their families.

### Database Resources

- Citations to articles on rare diseases have long been available in the MEDLINE database, now accessible to researchers, health professionals, and the public through NLM's free Web-based PubMed system, and also in the TOXNET system. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi and http://toxnet.nlm.nih.gov/.

- MedlinePlus, NLM's consumer health information service, has a general rare diseases page, which has been effective in referring members of the public to the NIH Office of Rare Diseases at http://www.nlm.nih.gov/medlineplus/rarediseases.html, and was accessed 13,097 times during FY 2005. MedlinePlus also incorporates links on health topic pages to Genetics Home Reference, a new NLM database which includes many rare diseases. Currently there are 174 links from MedlinePlus to Genetics Home Reference topics such as amyotrophic lateral sclerosis, Gaucher disease, and Marfan syndrome. In addition, MedlinePlus has continued to add topics on specific rare diseases requested by consumers; examples during FY 2005 included Lewy body disease, Mesothelioma, and Childhood Brain Tumors, as well as a related page on Newborn Screening. MedlinePlus also acquired new interactive health tutorials about the rare diseases Duchenne muscular dystrophy and phenylketonuria.

- The Genetics Home Reference (GHR) is the NLM's Web site for consumer information about genetic conditions and the genes and chromosomes responsible for those conditions. GHR's integrated Web-based approach provides brief, consumer-friendly summaries of genetic conditions and related genes and chromosomes. Understanding is enhanced by direct links to glossary definitions and a handbook called "Help Me Understand Genetics" that explains fundamental genetic concepts. Additional links to consumer information from MedlinePlus, applicable clinical trials, and relevant patient support groups are provided. Each summary also includes links to advanced information from the NLM and other authoritative sources. GHR currently offers summaries for more than 168 genetic conditions, including numerous rare diseases and disorders; 263 genes related to these conditions; and each of the 23 pairs of human chromosomes. GHR's content includes information for all the rare metabolic disorders listed in the Health Resources and Services Administration's recommended core-testing panel for newborn screening. New content is added and updated on a regular basis, and reviewed by experts in human genetics. http://ghr.nlm.nih.gov/.

- ClinicalTrials.gov, the NLM consumer health information system for linking patients to medical research, currently includes over 25,500 studies. Of these, about 9,460 represent

approximately 970 rare disease conditions. Also, since FY 2003, study records in ClinicalTrials.gov, including those investigating rare diseases, were linked to relevant genetic condition summaries in Genetic Home Reference. Such links provide consumers seeking information about clinical trials with additional background about the conditions and the genes responsible for the conditions. http://clinicaltrials.gov/ and http://ghr.nlm.nih.gov/

**Research Support**

- NLM, working in partnership with organizations in Africa, the United States, the United Kingdom and Europe, has created MIMCom.Net, the first electronic malaria research network in the world. Using satellite technology, the network provides full access to the Internet and the resources of the World Wide Web, as well as access to current medical literature, for scientists working in Africa. The African research sites are of recognized high quality, require improved communications to accomplish ongoing research, and have the necessary resources to purchase equipment and sustain the system. http://www.mimcom.net/

- NLM is assisting NCRR in establishing the NIH-funded Rare Disease Clinical Research Network as an early test-bed for the use of standard clinical vocabularies to improve the efficiency of clinical research.

- A Multicenter Clinical Trial Using Next Generation Internet (NGI) Technology. NGI technology was applied to provide the infrastructure of a multicenter clinical trial of new therapies for adrenoleukodystrophy (ALD), a fatal neurologic genetic disorder. This project involved the formation of a worldwide imaging network of clinical institutions to evaluate ALD therapies. This network was required to provide a sufficient number of patients for evaluating ALD therapies. This can serve as a model for many other disorders. Three centers collaborated on this project. The Imaging Science and Information Systems (ISIS) Center at Georgetown University Medical Center, the Kennedy Krieger Institute and the Department of Radiology at Johns Hopkins University. NGI technology was used to speed the transmission and evaluation of high quality MRI images. Another important feature of this project was to gain insight into procedures that ensure medical data privacy and security. http://www.nlm.nih.gov/research/ngisumphase2.html

**Grants**

- Kawasaki disease (KD) is an acute, self-limited illness of infancy and early childhood that has now replaced rheumatic fever as the leading cause of acquired heart disease in children in the United States and Japan. Although the acute illness resolves spontaneously, permanent damage to the coronary arteries occurs in 20-25 percent of untreated children. The cause of KD remains unknown and there is no specific laboratory test to identify affected children. Nonetheless, an effective treatment exists that significantly reduces the risk of coronary artery damage. KD thus presents a unique dilemma: the disease may be difficult to recognize, there is no diagnostic laboratory test,

there is an extremely effective therapy, and there is a 25 percent chance of serious cardiovascular damage or death if the therapy is not administered. NLM is funding a publications grant to the Kawasaki Disease Foundation to support the continued collaboration of an unusual multidisciplinary team with expertise in documentary film making, parent advocacy, pediatric medicine, anthropology, and the history of medicine to produce a Web-based archive of interviews and a television documentary to increase public awareness of KD and to support scholarly research on the origins of this emerging pediatric disease. Funds from this application will support three major interviewing sessions in Japan, Hawaii, and San Diego. The film will focus on 1) the importance of informed parents in establishing the timely diagnosis of KD, which permits effective treatment and prevention of complications and 2) the history of KD, showing that the ways in which it emerged as an internationally recognized disease mirror the ways in which it is now diagnosed or misdiagnosed in our contemporary health care system. In the case of KD, informed parent advocacy can mean the difference between life and death for an affected child.

- Hepatitis B is the world's most common serious liver infection, and is associated with more than 80 percent of liver cancer worldwide. Those that are affected are in urgent need of information to help them to successfully deal with their disease and maintain a high quality of life. The long-range goal of this information system grant to the Hepatitis Foundation is to provide information and support to the millions of individuals worldwide that are affected by hepatitis B. In particular, this project is developing a comprehensive hepatitis B Web site to bring high-quality health information to consumer users and health care professionals. The creation of this "virtual community" includes high-technology features such as an e-newsletter and expert forums, delivered in a user-friendly package. Additionally, Web site content and features will be personalized in response to the specific needs and preferences of the target audiences. This project also focuses on creating an important connection between information seekers and the National Library of Medicine databases, including MEDLINE, MedlinePlus, and ClinicalTrials.gov. The Web site will be evaluated throughout the three-year timeline, to incorporate user-feedback into the development process, to track Web site usage statistics, and to determine the impact of the Web site on the targeted users. Ultimately, the completed Web site can be utilized as a model for the interactive dissemination of high-quality health information through the Internet.

**Rare Disease Research Initiatives**

The National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM), serves as a national public resource for molecular biology information. In this capacity, NCBI establishes and maintains various genomic databases and develops software tools for mining and analyzing this data, all of which is freely available to the biomedical community to support research into the processes affecting human health and disease.

*The Human Genetic Map*

NCBI is responsible for collecting, managing, and analyzing the growing body of data being generated from the sequencing and mapping initiative of the Human Genome Project. NCBI makes the sequence of the entire human genome, with its complement of over 28,000 known and predicted genes, available without restriction to the research community and to the general public. This unrestricted access has expedited the decoding by the scientific community of important human genes, and as a result, scientists are beginning to understand the causes of many rare diseases. Access to the complete human genome and the related genetic data at NCBI helps scientists determine the organization of the genes on a chromosome, study how these genes produce their protein products, investigate how changes in a gene's DNA sequence give rise to a disease-causing mutation, and study how chromosomes are duplicated and inherited. Scientists have used these strategies to study gene defects on chromosomes 21 and 22 that lead to a variety of rare diseases, including Downs syndrome, Usher syndrome, DiGeorge syndrome, and Ewing's sarcoma. NCBI investigators have also played an instrumental role in the identification and analysis of other disease genes and genetic loci, and have analyzed genetic data leading to scientific advances in the understanding of several rare diseases and disorders, such as the identification and analysis of the genes for Kallmann syndrome and neurofibromatosis (NF1). Examples of the many rare diseases currently being studied by NCBI investigators include ataxia telangiectasia, breast cancer, hyper-IgE syndrome, nemaline myopathy, obesity, Fukuyama's muscular dystrophy, type II xanthinuria, xeroderma pigmentosum, kyphoscoliosis, sporadic hyperekplexia, autosomal-recessive polycystic kidney and hepatic disease, Imerslud-Grasbeck syndrome, amish microcephaly, common variable immudeficiency (CVID), nasopharyngeal carcinoma, and autoimmune lymphoproliferative syndrome (ALPS).

*Genetic analysis software*

NCBI investigators are working to develop, implement, and disseminate high performance computational tools and application software packages for the analysis of genetic data and its linkage to disease. Several of these software packages are described below.

FASTLINK is a computer program designed to analyze the associations between genes and genetic markers that lie near each other on a chromosome, a process called "genetic linkage analysis". Genes and other genetic markers that are linked are often inherited together and, therefore, can be used to map the location of a disease gene. NCBI scientists have used FASTLINK to study hyper-IgE syndrome, a rare immunodeficiency characterized by recurrent skin abscesses, pneumonia, and highly elevated levels of serum IgE. Using FASTLINK, researchers were able to find evidence linking this syndrome to chromosome 4. FASTLINK has been cited in over 400 other published genetic studies, including studies of macular dystrophy, type 1 hereditary sensory neuropathy, and Alstrom’s syndrome.

CASPAR (Computerized Affected Sibling Pair Analyzer and Reporter) is a computer program designed at NCBI to study the genetics of complex diseases, or diseases involving the interaction of multiple genes. It allows a scientist to explore various hypotheses about how different factors may be involved in disease susceptibility. NCBI investigators have used CASPAR to study linkage analysis in patients with a form of diabetes.

The PedHunter computer program was developed to query genealogical databases to uncover connections between relatives that are afflicted with the same disease and to construct a pedigree suitable for genetic linkage analysis. NCBI scientists are using PedHunter to query the Amish Genealogy database to collect information on various genetic diseases, including nemaline myopathy, a rare genetic neuromuscular disorder that is usually apparent at birth and is characterized by extreme muscle weakness. Using PedHunter, in combination with other genetic analysis software, NCBI investigators have demonstrated that, in the Amish, this disorder is caused by a mutation in the gene for the sarcomeric thin-filament protein, slow skeletal muscle troponin T (TNNT1). TNNT1 maps to chromosome 19 and has been previously sequenced. Further analysis resulted in the identification of a stop codon that segregated with the disease. Researchers concluded that Amish nemaline myopathy is a distinct, heritable, myopathic disorder caused by a mutation in TNNT1.

The Comparative Genomic Hybridization (CGH) analysis software package is being used by NCBI investigators for modeling the process of tumor formation in various forms of cancer. The function of the software is to develop models that relate genetic aberrations with tumor progression. Investigators have used CGH as part of a larger project to search for and identify possible susceptibility loci involved in both breast and bladder cancer. Investigators have also published the results of a case study in which CGH was used to analyze chromosomal abnormalities in a large collection of ovarian cancer samples.

*Three-Dimensional Structure Database*

NCBI's Structure Research Group maintains a database of experimentally determined three-dimensional biomolecular structures, as well as tools for visualizing and analyzing these structures. Three-dimensional structures provide a wealth of information on the biological function of a molecule, on mechanisms linked to function, and on evolutionary history of and relationships between macromolecules; all valuable clues leading to a better understanding of rare diseases.
For example, in 1995, the structure of leptin-the protein coded within a gene linked to obesity and diabetes-was predicted by NCBI investigators using the structure database. After the discovery of leptin, researchers analyzed the protein's sequence and determined that it exhibited no similarities to other known proteins. NCBI investigators hypothesized that leptin was ancestrally related to at least one other protein whose sequence had diverged such that only a comparison of three-dimensional structures might detect a relationship. Investigators conducted a search of the database to determine whether this protein might adopt a similar fold pattern, or structure, to that of a protein structure already stored in the database. They discovered that leptin's sequence was compatible with the structure of a family of known proteins and predicted a structural model based on these results. Subsequently, this early prediction was confirmed by cloning of the protein's receptor, and more recently, by X-ray structure determination. Now that the structure of leptin has been confirmed, future studies of leptin, as well as other leptin-regulated genes, may reveal the mechanisms by which leptin exerts its effect on the body.

*Malaria genetics and genomics*

Human malaria is caused by four Plasmodium parasites, P. falciparum, P. vivax, P. ovale, and P. malariae. Although P. vivax is widespread, P. falciparum is the most severe and lethal tropical parasite, leading to an estimated 1-2 million deaths each year, mostly of children in Africa.

The resurgence of malaria in recent years is mainly attributed to the emergence and spread of multiple-drug-resistant parasites and insecticide-resistant mosquito vectors, presenting a serious problem for travelers and the military in malaria-endemic regions as well as for the resident populations. Accordingly, much research at the NIH focuses on the treatment and prevention of malaria, which is a curable disease if promptly diagnosed and adequately treated.
The NCBI, in collaboration with the NIAID, has supported the efforts to sequence and analyze the complete genome of P. falciparum and related parasites, thereby providing researchers with access to information relating to all of the genes found in these parasites. Analyses of these genomic data by NCBI researchers are contributing to enhanced understanding of this complex disease and attempts to develop improved anti-malarial drugs, vaccines, and other control strategies. Moreover, a collaborative team of NIH investigators, including researchers from the NCBI, have constructed a genome-wide, high-resolution genetic linkage map of P. falciparum. The computer analyses based on these genetic parameters and markers have facilitated genome sequence assembly and are currently helping to define the genes involved in parasite resistance to multiple drugs and to trace the evolution and spread of these genes in parasite field populations in Africa, Asia and the Americas.

NCBI's Malaria Genetics and Genomics Web page serves as an information and data resource covering Plasmodium and related parasites, including rodent malarias, and also the Anopheles gambiae mosquito vector. This resource includes links to the sequence and genomic data in Entrez Genomes and other NCBI databases, together with unique information on genome maps, linkage markers, genetic studies. Links are provided to various malaria research projects being conducted at NIH and to NIAID's Malaria Research and Reference Reagent Resource Center, and other malaria-related sites.

The NCBI, in collaboration with the WHO's Special Programme for Research and Training in Tropical Diseases (TDR) and other international partners, has continued to support the international outreach efforts to train scientists in developing countries to use current bioinformatics tools and genomic data, such as the mosquito and malaria genome, for their own research. NCBI staff have provided coordination and instruction at several international bioinformatics training courses and centers in Africa, Asia, and South America, including the WHO/TDR sponsored Regional Training Course in Bioinformatics Applied to Tropical Diseases.

*Database of the major histocompatibility complex*

The NCBI dbMHC database provides an open, publicly accessible platform for DNA and clinical data related to the human Major Histocompatibility Complex (MHC). MHC research and clinical data generated at meetings such as the International HLA Workshop and Congress has proven valuable to the international research community. NCBI makes these data available along with tools for submission and analysis of research data linked to the MHC. The dbMHC contains

reagent data used for tracing DNA typing and a section with anonymous clinical data from MHC-related research projects related to diseases such as celiac disease, narcolepsy, ankylosing spondylitis, and hemochromatosis.

*Additional human genome resources*

NCBI makes available a number of other resources to facilitate the widespread use of human sequence data. The Human Genome Resources Web page serves as a focal point for biomedical investigators from around the world enabling them to use this data in their research. From the Human Genome Resources Web page, researchers can access the NCBI Map Viewer, which presents a graphical view of the available human sequence data in conjunction with cytogenetic, genetic, and physical maps. Researchers may quickly search for a gene or a gene marker of interest by querying against the entire human genome. Query results link to a graphical display of the gene or gene marker within the context of additional data. The coupling of the human genome sequence with genetic and physical maps bearing markers associated with disease helps researchers to identify candidate genes for further research. The NCBI Map Viewer also allows the maps and genomic sequences of organisms used in models of human disease, such as mouse and rat, to be viewed alongside the human maps. The ability to compare sets of genomes in this manner allows researchers to use the results obtained in their laboratories with these model organisms to better understand the roots of human disease.

NCBI's Genes and Disease Web page is designed to introduce visitors to the relationship between genetic factors and human disease. Genes and disease provides information for more than 80 genetic diseases, including many rare diseases. A resource for both researchers and the general public, the Online Mendelian Inheritance in Man database, (OMIM), is a continuously updated catalog of inherited human disorders and associated sequence mutations, authored and edited by Dr. Victor A. McKusick and colleagues and developed for the Web by NCBI. OMIM now contains over 15,000 entries for diseases linked to over 9,000 locations on the human genome. Over 2000 records in OMIM are linked to GeneTests, a portal to information on genetic testing and its use in disease diagnosis, disease management, and in genetic counseling. Screens for a wide array of rare diseases are detailed in GeneTests. A few of interest include tests for cystic fibrosis, marfan syndrome, spinal muscular atrophy, hereditary non-polyposis colon cancer, and neurofibromatosis.

One of the primary reasons for sequencing the human genome was to gain an understanding of the role of genes in human disease. By studying the gene sequences associated with a human or model organism disease, researchers can gain important insights into the genetic and environmental basis of disease. The advances outlined here demonstrate the importance and utility of NCBI's computer databases, data analysis tools, and software algorithms in identifying and understanding human disease genes; they will pave the way for the development of novel strategies to diagnose, treat, and ultimately, prevent, disease.

## OFFICE OF RESEARCH ON WOMEN'S HEALTH (ORWH)

### Overview of Rare Disease Activities

The Office of Research on Women's Health (ORWH) was established in 1990 within the Office of the NIH Director. Since its establishment, the ORWH has served as the focal point for women's health research at the NIH to strengthen and enhance research related to diseases, disorders, and conditions that affect women across all the Institutes and Centers. ORWH also ensures that women are appropriately represented in biomedical and biobehavioral research studies supported by the NIH, and develops opportunities for and supports biomedical careers for women and investigators interested in women's health research.

The report, *An Agenda for Research on Women's Health for the 21st Century*, provides the basis for ORWH collaboration with the scientific and advocacy communities to encourage and address interdisciplinary scientific initiatives. Each year, these recommendations are reviewed and revised by an *ad hoc* subcommittee of the Coordinating Committee on Research for Women's Health (CCRWH). This new set of recommendations, once approved by the CCRWH and the Advisory Committee for Research on Women's Health are published and shared with the scientific community. Although the ORWH does not have direct funding authority, it supports these interdisciplinary research initiatives through its partners throughout the appropriate NIH Institutes and Centers.

During fiscal year 2005, ORWH supported research for disorders that might be considered rare diseases totaled $2,340,796. This research consisted of studies on Sjögren's syndrome, systemic lupus erythmatosus (SLE), antiphospholipid antibody syndrome (ASL), age-related macular degeneration (ARM), scleroderma, and interstitial cystitis (IC) and was funded in collaboration with the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Eye Institute (NEI), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the National Institute of Dental and Craniofacial Research (NIDCR). The ORWH is particularly pleased that the major scientific advance to be presented was a product of its own SCOR (Scientific Centers of Research on Sex and Gender Factors influencing Women's Health) program.

### Fiscal Year 2005 Scientific Advances

Urinary tract infections (UTIs) are more common in women than men. The Gram-negative bacterium, Escherichia coli (E. coli), is the most common cause of these infections that account for significant morbidity with frequent recurrences. It has been assumed that recurrent infections are due to re-inoculation of the urinary tract with E. coli. Recent evidence suggests this may be incorrect. Investigators from an ORWH and NIDDK funded Specialized Center of Research on Sex and Gender Factors Affecting Women's Health (SCOR) have discovered that as few as only one bacterium can penetrate the urinary tract mucosa and host defense mechanisms triggering the formation of intracellular bacterial colonies that result in reinfection and recurrent UTIs. Results of this research could revolutionize the way UTIs are prevented, evaluated and treated. Further, this concept may be applicable to infections of other mucosal surfaces (1 P50DK06450).

1. Kau AL, Martin SM, Lyon W, Hayes E, Caparon MG, Hultgren SJ, Enterococcus faecalis tropism for the kidneys in the urinary tract of C57BL/6J mice. Infect. Immun. 73: 2461-2468, 2005.
2. Hunstad DA, Justice SS, Hung CS, Lauer SR, Hultgren SJ, Suppression of bladder epithelial cytokine responses by uropathogenic Escherichia coli. Infect. Immun. 73: 3999-4006, 2005.
3. Kau AL, Hunstad DA, Hultgren SJ, Interaction of uropathogenic Escherichia coli with host uroepithelium. Curr. Opin. Microbiol. 8: 54-59, 2005.

**Fiscal Year 2005 Scientific Activities**

In addition to the scientific advance reported above, ORWH also cofunded with NIDDK on an epidemiological study to develop methods for and estimate the prevalence of interstitial cystitis (IC) in the U.S. This study also plans to develop and field a survey to assess the impact of IC on all aspects of patients' lives (5 U01 DK 070234-02).

The incidence of macular degeneration in older women is a case control study cofunded with the NEI that will provide an accurate estimate of the number of individuals over 80 years of age who suffer with this condition. They will be identified among the population in the Study of Osteoporotic Fractures (SOF) and studied over a 14-year period. Rates of progression in the disease as well as associated risk factors will be also be determined (5 U10 EY13626-04).

ORWH also cofunds two studies of scleroderma with NIAMS. One utilizes a murine model to dissect the molecular pathways involved in the onset of scleroderma with the potential goal of developing therapies to control extracellular matrix metabolism (5 R01 AR48082-05). The other, a translational research project, will apply the well-honed techniques that have identified the peptide-induced model of lupus autoimmunity and a similar scientific strategy to analyze the fine specificity of the anti-nRNP and anti-topoisomerase autoantibodies found in scleroderma. The common humoral epitopes of the anti-nRNP and anti-topoisomerase autoantibodies will be identified; and their development over time, and with treatment, will be described to both understand their roles in the etiology and pathogenesis in scleroderma and primary Raynaud's disease (5 R01 AR48045-05).

A basic study of the mechanisms of antiphospholipid (aPL) antibody-induced pregnancy loss and a clinical study of the predictors of pregnancy outcome in SLE and APS are also cofunded with NIAMS. The basic study uses a murine module of APS to determine how this complement that plays an essential role in fetal loss and growth restriction is activated, which complement products mediate the clinical complications associated the aPL antibody, and what role complement activation has in the overall inflammatory cascade. It also tests the hypothesis that activation of the complement at the maternal-fetal interface plays an etiological role in intrauterine growth restriction (2 R01 AR38889-14). An observational experiment of over 400 pregnant women, grouped and analyzed according to the presence or absence of an aPL and preexisting SLE, is the method through which findings from the murine study are translated to determine whether elevations of split products generated by activation of the complement pathways predict poor fetal outcomes in actual patients with aPL antibodies and/or SLE (5 R01 AR049772-03).

The ORWH also co-funded with NIDCR an international registry network for Sjögren's syndrome that will develop standardized criteria to identify patients; collect, process, store, analyze and ship data from patients and their families; and disseminate to researchers the clinical and biological specimens from these patients with Sjögren's syndrome (N01 DE32636).

**New/Planned Project**

The ORWH is committed to seeing these projects through completion and will continue to plan and encourage new research proposals and activities on rare diseases in collaboration with the NIH Institutes and Centers.

## OFFICE OF TECHNOLOGY TRANSFER (OTT)

### Overview

The NIH Office of Technology Transfer evaluates, protects, monitors, and manages the wide range of NIH and FDA discoveries, inventions, and other intellectual property as mandated by the Federal Technology Transfer Act and related legislation.

To accomplish its mission, OTT oversees patent prosecution and negotiates and monitors licensing agreements. OTT performs similar functions for patenting and licensing activities for the Food and Drug Administration (FDA), another component of the Department of Health and Human Services (HHS).

Other major functions within OTT include the development of technology transfer policies for NIH and with the other two major research components of HHS (FDA and the Centers for Disease Control and Prevention [CDC]) and the implementation of decisions by the Technology Transfer Policy Board.

### Overview of Rare Diseases Activities

**Vaccines Against Crimean-Congo Hemorrhagic Fever**
Dimiter Dimitrov and Xiadong Xiao (NCI)
U.S. Provisional Patent Application filed 03 Nov 2004 (DHHS Reference Nos. E-299-2004/0-US-01 and E-299-2004/1-US-01)

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne public health concern in many regions of the world including Africa, the Middle East, Europe, and Western Asia. The disease is etiologically linked to Crimean-Congo hemorrhagic fever virus (CCHFV) from the Nairovirus genus of the Bunyaviridae family of viruses and is transmitted primarily through the bite of Ixodid ticks. Available for licensing and commercial development are antigens, immunogens, and nucleic acid constructs for the development of vaccines against CCHFV. The antigens and immunogens are peptides corresponding to the soluble ectodomains of CCHFV G1 (Gc) and G2 (Gn) glycoproteins. Also provided are coupled proteins that include soluble peptide fragments derived from the G1 (Gc) or G2 (Gn) ectodomains or portions thereof; peptidomimetics; vaccines; immunogenic compounds methods for vaccination and inhibitors of CCHFV cell entry. Expression vectors and DNA vaccines encoding these peptides are also within the scope of the invention as well as antibodies, aptamers and kits containing antibodies or aptamers that bind to these peptides. CCHFV has been implicated as a pathogen of biodefense significance.

The patent rights to the CCHFV vaccines have been abandoned; however, OTT is licensing the anti-Gc and anti-Gn antibodies that the inventor generated to Chemicon for sale as reagents and for research services.

**Human Monoclonal Antibodies against Hendra and Nipah Viruses**
Dimiter S. Dimitrov et al. (NCI)
U.S. Provisional Application filed 01 Nov 2004 (DHHS Reference No. E-004-2005/0-US-01); Related to the Phage Display Library described in DHHS Reference No. E-005-2005/0

Available for licensing are neutralizing human monoclonal antibodies against the envelope proteins (Envs) of Hendra virus (HeV) and Nipah virus (NiV) for uses in immunotherapy, vaccine development and as diagnostic or research reagents. Monoclonal antibody variable region fragments (Fabs and scFvs) have been isolated from screening a human phage display library against the Envs. The phage display library (DHHS Ref. No. E-005-2005) is useful for screening other viral or cancer antigens and can be licensed from HHS under a biological materials license.

The patent rights in Hendra and Nipah Virus were scheduled for abandonment until the Henry Jackson Foundation (co-inventor/co-owner) expressed some interest in assuming prosecution of the case. OTT, therefore, filed a PCT application preserving the rights until a decision is reached. Thus far, this invention is unlicensed.

**Mesothelium Antigen and Methods and Kits for Targeting It**
Ira Pastan et al. (NCI)
The related technology includes PCT Patent Application No. PCT/US97/00224, filed January 3, 1997, entitled “Mesothelium Antigen and Methods and Kits for Targeting It” [E-002-1996/0].

CAK1, or "mesothelin", is an antigen present on the cell surface in mesothelium and on many mesotheliomas and ovarian cancers. While the role of this differentiation antigen has not yet been determined, it is postulated that it may be implicated in adhesion and in the dissemination of mesotheliomas and of ovarian cancers. CAK1, therefore, is a potential target for monoclonal antibodies to be used in the diagnosis and treatment of these cancers. The gene for CAK1 has been cloned and sequenced, as embodied in the current invention. The invention, therefore, should provide a valuable research tool for use in the development of diagnostics and/or therapeutic agents toward mesotheliomas and ovarian cancers.

There are two main licenses for this technology: a) Morphotek (L-032-2006/0) and b) Enzon (L-153-2004/0). Both licenses include other uses (ovarian, pancreatic, etc.) in addition to mesothelioma.

**Anthrax**
Klinman et al.
E-184-2002, "CpG Oligonucleotides (ODN) to Improve Host Resistance to Anthrax", US and foreign rights pending, licensed.

Sternberg et al.
E-247-2002, "Anthrax Lethal Factor Represses the Glucocorticoid Receptor And...", US and foreign rights pending, not licensed.

Kopecko et al.
E-345-2003, "Vaccine for Protection against the Bioterrorist Threat Agent Anthrax", US and foreign rights pending, not licensed.

Klinman et al.
E-302-2005, "Immunogenic Compositions Containing Anthrax Antigen . . .", US and foreign rights pending, not licensed.

Shiloach et al.
E-023-2002, "Methods for Preparing Bacillus Anthracis Protective Antigen for use . . .", US rights pending, licensed.

Robbins et al.
E-343-2002, "Poly-gamma-D-Glutamic Acid Capsule (PGA)-Bacillus Anthracis Protective Antigen . . .", US and foreign rights pending, licensed.

Robbins et al.E-040-2005, "Addtional Methods for Preparing Conjugates of Poly-gamma-D-Glutamic Acid Capsule (PGA)-Bacillus Anthracis . . ." US and foreign rights pending, licensed.

**Cholera O139**
Robbins et al.
E-274-2000, "Vibrio Cholerae O139 Conjugate Vaccines", US patent rights pending, not licensed,

**Dengue**
Murphy et al.
E-089-2002, "Dengue Tetravalent Vaccine Containing a Common 30 Nucleotide Deletion . . .", US and foreign rights pending, licensed.

Murphy et al.
E-120-2001, "Development of Mutations Useful for Attenuating . . .", US and foreign rights pending, licensed.

Lai et al.
E-171-1988/E-170-1991, "Chimeric Dengue Viruses", US and foreign rights pending, licensed.

Zeng et al.
E-067-1998, "Replication-Defective Dengue Viruses that are Replication-Deficient . . .", US and foreign rights pending, not licensed.

Pang et al.
E-228-2000, "Subgenomic Replicons of Flavivirus Dengue", US and foreign rights pending, not licensed.

**E. Coli O157**

Robbins et al.
E-158-1998, "Vaccine against Escherichia Coli O157 Infection", US and foreign rights pending, licensed for veterinary use to Fort Dodge Animal Health,

**Lyme Disease**

Robbins et al.
E-107-2003, "A New Vaccine for Lyme Disease (Borrelia Burgdorferi)", US and foreign rights pending, not licensed.

**Tick-Borne Encephalitis Virus (TBEV)**

Pletnev et al.
E-221-1997, "Chimeric Vaccine against Tick-Borne Encephalitis Virus", US and foreign rights pending, not licensed.

Pletnev et al.
E-281-1998, "Full-Length Infectious cDNA Clones of Tick Borne Flavivirus", US and foreign rights pending, not licensed.

**West Nile Virus (WNV)**

Murphy et al.
E-357-2001, "Construction of West Nile Virus and Dengue Virus Chimeras for Use in a Live Virus Vaccine to Prevent Disease Caused by West Nile Virus", US and foreign rights pending, licensed.

Liang et al.
E-352-2003, "Expression of Structural Proteins of West Nile Virus in Insect Cells", US and foreign rights pending, licensed.

Markoff et al.
E-022-2004, "West Nile Viruses with Mutations In The 3' Terminal Stern and Loop Secondary Structure for Use as Live Virus Vaccine", US and foreign rights pending, licensed.

**West Nile Virus**

Provisional Patent application USSN 60/667,064 (E-164-2005), filed April 1, 2005, for technology from the VRC; jointly owned with CRADA partner Vical Incorporated
Vical declined to exercise exclusive license option (has non-exclusive rights through co-ownership) in FY 2006. OTT proposed entering into patent cost sharing agreement and currently waiting for response.

OTT will market this technology in near future.

**Neisseria Meningitidis**
U.S. Patent No. 6,531,131 issued March 11, 2003 for conjugate vaccine technology
Received licensing interest in May 2005 from Sanofi Aventis. Due to turnover in personnel at Sanofi, however, no response to a draft license agreement sent in June 2005 has been received to date. OTT actively following up on monthly basis.

**Bordatella pertussis**
Technology from NIDCR
U.S. 371 application Serial Number 10/312,272 (E-159-1999/0-US-03), filed December 20, 2002, related to B. bronchiseptica strain that produced B. pertussis toxin in high yield and without filamentous hemaglutinin. Patent prosecution on going.

Technology licensed to Serum Institute of India during FY 2005. Previously licensed to 3 other Indian companies.

Received license application from Saskatchewan Research Council in FY 2006, who intends to use technology in performance of a contract for a third party developing a pertussis vaccine for the developing world. SRC still waiting for response from their third party before moving ahead with license agreement with NIH.

**Ebola and Marburg viruses**
Filed four provisional patent applications related to point mutations in genes encoding the glycoproteins of Ebola and Marburg viruses and use of these genes in plasmids and other vectors (e.g. adenoviral vector) as vaccines from the VRC:
60/613,883 (E-319-2004/0-US-01), filed September 27, 2004
60/677,606 (E-319-2004/1-US-01), filed May 3, 2005
60/679,767 (E-198-2005/0-US-01), filed May 10, 2005
60/701,694 (E-284-2005/0-US-01), filed July 22, 2005

Co-owned with CRADA partner Crucell; determined to be a CRADA subject invention; OTT currently working with contract law firm and Crucell inventors to determine Crucell inventors' contribution

Above applications combined into single provisional patent application, Serial number 60/715,874, filed Sept. 9, 2005; PCT application filed from provisional application on Sept. 27, 2005

Invention non-exclusively to Crucell; will be converting to exclusive.
OTT and Crucell executed exclusive license (L-093-2005/0, effective March 16, 2005) for Ebola, Marburg and Lassa vaccine technologies from the VRC: E-241-2001/0, E-246-2001/0, E-090-2002/0,1, E-317-2003/0, E-319-2004/0 (non-exclusive), Field of use: Ebola, Marburg, or Lassa vaccine in which at least one component is adenoviral based

**Lassa Virus**
Exclusive license to Crucell for Lassa technology from the VRC as noted above

**Botulinum**
Inventors at the NCI
EIR E-276-2005, entitled "Novel Small Molecule Inhibitors of Botulinum Neurotoxin Metalloprotease Activity"; Technology co-owned with USAMRIID, who is taking lead in patent filing and prosecution as well as licensing.

USAMRIID has a company interested in an exclusive license for this technology, among others.

**Epstein-Barr Virus**
Jeffrey Cohen et al. (NIAID)
E-312-2003, entitled "Use of Statins to Kill EBV-Transformed B Cells" PCT application pending, approaching National Stage filing deadline (April 28, 2006)

There are EBV associated tumors that meet the "rare diseases" definition such as nasopharyngeal carcinoma, Hodgkin's disease, non-Hodgkin's lymphoma, and T-cell lymphoma

Following abstract published in Federal Register in September 2005 (2nd advertisement):
Methods for Treating Viral-Associated Tumors with LFA-1 Inhibiting Statins
U.S. Provisional Application No. 60/515,013 filed 28 Oct 2003 (HHS Reference No. E-312-2003/0-US-01); PCT Application No. PCT/US2004/035829 (publication WO2005/042710) filed 28 Oct 2004 (HHS Reference No. E-312-2003/0-PCT-02)

This technology describes the use of certain natural and synthetic statins, including simvastatin, other leukocyte function antigen-1 (LFA-1) inhibiting statins, and compounds derived from LFA-1 inhibiting statins and statin-like compounds, for treatment or prevention of Epstein-Barr Virus (EBV) associated tumors, including lymphomas that express LFA-1 and transforming proteins. Such compounds could also be used to treat tumors associated with other viruses that express LFA-1. Cancers associated with EBV that could be treated with the statins by methods described herein include gastric carcinoma (the second leading cause of cancer deaths worldwide), nasopharyngeal carcinoma, Hodgkin's disease, lymphoproliferative disease, T-cell lymphoma, and non-Hodgkin's lymphoma. These compounds could potentially be used as chemotherapeutics with possibly less severe side effects than currently employed chemotherapies.

This technology is further described in Katano et al., "Simvastatin induces apoptosis of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines and delays development of EBV lymphomas," PNAS 2004 Apr 6, 101(14:4966-4971, doi 10.1073/pnas.0401064101.

In addition to licensing, the technology is available for further development through collaborative research opportunities with the inventors.

OTT directly marketed technology in FY 2005 to companies with statin products or research programs (Merck, Bristol-Myers Squibb, Pfizer, Genentech, Novartis, ICOS Pharmaceuticals (no response), KOS Pharmaceuticals, Schering-Plough, Andrx Pharmaceuticals, Roche, Boehringer-

Ingelheim (no response), New Horizon Pharmaceuticals (no response), Takeda Pharmaceuticals (no response), and Astellas (no response)). All declined to license except as noted.

**Lyme diagnostic**

Received invention report E-075-2006 for a hybridoma cell line producing an anti-Lyme antibody. The EIR was submitted after the inventor received a request from Bio-Rad regarding obtaining the materials.

Initial contact has been made with the company, who indicated they have another source of the antibody but are exploring options. OTT responded expressing a desire to become the source of materials and willingness to negotiate accordingly.

**Genes Coding For Melanoma Tumor Antigens**

Kawakami, Y., Rosenberg, S.A. (NCI)
Filed 22 Apr 94, Serial No. 08/231,565, U.S. Patent 5,844,075 issued 10 Dec 1998

The technology was designated by the FDA for treatment of an orphan disease. The invention encompasses various GP100 peptides that are currently being used in combination with a Mederax and a Bristol-Myers Squibb (BMS) proprietary antibody to treat stage III and IV melanomas. GP100 is a tumor specific melanoma antigen that has been shown to be successful in stimulating the immune response to melanoma in humans.

Genes have been isolated that code for melanoma tumor antigens, which may provide an important new method for the prevention or treatment of this deadly form of cancer. Melanomas are aggressive, frequently metastatic tumors, and even when the melanoma is apparently localized to the skin, up to 30 percent of patients eventually will have the tumor spread to other organs and tissues of the body. The majority of these individuals will die. Recent studies have shown that many melanoma patients mount cellular and humoral responses against these tumors and that melanomas express both MHC antigens and tumor-associated antigens. These newly discovered melanoma antigen genes may be used in gene therapy protocols, or peptides derived from the gene product may be used in vaccines to help the recipient mount a T-cell-mediated immune response against the melanoma.

DHHS announces the availability of select gp100 cancer antigens for licensing. These antigens are composed of a class that fall under the following definition: gp100 P Core Peptide(s), meaning any gp100 peptide of nine (9) to fifteen (15) amino acids in length which is capable of eliciting an HLA-A2.1-restricted cytotoxic T cell response, and which comprises the formula X1X2X3PGPX5TX4, where X1 is any naturally occurring amino acid, X2 is any hydrophobic aliphatic amino acid; X3 is any naturally occurring amino acid; X4 is any hydrophobic aliphatic amino acid, and X5 is the amino acid V, C, I, L, or M.

The two license application numbers covered under the IND filing are with BMS and Medarex. Medarex is in late stage clinical trials.
(See BMS-Mederax press release for the clinical trials
http://www.bms.com/news/press/data/fg_press_release_5531.html)

**Treatment of Cardiovascular Conditions with Nitrite Therapy**
M. Gladwin (CC), R. Cannon (NHLBI), A. Schechter (NIDDK), C. Hunter (CC), R. Pluta (NINDS), E. Oldfield (NINDS) et al.

PCT Applications filed 09 Jul 2004 (priority date 9 July 2003): PCT/US04/21985, International Publication No. WO 2005/007173, Publication Date 27 January 2005 [DHHS Reference No. E-254-2003/2-PCT-01] and PCT/US04/22232, International Publication No. WO 2005/004884, Publication Date 20 January 2005 [E-254-2003/3-PCT-01].

A wide variety of disease indications, including cardiovascular and respiratory diseases, have been treated by different therapeutic classes of compounds that are able to increase blood flow and act as vasodilators. Endothelium-derived factors, such as nitric oxide (NO), play a crucial role in the maintenance of vascular homeostasis and NO-enhancing compounds have been administered alone or in combination with an approved pharmaceutical agent in order to provide an effective therapeutic treatment. However, many of these therapies are very costly and there remains a strong need for an affordable treatment. Recent scientific work by the inventors provided the first evidence that the anion nitrite represents a circulating and tissue storage form of nitric oxide whose bioactivation is mediated by the nitrite reductase activity of deoxyhemoglobin (Nature Medicine 2003 9: 1498-1505).

NIH scientists and their collaborators have now shown that low, physiological and non-toxic concentrations of sodium nitrite are able to increase blood flow and produce vasodilation by infused and nebulised routes of administration. Proof of concept data has been obtained in animal models for myocardial and hepatic ischemia and reperfusion injury, in a neonate lamb model for neonatal pulmonary hypertension, and in a primate model for control of delayed cerebral vasospasm following sub-arachnoid haemorrhage. The implications of these results point to the use of nitrite as a potential cost-effective platform therapy for a wide variety of disease indications characterized broadly by constricted blood flow or hypoxia.

Subarachnoid hemorrhage affects approximately 30, 000 individuals/year, of the approximate 50percent who survive the initial hemorrhage an estimated half will develop delayed cerebral vasospasm. However, DCV is not listed as a rare disease on the rare disease Web site.

**Therapeutic Application of Intra-Vascular Nitrite for Sickle Cell Disease**
Mark Gladwin, M.D., CCMD/CC, Richard Cannon, M.D., NHLBI, Gregory Kato, M.D., CCMD/CC, Kyle Mack, M.D., CCMD/CC, Alan Schechter, M.D., NIDDK, Xunde Wang, Ph.D., CCMD/CC, Rong-Fong Shell, Ph.D., NHLBI, Angel Aponte, NHLBI

Sickle cell disease is an autosomal-recessive disorder and the most common genetic disease affecting African Americans. Approximately 0.15percent of African Americans are homozygous for sickle cell disease and 8 percent have sickle cell trait. Hemoglobin S polymerization leads to red cell rigidity, microvascular obstruction, inflammation, and end-organ ischemia-reperfusion injury and infarction. Previously published data indicate that up to 50 percent of sickle cell patients have endothelial dysfunction due to impaired bioavailability of endogenous nitric oxide

(NO) due in large part to scavenging of nitric oxide by cell-free plasma hemoglobin. These data suggest that therapies directed at restoring NO bioavailability might prove beneficial. We have recently discovered that the nitrite anion, available currently for human use as a component of the cyanide antidote kit, is a vasodilator *in vivo* by generating NO in tissues with lower oxygen tension and pH. The mechanism involves a novel physiological function of human hemoglobin as an oxygen- and pH-dependent nitrite reductase. To date we have observed that nitrite infusions in animal models significantly reduce liver and cardiac ischemia-reperfusion injury and infarction in mouse models, prevent cerebral vasospasm after subarachnoid hemorrhage in primates, and decrease pulmonary hypertension in newborn hypoxic sheep. This protocol is designed as a phase I/II trial to address the hypothesis that nitrite infusions will vasodilate the circulation in patients with sickle cell disease at rest and during vaso-occlusive pain crisis, inactivate circulating cell-free plasma hemoglobin, reduce pulmonary artery pressures, and reduce ischemia-reperfusion injury.

**Acronyms**

| | |
|---|---|
| a-1 | alpha-one |
| AA | aplastic anemia |
| AAMDSIF | Aplastic Anemia and MDS International Foundation |
| AAT | a1-antitrypsin, |
| AATD | a1-antitrypsin deficiency |
| ABPA | allergic bronchopulmonary aspergillosis |
| ACCESS | A Case Control Etiologic Study of Sarcoidosis (NHLBI) |
| ACMG | American College of Medical Genetics |
| ADCC | Autoimmune Diseases Coordinating Committee |
| ADHD | attention deficit hyperactivity disorder |
| AFSP | American Foundation for Suicide Prevention |
| AGS | Alagille syndrome |
| ALD | adrenoleukodystrophy (NLM) |
| ALL | (childhood) acute lymphoblastic leukemia |
| ALPS | autoimmune lymphoproliferative syndrome |
| ALS | amyotrophic lateral sclerosis |
| A/M | anophthalmia/microphthalmia |
| APS | antiphospholipid syndrome |
| ARDS | acute respiratory distress syndrome |
| ARND | alcohol-related neurodevelopmental disorder |
| ARVD | arrhythmogenic right ventricular dysplasia |
| AS | Angelman syndrome |
| ASCUS | atypical squamous cells of undetermined significance |
| ASF | Angelman syndrome Foundation |
| ASPS | advanced sleep phase syndrome |
| AT | ataxia telangiectasia |
| BAA | broad agency announcement (NHLBI) |
| BBS | Bardet-Biedl syndrome |
| BDNF | brain-derived neurotrophic factor |
| BE | Barrett's esophagus |
| BH4 | tetrahydrobiopterin |
| BIBIN | bilirubin-induced brain injury in the newborn |
| BLM | human gene encoding Bloom syndrome |
| BN | bulimia nervosa |
| BPD | bronchopulmonary dysplasia |
| BS | Bloom syndrome |
| BSE | bovine spongiform encephalopathy |
| CAG (triplet repeat) | nucleotides (CAG) consecutively repeated within a region of DNA |

| | |
|---|---|
| CAM | complementary and alternative medicine |
| CASG | Collaborative Antiviral Study Group |
| CASPAR | computerized affected sibling pair analyzer and reporter |
| CBV | coxsackie virus B |
| CC | Warren Grant Magnuson Clinical Center, NIH |
| CCHS | congenital central hypoventilation syndrome |
| CDC | Centers for Disease Control and Prevention |
| CDG | congenital disorders of glycosylation |
| CDH | congenital diaphragmatic hernia |
| CF | cystic fibrosis |
| CFS | chronic fatigue syndrome |
| CFTR | cystic fibrosis (CF) transmembrane conductance regulator |
| CGD | chronic granulomatous disease |
| CHD | coronary heart disease |
| CHID | Combined Health Information Database |
| CHOP | Children's Hospital of Philadelphia |
| CIN | cervical intraepithelial neoplasia |
| CJD | Creutzfeldt-Jakob disease |
| CLL | chronic lymphocytic leukemia |
| CL/P | cleft lip and cleft palate |
| CMV | congenital cytomegalovirus |
| CNS | central nervous system |
| COPD | chronic obstructive pulmonary disease |
| CRADA | cooperative research and development agreement |
| CRC | clinical research center |
| CRD | cannabis-related disorder |
| CRF | corticotropin-releasing factor |
| CS | Cockayne syndrome |
| CVB3 | Cocksackie virus B3 |
| CWD | chronic wasting disease |
| DCIPS | Developing Centers on Interventions for the Prevention of Suicide (NIMH) |
| DDG | Drug Development Group (NIH) |
| DeNOVO | delivery of NO for vaso-occlusion (clinical trial title) |
| DHHS | Department of Health and Human Services |
| DMAC | disseminated infection with mycobacterium avium complex |
| DNA | deoxyribonucleic acid |
| DOE | Department of Energy |
| DSRCT | desmoplastic small round-cell tumor |
| DTCC | Data and Technology Coordinating Center |
| EB | epidermolysis bullosa, severe blistering skin diseases |

| | |
|---|---|
| EBV | Epstein-Barr virus |
| ECMO | extracorporeal membrane oxygenation |
| EDS | Ehlers-Danlos syndrome |
| ES | Ewing's sarcoma |
| FA | Fanconi anemia |
| FAS | fetal alcohol syndrome |
| FASD | fetal alcohol spectrum disorders |
| FBN1 | fibrillin 1 |
| FDA | Food and Drug Administration |
| FENIB | familial encephalopathy with neuronal inclusion bodies |
| FGFR3 | fibroblast growth factor receptor 3 |
| FH | familial hypercholesterolemia |
| FHBL | familial hypobetalipoproteinemia |
| FMR1 | fragile X mental retardation gene |
| FRDA | Friedreich ataxia |
| FRX | fragile X syndrome |
| FSHD | facio-scapulo-humeral dystrophy |
| GCPS | Greig cephalopolysyndactyly syndrome |
| GCRC | General Clinical Research Center (NCRR) |
| GHR | Genetics Home Reference (NLM) |
| GLP | good laboratory practice |
| GMP | good manufacturing practice |
| GPS | Gray platelet syndrome |
| HAART | highly active anti-retroviral therapy |
| HbF | fetal hemoglobin |
| HD | Huntington disease |
| HDL | high-density lipoprotein |
| HEV | hepatitis E virus |
| HGP | human genome project |
| HGPS | Hutchinson-Gilford progeria syndrome |
| Hh | hedgehog (signaling pathway) |
| HHT | hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) |
| HHV-8 | human herpesvirus 8 |
| HIBM | hereditary inclusion body myopathy |
| hIPF | hereditary idiopathic pulmonary fibrosis |
| HIV/AIDS | human immunodeficiency virus/acquired immune deficiency syndrome |
| HPP | health partnership program (NIAMS) |
| HPT-JT | hyperparathyroidism-jaw tumor ( syndrome) |
| HPV | human papillomavirus |
| HRSA | Health Resources and Services Administration |
| HTLV | human T cell leukemia virus |

| | |
|---|---|
| IBMFS&C | idiopathic bone marrow failure states and cytopenias |
| IBS | irritable bowel syndrome |
| ICBG | International Cooperative Biodiversity Groups (NHLBI) |
| ICs | (NIH) institutes and centers |
| IDDM | insulin-dependent diabetes mellitus |
| IFN | interferon |
| IGFs | insulin-like growth factors |
| IL | interleukin |
| IND | investigational new drug |
| IPF | idiopathic pulmonary fibrosis |
| IRSA | International Rett syndrome Association |
| ISIS | Imaging Science and Information Systems Center (NLM) |
| JDRF | Juvenile Diabetes Research Foundation International |
| JRA | juvenile rheumatoid arthritis |
| KTWS | Klippel-Trenaunay-Weber syndrome |
| LAM | lymphangioleiomyomatosis |
| LDL | low-density lipoprotein |
| LMNA | lamin A (gene) |
| LQTS | long QT syndrome |
| LVAD | left ventricular assist device |
| mAB | monoclonal antibodies |
| MADGC | Multiple Autoimmune Diseases Genetics Consortium |
| MALD | mapping by admixture linkage disequilibrium |
| MATT | Methamphetamine Addiction Treatment Think Tank (NIDA) |
| MCA/MR | multiple congenital anomaly/mental retardation |
| MD-CARE | P.L. 107-84, Muscular Dystrophy Community Assistance, Research, and Education Amendments of 2001 |
| MDCC | Muscular Dystrophy Coordinating Committee |
| MDS | myelodysplastic syndrome |
| MDA | Muscular Dystrophy Association |
| MDD | Medications Development Division (NIDA) |
| MDMA | Methylene-dioxy-meth-amphetamine (ecstasy) |
| MEN1 | multiple endocrine neoplasia type 1 |
| MHC | major histocompatibility complex |
| MKS | McKusick-Kaufman syndrome |
| MMP | matrix metalloproteinase |
| MOU | Memorandum of Understanding |
| MPD | myeloproliferative disease |
| MR4 | Malaria Research and Reference Reagent Resource (Center) |
| MS | multiple sclerosis |
| MSC | mesenchymal stem cell |

| | |
|---|---|
| MSH | Multicenter Study of Hydroxyurea |
| MTA | material transfer agreement |
| NBLs | National Biocontainment Laboratories |
| NBN | National Biospecimen Network |
| NBTT | New Approaches to Brain Tumor Therapy Consortium (NCI) |
| NCBI | National Center for Biotechnology Information |
| NCCAM | National Center for Complementary and Alternative Medicine |
| NCI | National Cancer Institute |
| NCL | neuronal ceroid lipofuscinosis (Batten disease) |
| NCMHD | National Center on Minority Health and Health Disparities (Office of the Director, NIH) |
| NCRR | National Center for Research Resources |
| NCS | National Children's Study |
| NDA | new drug application |
| ND-BD | non-dementing brain disorders |
| NEI | National Eye Institute |
| NF1 | neurofibromatosis type 1 |
| NF-kappaB | nuclear factor kappaB |
| NGI | next generation Internet |
| NHGRI | National Human Genome Research Institute |
| NHL | non-Hodgkin lymphoma |
| NHLBI | National Heart, Lung, and Blood Institute |
| NIA | National Institute on Aging |
| NIAAA | National Institute on Alcohol Abuse and Alcoholism |
| NIAID | National Institute of Allergy and Infectious Disease |
| NIAMS | National Institute of Arthritis and Musculoskeletal and Skin Diseases |
| NICHD | National Institute of Child Health and Human Development |
| NIDA | National Institute on Drug Abuse |
| NIDCD | National Institute of Deafness and Other Communication Disorders |
| NIDCR | National Institute of Dental and Craniofacial Research |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases |
| NIEHS | National Institute of Environmental Health Sciences |
| NIH | National Institutes of Health |
| NIMH | National Institute of Mental Health |
| NINDS | National Institute of Neurological Disorders and Stroke |
| NINR | National Institute of Nursing Research |
| NLM | National Library of Medicine |
| NNFF | National Neurofibromatosis Foundation |
| NO | nitric oxide |
| NOMID | neonatal onset multisystem inflammatory disease |
| NPA | Niemann-Pick type A disease |

| | |
|---|---|
| NPB | Niemann-Pick type B disease |
| NPC | Niemann-Pick type C disease |
| NPD | Niemann-Pick type D disease |
| OA | osteoarthritis |
| OCRL | oculo-cerebro-renal syndrome (Lowe syndrome, LS) |
| ODS | Office of Dietary Supplements (Office of the Director, NIH) |
| OI | Osteogenesis imperfecta |
| ORD | Office of Rare Diseases (Office of the Director, NIH) |
| ORWH | Office of Research on Women's Health (Office of the Director, NIH) |
| OTP | opiate treatment program (NIDA) |
| PA | program announcement |
| PACT-G | Pediatric AIDS Clinical Trial Group |
| PAP | pulmonary alveolar proteinosis |
| PCD | primary ciliary dyskinesia |
| PCP | phencyclidine |
| PCP | pneumocystis carinii pneumonia |
| PEGT | Programs of Excellence in Gene Therapy (NHLBI) |
| PGA | Programs for Genomic Applications (NHLBI) |
| PHS | Pallister-Hall syndrome |
| PKC | protein kinase C |
| PKU | phenylketonuria |
| PML | progressive multifocal leuceoencephalopathy |
| POF | premature ovarian failure |
| PPH | primary pulmonary hypertension |
| PPHN | persistent pulmonary hypertension of the newborn |
| PTLD | post-transplant lymphoproliferative disease |
| PTS | post-traumatic stress syndrome |
| PWS | Prader-Willi syndrome |
| PXE | pseudoxanthoma elasticum |
| RA | rheumatoid arthritis |
| RAID | Rapid Access to Intervention Development Program (NCI) |
| RBLs | regional biocontainment laboratories |
| RDCRC | Rare Disease Clinical Research Consortium |
| RDCRN | Rare Diseases Clinical Research Center Network |
| REM | rapid eye movement (characteristic of deep sleep) |
| RFA | request for applications |
| RFP | request for proposals |
| RLGS | restriction landmark genome scanning |
| RNA | ribonucleic acid |
| RS | Rett syndrome |
| RTH | resistance to thyroid hormone |

| | |
|---|---|
| RTOG | Radiation Therapy Oncology Group |
| RTS | Rothmund-Thompson syndrome |
| SADDAN | severe achondroplasia with developmental delay and acanthosis nigricans |
| SAMHSA | Substance Abuse and Mental Health Services Administration |
| SAGA | Sarcoidosis Genetic Analysis Consortium |
| SARS | severe acute respiratory syndrome |
| SBIR | small business innovative research |
| SCD | sickle cell disease |
| SCD | sudden cardiac death |
| SCID | severe combined immunodeficiency disorder |
| SCOR | specialized center of research |
| SGBS | Simpson Golabi Behmel syndrome |
| SIDS | sudden infant death syndrome |
| SLE | systemic lupus erythematosus |
| SLOS | Smith-Lemli-Opitz syndrome |
| SMA | spinal muscular atrophy |
| SPORE | Specialized Program of Research Excellence |
| SUD | substance use disorder |
| SS | sickle cell |
| SS | Sjögren syndrome |
| SVAS | supravalvular aortic stenosis |
| TB | tuberculosis |
| TCR | transcription-coupled repair |
| TIGR | The Institute for Genomic Research |
| TMAU | trimethylaminuria |
| TMD | temporo-mandibular disorders |
| TMJ | temporomandibular joint |
| TSC | tuberous sclerosis complex |
| TSEs | transmissible spongiform encephalopathies |
| TTP | thrombotic thrombocytopenic purpura |
| UCD | urea cycle disorder |
| UIP | usual interstitial pneumonitis |
| UPD | uniparental disomy |
| UV | ultraviolet |
| VA | (Department of )Veterans Affairs |
| vCFD | variant Creutzfeldt-Jakob disease |
| VCFS | velo-cardio-facial syndrome |
| VCRN | Vasculitis Clinical Research Network |
| VEG5Q | vascular endothelial gene on chromosome 5q |
| VEGF | vascular endothelial growth factor |
| VLBW | very low birth weight |

| | |
|---|---|
| VLDL | very low-density lipoprotein |
| VTUs | vaccine treatment and evaluation units |
| VWD | von Willebrand disease |
| VWF | von Willebrand factor |
| WAS | Wiscott-Aldrich syndrome |
| WNV | West Nile virus |
| WRN | defective gene for Werner syndrome |
| WS | Waardenburg syndrome (NIDCD) |
| WS | Werner syndrome (NIA) |
| WS1 | Wilm's tumor suppressor |
| XPD | a human DNA repair protein |

**Index**