

Progress Review for 2005 (Year One Analysis):
Action Plan for Liver Disease Research

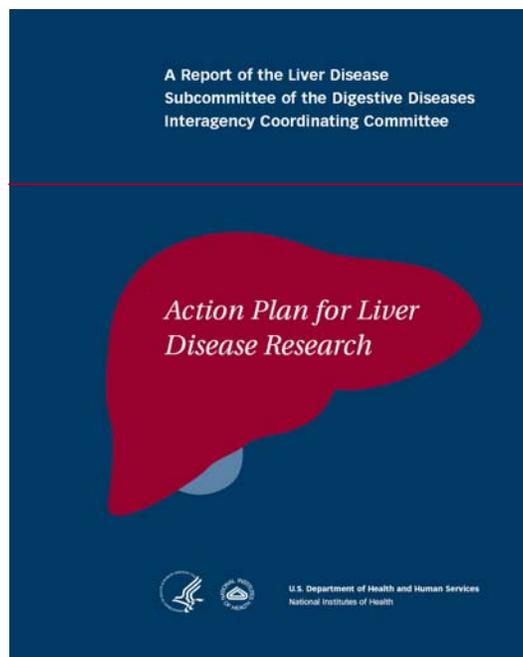


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Objective

This Progress Review of the trans-NIH Action Plan for Liver Disease Research describes progress made toward reaching the Action Plan's research goals during 2005, the first year following its release. The objective of the annual Progress Review is to aid in the implementation of the Action Plan through an ongoing assessment of progress and the need for further efforts to promote liver and biliary disease research.

Introduction

The trans-NIH Action Plan for Liver Disease Research was developed by a broad consortium of basic and clinical research investigators, physicians, health care providers and concerned lay persons, with input from the 17 Institutes and Centers at the National Institutes of Health (NIH) involved in liver disease research. The primary purpose of the Action Plan is to advance research on liver and biliary diseases with the ultimate aim of decreasing the burden of these diseases in the United States. The final document of the Action Plan summarizes the status of liver disease morbidity and mortality in the United States, the status of liver disease research, and lists 214 specific research goals for the future, with background and justification for each. The research goals are organized into 16 topic areas, and each research goal is categorized for its degree of difficulty (low, medium or high risk) and the estimated time for its completion (short, medium or long term). The final Action Plan was made available on the NIH web site (<http://liverplan.niddk.nih.gov>) in December 2004 and was published as a monograph in February 2005. The Action Plan is an ambitious and optimistic document that provides a structure and focus for research on liver and biliary diseases, which together rank among the top 10 causes of death in the United States.

The ultimate purpose of the Action Plan is to affect a decrease in the morbidity and mortality from liver and biliary diseases. The 214 research goals are focused on areas that would materially advance knowledge about liver diseases and improve means for their diagnosis, monitoring, treatment, and prevention. The research goals are specific enough to be measurable, but broad enough to affect an advance in the field.

The Action Plan document also includes plans for implementation. Attainment of the research goals is to be promoted through: (1) broad distribution of the document, (2) encouragement of its use in grant applications and in peer review, (3) promotion of collaborations between research funding entities (including industry), and (4) specific initiatives from the NIH and other Federal Agencies concerned with liver disease research, such as the Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), Department of Veterans Affairs, and the Department of Defense. Primary responsibility for the Action Plan was placed on the Liver Disease Research Branch of the National Institute of Diabetes and Digestive and Kidney Diseases

(NIDDK). Coordination of activities related to the Action Plan was the charge of the Liver Disease Subcommittee of the statutory Digestive Diseases Interagency Coordinating Committee (DDICC).

The current document is a Progress Review of the Action Plan for the year 2005, approximately 1 year after its release. The Progress Review was prepared by the Liver Disease Research Branch with the assistance of the chairperson and members of the 16 Working Groups that initially established the research goals for the Action Plan, as well as input from members of the Liver Disease Subcommittee of the Digestive Diseases Interagency Coordinating Committee. This Progress Review provides a concise analysis of the progress made toward reaching each of the 214 goals and, when appropriate, a brief description of initiatives focusing on the specific research goal. The Progress Review is not comprehensive, but provides specific examples of important advances made in 2005 that are apropos to each research goal. Finally, the degree of progress made toward each research goal is estimated on a scale of 0 percent (no progress) to 100 percent (full attainment of the goal) in increments of 10 percent. These percentages are purely estimates but are made on the basis of agreement among experts serving on the Working Groups. The estimated degree of progress is also demonstrated graphically for each of the 16 topic areas. These assessments of progress are presented later in this document and are grouped by the 16 topic areas of the Action Plan.

The Action Plan also includes a section on Summary Recommendations, including a series of 10 overarching “benchmark” research goals. These 10 research goals are important cross-cutting goals that are mentioned in several of the 16 topic areas and would constitute clear major advances in liver disease management, control and prevention by which to assess the overall success of the Action Plan. A discussion of the status of these benchmark goals concludes this Progress Review.

Action Plan: 16 Topic Areas of Liver Disease Related Research

The titles of the 16 topic-specific chapters are listed below, along with the many individuals who contributed to this assessment of progress made toward achieving research goals in these areas. These individuals include members of the original 16 Working Groups that developed the goals, as well as others with expertise in these fields.

- **Cell and Molecular Biology of the Liver:** Drs. Allan Wolkoff, Irwin Arias, Laura Beretta, David Cohen, Anne Hubbard, Mark McNiven, Michael Nathanson, and Jose Serrano
- **Liver Injury, Inflammation, Repair, and Fibrosis:** Drs. Gregory Gores, Mark Czaja, Scott Friedman, Jacquelyn Maher, John Lemasters, Don Rockey, and Jay Hoofnagle
- **Developmental Biology and Regeneration:** Drs. Nelson Fausto, Markus Grompe, Mark Kay, George Michalopoulos, Kenneth Zaret, David Shafritz and Jose Serrano
- **Bile, Bilirubin and Cholestasis:** Drs. James Boyer, Sawkat Anwer, John Chiang, David Cohen, Norman Javitt, and Jay Hoofnagle
- **Viral Hepatitis:** Drs. Stanley Lemon, Harvey Alter, Francis Chisari, Jeffrey Glenn, William Mason, Charles Rice, Rajen Koshy, and Leonard Seeff
- **HIV and Liver Disease:** Drs. David Thomas, Margaret Koziel, Jules Levin, Marion Peters, Ken Sherman, Katherine Davenny, and Fulvia Veronese
- **Fatty Liver Disease:** Drs. Anna Mae Diehl, David Crabb, Joannes Hoek, Craig McClain, Arun Sanyal, and Sam Zakhari
- **Drug- and Toxicant-Induced Liver Disease:** Drs. Neil Kaplowitz, Timothy Macdonald, Sidney Nelson, Lance Pohl, Robert Roth, John Senior, Paul Watkins, and Carol Shreffler
- **Autoimmune Liver Disease:** Drs. John Vierling, Nora Bergasa, Nicholas Crispe, Eric Gershwin, James Gorham, Keith Lindor, Barbara Rehmann, and Stephen James
- **Pediatric Liver Disease:** Drs. Jorge Bezerra, Saul Karpen, David Perlmutter, Ron Sokol, Frederick Suchy, and Tonse Raju
- **Genetic Liver Disease:** Drs. Bruce Bacon, Nancy Andrews, Herbert Bonkovsky, Joseph Bloomer, Jonathan Gitlin, Caroline Philpott, and Alan Guttmacher
- **Liver Transplantation:** Drs. Jean Emond, Michael Lucey, Sue McDiarmid, Kim Olthoff, John Roberts, Hugo Rosen, and James Everhart
- **Complications of Liver Disease:** Drs. Thomas Boyer, Andres Blei, Michael Fallon, Roberto Groszmann, Michael Henderson, William Lee, and Leonard Seeff
- **Liver Cancer:** Drs. Adrian Di Bisceglie, Michael Abecassis, Brian Carr, Greg Gores, Snorri Thorgeirsson, Jack Wands, and Jaye Viner
- **Gallbladder and Biliary Disease:** Drs. Sum Lee, Martin Carey, Michael Kimmey, Nicholas LaRusso, Henry Pitt, and James Everhart
- **Liver Imaging and Biotechnology:** Drs. King Li, Glenn Krinsky, Jonathan Kruskal, Fred Lee, and Alan McLaughlin.

Active Initiatives in Liver Disease Research

There are several means of promoting specific areas in NIH-supported liver disease research that go beyond the focus of investigator-initiated research. The major approaches include requests for applications (RFAs), program announcements (PAs), and scientific meetings. The meetings encourage research in specific areas by bringing together experts in the field to review the current status of understanding and outline areas of specific research opportunities for the future. RFAs and PAs are published by the NIH to encourage specific areas of research. RFAs usually have a single receipt date for grant applications and set aside funds. The applications are usually reviewed by a special review group. PAs generally have multiple receipt dates that may extend over several years; the applications are typically reviewed by standard initial review groups (IRGs, also known as Study Sections), and funds are not usually specifically set aside, although these applications receive special consideration for funding. Finally, either RFAs or PAs can call for a specific type of study, consortium, database, or clinical trial focusing on an area of greatest opportunity. Table 1 lists specific PAs and RFAs released in or applicable to 2005 that encourage research applications directed at specific research goals delineated in the Trans-NIH Action Plan for Liver Disease Research.

Table 1. Program Announcements and Requests for Applications Relevant to Action Plan

Initiative Number	Title	Sponsoring ICs
PAR-03-033 (Reissued as PAR-06-171)	Endoscopic Clinical Research in Pancreatic and Biliary Diseases	NIDDK, NCI
PA-04-043 (Reissued as PA-06-177)	Research Grants for Studies of Hepatitis C in the Setting of Renal Disease	NIDDK
PA-04-068	Development of Assays for High Throughput Drug Screening	NIDDK, NCI, NIAID
PA-04-081 (Reissued as PA-06-185)	Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases	NIDDK
PA-04-088 (Reissued as PA-06-143)	Non-Invasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological and Digestive Diseases	NIDDK
PA-05-049	Animal Models of NIDDK-Relevant Diseases	NIDDK, NIAID
PAR-05-056	Targeting Diseases Caused by Protein Misfolding or Misprocessing	NIDDK
PA-05-098 (Reissued as PA-06-147)	Development of Disease Biomarkers	NIDDK, NIBIB, NIAAA, ODS
PA-05-119	Mechanisms of Alcoholic and Nonalcoholic Fatty Liver	NIAAA, NIDDK
PA-05-137	Etiology, Prevention and Treatment of Hepatocellular Carcinoma	NCI, NIDDK, NIBIB, NIAAA
RFA-AI-04-028	Hepatitis C Cooperative Research Centers	NIAID, NIDDK, NIDA
RFA-AT-05-006	Phase I/II Trials of Silymarin for Chronic Liver Diseases	NCCAM, NIDDK
RFA-AI-05-030	Partnerships for Hepatitis C Vaccine Development	NIAID

The NIH also supports liver disease-related research through ongoing clinical and epidemiologic studies focused on specific diseases, procedures, and patient populations. Examples of current studies are listed in Table 2 below.

Table 2. Clinical and Epidemiologic Studies and Relevant Announcements

Short Title/ Initiative #	Full Title	Sponsoring ICs/Agencies
A2ALL	Adult-to-Adult Living Donor Liver Transplantation Cohort Study (including "LADR" study below)	NIDDK, HRSA
<i>LADR</i>	Low Dose Accelerating Regimen study	NIDDK, HRSA
AACTG	Adult AIDS Clinical Trials Group, Hepatitis Subcommittee	NIAID
PACTG	Pediatric AIDS Clinical Trials Group	NIAID
ALFSG	Adult Acute Liver Failure Study Group	NIDDK
PALFSG	Pediatric Acute Liver Failure Study Group	NIDDK
ARPKD Network	Autosomal Recessive Polycystic Kidney Disease and Congenital Hepatic Fibrosis Study	NHGRI
BARC	Biliary Atresia Research Consortium	NIDDK, ORD
CLiC	Cholestatic Liver Disease Consortium	NIDDK, ORD
DILIN	Drug-Induced Liver Injury Network	NIDDK, FDA
HALT-C	Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial	NIDDK, NCI, NIAID
HURSO	High Dose Ursodiol for Primary Sclerosing Cholangitis	NIDDK
LABS	Longitudinal Assessment of Bariatric Surgery	NIDDK
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network (including "PIVENS" and "TONIC" below)	NIDDK, NICHD
<i>PIVENS</i>	Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis study	NIDDK
<i>TONIC</i>	Treatment of Nonalcoholic Fatty Liver Disease in Children trial	NIDDK
OLT HBV	Orthotopic Liver Transplantation for Hepatitis B Study	NIDDK
PEDS-C	Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C	NIDDK, FDA
PGRN	Pharmacogenetics Research Network	NIGMS
SPLIT	Study of Pediatric Liver Transplantation	NIDDK
Virahep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C	NIDDK
PAR-04-078 (Reissued as PAR-06-216)	Ancillary Studies to Major Ongoing NIDDK Clinical Research Studies	NIDDK
PAR-04-081	Small Clinical Grants in Digestive Diseases, Nutrition and Obesity	NIDDK

NIH Funding of Liver Disease Research

The Trans-NIH Action Plan for Liver Disease Research provides a background that includes NIH funding levels for liver disease research through Fiscal Year (FY) 2003. Since then, funding in liver disease research has continued to grow commensurate with the growth in the overall NIH budget. The total amount of funding designated as “Liver Disease Related” for FY 2005 was approximately \$454 million, which represented an 8.4 percent increase from FY 2004—above the average NIH growth rate of 1.3 percent. The growth in Liver Disease Related research funding is shown graphically in the Figure 1 below expressed as percent growth from a baseline in 1993. The proportion of Liver Disease Related funding by NIH Institutes and Centers for FY 2005 is shown in Figure 2. The majority of funding in liver disease research continues to derive from NIDDK (38%), NIAID (19%), NCI (13%), NIAAA (8%), NIEHS (6%), NIDA (6%), NHLBI (4%), and the NCCR (3%). Another 10 NIH Institutes or Centers provide 3 percent more of the liver disease research budget.

Figure 1. Growth in Liver Disease Research Funding: FY 1993-2005

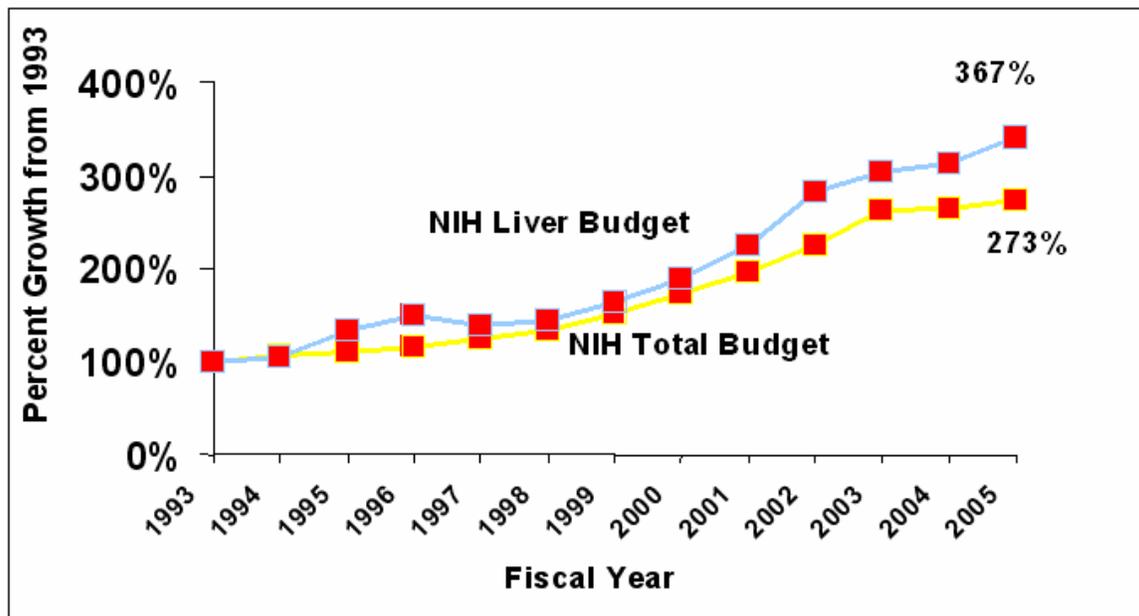
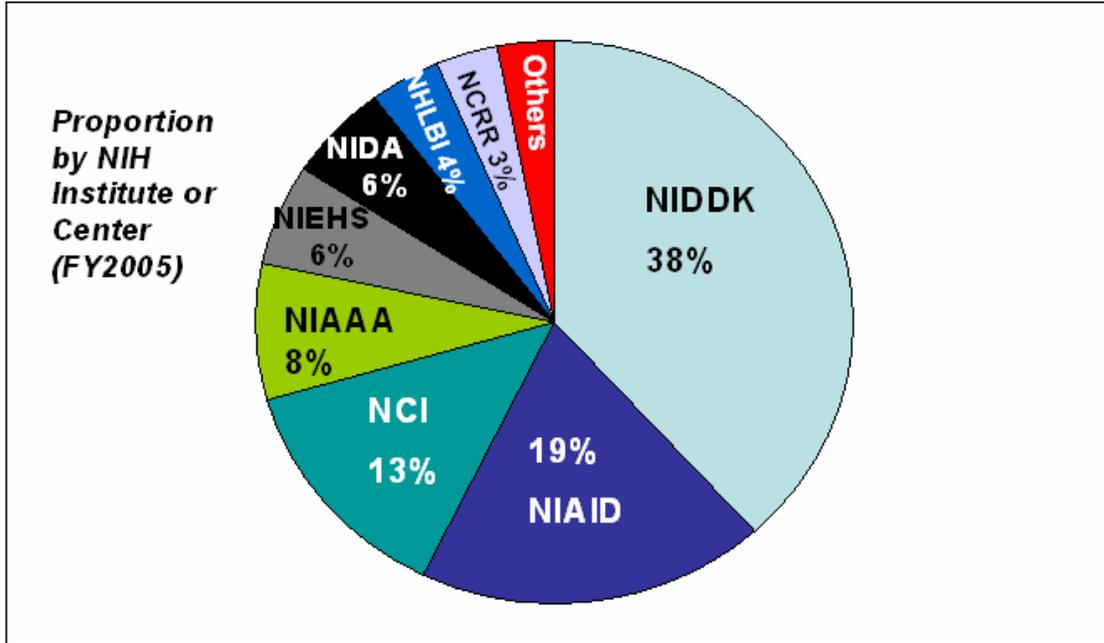


Figure 2. NIH Support of Liver Disease Related Research



Progress Review: Year One Analysis (2005)

The following 16 sections describe the first year of progress made toward reaching each of the research goals in the Action Plan's 16 topic-specific chapters, with research support provided by the NIH, other agencies, and industry. Goals are identified by the original letter-number combinations used in the Action Plan, which indicate the estimated time and degree of difficulty involved in their completion.

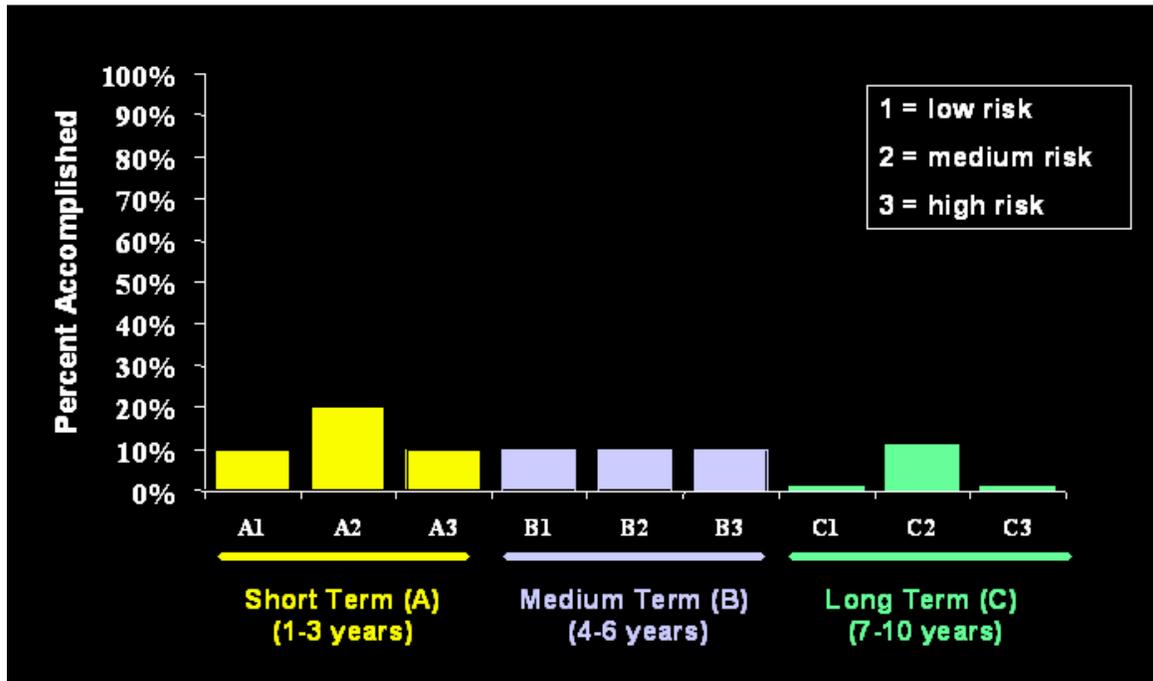
Chapter 1

Cell and Molecular Biology of the Liver:

- A1. Define major pathways and molecular participants in signal transduction in liver cells.** New signaling pathways have been identified in liver during the past year. Signaling events generally begin with plasma membrane receptors, so receptor desensitization and resensitization are critical gatekeepers. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) redirects certain internalized G protein-coupled receptors back to the plasma membrane to prevent lysosomal degradation (Hanyaloglu AC. *EMBO Journal* 2005; 24:2265). Calcium is a versatile second messenger, but excessive increases can lead to cell death. Cyclophilin D forms the mitochondrial permeability transition pore that results in the death of hepatocytes damaged by excessive increases in calcium or by oxidative stress; consequently, mice lacking cyclophilin D are resistant to these forms of cell death (Baines CP. *Nature* 2005; 434:658). Polycystin-2 is a cholangiocyte protein defective in autosomal dominant polycystic kidney and liver disease and has been linked to regulation of secretion by cholangiocytes and cholangiocyte proliferation (Li X. *Nature Cell Biology* 2005; 7:1102) (10%).
- A2. Elucidate the mechanisms of lipid metabolism and transport in liver as it relates to whole body lipid homeostasis.** This is an area of active investigator-initiated research. In the past year, the scavenger receptor class B type I (SR-BI) has been shown to be a regulator of cholesterol efflux from macrophages for excretion by the liver into bile and feces (Zhang YZ. *J Clin Invest* 2005;115:2870). Furthermore, the ABCG1 and ABCA1 transporters act synergistically in mediating cholesterol efflux from macrophages and incorporation into HDL particles (Gelissen IC. *Arterioscler Thromb Vasc Biol* 2005; In press). Biliary cholesterol secretion is regulated largely through the ABCG5/G8 transporters, which in turn are regulated by the nuclear receptors LXR, FXR and PXR (Yu L. *J Biol Chem* 2005;280:8742). (20%)
- A3. Determine how intra- and inter-cellular signals are integrated *in vivo* to regulate liver function.** The importance of cross-talk between Kupffer cells and hepatocytes was shown in studies of site specific deletion of I κ B kinase (IKK β) activity (required for NF- κ B activation) in mice. When IKK β was deleted from hepatocytes only, chemical-induced hepatocarcinogenesis was enhanced; whereas when it was deleted from both hepatocytes and Kupffer cells, hepatocarcinogenesis was decreased (Maeda S. *Cell* 2005;121: 977). (10%)
- B1. Elucidate physiological importance of liver plasma membrane transporters and mechanisms of action.** The organic solute and drug membrane uptake transporters located in liver cell plasma membranes include NTCP, and several organic anion and cation transporters (OATPs and OCTs), while the export transporters include multiple ATP-binding cassette (ABC) proteins (BSEP, MDR1 and 2, MRP2, 3 and 6). These transporters can be regulated by the orphan nuclear receptors, but also by several hormones (prolactin, growth hormone) and inflammatory signals (TNF- α , IL-1 β) (Wood M. *Mol Pharmacol* 2005;68:218;

- Geier A. *Am J Physiol GI* 2005;289:831). The binding of OATP1A1 to cell surface membranes is mediated in part by the chaperone protein PDZK1 (Wang P. *J Biol Chem* 2005;280:30143). (10%)
- B2. Develop cell culture model that reflects different liver cell interactions (e.g., hepatocyte with Kupffer cell, cholangiocyte, stellate cell, or endothelial cell).** Several groups of investigators are working on developing methods for co-culture of hepatocytes with Kupffer cells, bone marrow stromal cells and biliary epithelial cells (Zinchenko YS. *J Biomed Mater Res A*. 2005;75:242; Auth MK. *Liver Transpl* 2005;11:410; Takeda M. *J Biosci Bioeng* 2005;100:77). (10%)
- B3. Elucidate intra- and extra-cellular events that determine hepatocyte polarity.** Recent studies have shown that polarization of hepatocytes and development of canaliculi require recruitment of rab11a and myosin Vb to intracellular membranes that contain apical ABC transporters and transcytotic markers, permitting their appropriate targeting (Wakabayashi Y. *PNAS* 2005;102:15087). Delivery of rab11a-myosin Va-containing membranes to the cell surface leads to their differentiation into a bile canaliculus. (10%)
- C1. Elucidate major elements in process of transcellular vesicle trafficking in the hepatocyte.** Flow cytometry and fluorescent labeling has been used to directly visualize the binding and movement of vesicles along microtubules towards apical and basolateral membranes in the hepatocyte (Murray JW. *Meth Enzymol* 2005;403:92). The factors that determine binding, motion and fission of vesicles to membranes are now being elucidated. (0%)
- C2. Elucidate how cells interact with each other (e.g., via gap junctions, ECM, paracrine, and endocrine signaling).** Caveolin regulates the organization and activity of signaling molecules concentrated in caveolae in the plasma membrane. Dynamin-2 interacts directly with caveolin-1 in hepatocytes and mediates the scission of caveolae from the plasma membrane (Yao Q. *J Mol Biol*. 2005;348:491). (10%)
- C3. Develop knowledge base of normal liver proteome, including analysis of individual cell types, subcellular compartments, and changes along hepatic acinus.** Research to characterize the proteomes of liver and biliary cell types is encouraged by the NIH through program announcements, such as "Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases" (PA-04-081). International research efforts in this area are being coordinated through the Human Liver Proteome Project (HLPP). The HLPP pilot phase (2004-2006) is ending. The data generated by worldwide participating laboratories will be publicly available in Summer 2006 and will be presented at that time in a special issue of *Proteomics*. A major effort to generate antibodies against all liver proteins is underway. (0%)

Figure 3. Estimated Progress on Cell and Molecular Biology Research Goals, 2005 (Year 1)



Chapter 2

Liver Injury, Inflammation, Repair, and Fibrosis

- A1a. Identify individual liver cell type-specific responses to inflammatory mediators.** Multiple publications have documented liver cell responses to cytokines, chemokines, adipokines and growth factors including PDGF-C (Campbell JS. *PNAS* 2005;102:3389), leptin and adiponectin (Ding X. *Am J Pathol* 2005;166:1655), angiotensin, TGF β and others. (20%)
- A1b. Elucidate whether and how nutritional factors affect liver cytotoxic and fibrotic pathways.** Numerous studies have identified how dietary fat results in NF- κ B activation in both hepatocytes and Kupffer cells (Dela Pena A. *Gastroenterology* 2005; 129:1663; She H. *J Biol Chem* 2005;280:4959). (10%)
- A2a. Define the role of anti-apoptotic therapy in liver injury, fibrosis, and regeneration.** Apoptosis is the underlying mechanism of hepatocyte cell death in many liver diseases, and its inhibition results in lessening of injury and acceleration of recovery in some animal models. Caspase inhibitors have been developed for this purpose that are now in phase I/II trials in humans (Linton SD. *J Med Chem* 2005; 48:6779). (10%)
- A2b. Identify the impact of individual leukocyte sub-populations and their mediators on liver injury, fibrosis, and regeneration.** Recent research indicates that T- and B-lymphocytes and macrophages, all contribute to hepatic fibrosis, probably mediated by their effects on hepatic stellate cells (Novobrantseva TI. *J Clin Invest* 2005;115:3072). (10%)
- A3. Develop noninvasive biomarkers for fibrosis.** A variety of serum panels with potential biomarkers as well as noninvasive imaging techniques (such as elastography) for assessing hepatic fibrosis have been described, but none have been widely accepted. This area of research is encouraged through the following program announcements: “Development of Disease Biomarkers” (PA-05-098) and “Noninvasive Methods for Diagnosis and Progression of Diseases” (PA-04-088). (10%)
- B1. Identify individual liver cell type-specific extrinsic (e.g., mediator-based) and intrinsic (e.g., organelle-based) cytotoxic signaling pathways.** Progress has been made in identifying several cytotoxic signaling pathways that lead to liver cell apoptosis, but the relative role and interactions of these pathways needs further elucidation, particularly in humans (Reinehr R. *J Biol Chem* 2005;280: 27179; Klein C. *J Clin Invest* 2005;115:860; Guicciardi ME. *Gastroenterology* 2005;129:269.) (10%)
- B2a. Identify the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemic-reperfusion injury and the role of sinusoidal cells.** The integrative mechanisms modulating oxidative, nitrosative, hypoxic and ischemic injury of endothelial cells still remains unclear. The NIH encourages research into the mechanisms of various types of liver injury through initiatives on

“Mechanisms of Alcoholic Hepatitis” (PA-02-078), “Preventing Mitochondrial Stress in Diabetes and Obesity” (RFA-DK-05-005), and “Ubiquitin and Ubiquitin-like Modifications Regulating Disease Processes” (PA-03-145). (0%)

B2b. Identify the proteomic response of the liver and liver-derived serum proteins as intermediate biomarkers for liver disease progression and response to therapy. The proteomic response during disease progression and with therapy of liver disease are the topic of intensive investigation. The NIH encourages research in proteomics of the liver in the program announcement PA-04-81 (“Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases”). (0%)

B3. Develop gene-, cell-, or pharmacology-based therapies for hepatic injury. Modulators of the activity of nuclear receptors (FXR, PSR, CAR, PPAR α and PPAR γ) are being developed and evaluated as therapies for liver disease. The role of the thiazolidinediones (PPAR γ agonists) in therapy for chronic hepatitis C and NASH is being actively pursued in several NIH-funded trials. (10%)

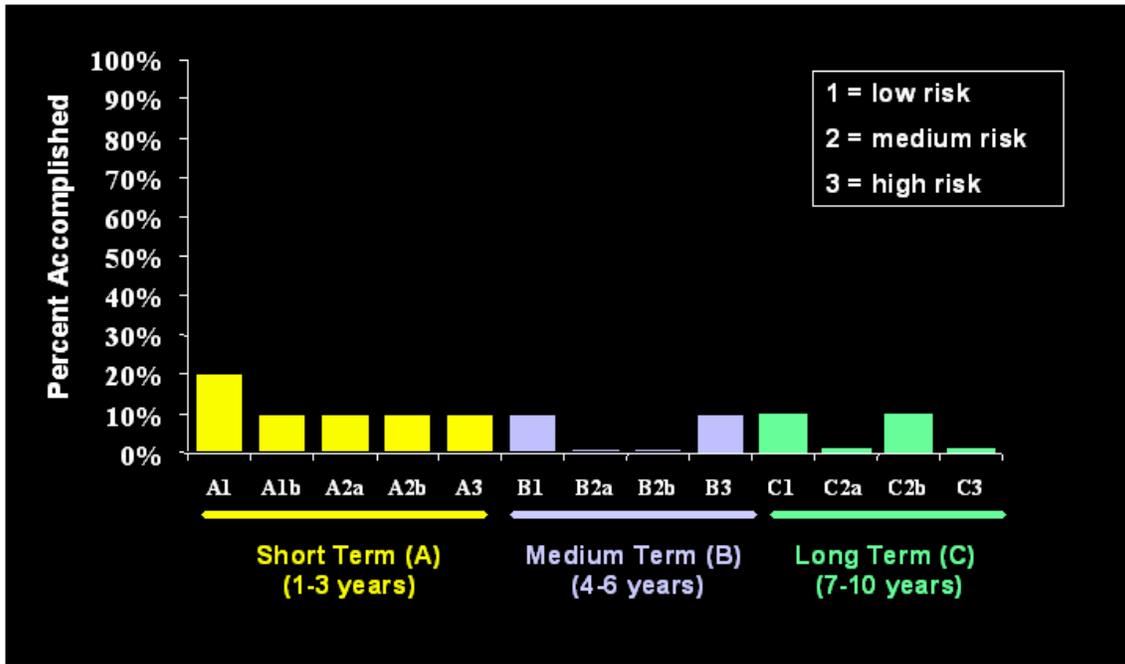
C1. Develop relevant and robust animal models of hepatic injury, inflammation, and fibrosis progression and resolution. The NIH encourages development of relevant animal models through its initiative on “Animal Models of NIDDK-relevant Diseases” (PA-05-049). The *mdr* knockout mouse develops biliary inflammation and fibrosis that resembles primary sclerosing cholangitis in humans (Popov Y. *J Hepatology* 2005;43:1045). (10%)

C2a. Using high-throughput screens, identify candidate small molecules that modify cytotoxic and fibrotic pathways in liver cells. Progress in this area will require development of methods for screening small molecules that augment or impede cell signaling pathways related to apoptosis and fibrosis. This area is the focus of NIH Roadmap initiatives, including the RFA “Pilot-Scale Libraries for High-Throughput Drug Screening” (RFA-RM-05-014) and a PA “Development of Assays for High-Throughput Drug Screening” (PA-05-068). (0%)

C2b. Define genetic determinants of disease risk and progression in acute/chronic liver injury, fibrosis, and regeneration. Genetic determinants of hepatic fibrosis are being intensively examined in cohorts of patients with various liver diseases. Genes associated with increased risk of disease progression include complement factor 5 (Hillebrandt S. *Nat Genet* 2005;37:835). Additional genes under scrutiny include TGF β , IFN γ , IL-10, Mx1, PKR and angiotensinogen. (10%)

C3. Develop mechanism-based drug therapy in fibrotic disease, targeting pro-fibrogenic and fibrosis resolution pathways. Both industry- and NIH-funded investigator-initiated research programs are active in this area. Approaches currently being evaluated in animal models include inhibitors of: angiotensin II, endothelin, TGF β signaling, cannabinoid receptors, and stellate cell activation. (0%)

Figure 4. Estimated Progress on Liver Injury, Inflammation, Repair, and Fibrosis Research Goals, 2005 (Year 1)



Chapter 3 Developmental Biology and Regeneration

- A1a. Identify and characterize hepatic stem cells in fetal and adult liver.** The presence of hepatic progenitor cells has been identified in adult liver by detection of Hedgehog signaling which is active during embryogenesis of the liver, but not found in fully differentiated hepatocytes and cholangiocytes (Sicklick JK, *Am J Physiol Gastrointest Liver Physiol*, 2005: In press). Two types of endodermal progenitor cells contribute to the liver bud in embryos (Tremblay KD. *Dev Biol*. 2005;280:87). (20%)
- A1b. Profile transcriptional network during endodermal specification, liver growth and regeneration.** Microarray analyses of different stages of liver development and regeneration are being evaluated by NIH-funded investigator-initiated research project grants, but there has yet to be a synthesis of results. (0%)
- A2a. Identify noninvasive biomarker or imaging method for assessing liver regeneration.** Whole liver imaging for liver volume is still used as a means of assessing human liver regeneration after hepatectomy and partial liver graft transplantation. Development of markers for regeneration is encouraged in the following program announcements: “Non-Invasive Methods for Diagnosis and Progression” (PA-04-088) and “Development of Disease Biomarkers” (PA-05-098). (0%)
- A2b. Define role of inflammation, fibrosis, and cell injury in regeneration.** Many pro-inflammatory signals play a role in liver regeneration, including IL6, TGF β , and lymphotoxin (Anders RA. *J Immunol* 2005;175:1295). The interplay of these signaling pathways and their modulation as well as the role of fibrogenic pathways in regeneration require further elucidation. (10%)
- A3a. Define role of nonparenchymal cells in liver regeneration and liver development.** The extracellular matrix, a major product of nonparenchymal cells, plays an essential role in normal liver regeneration (Serandour AL. *Hepatology* 2005;41:478). (10%)
- A3b. Develop new animal model systems to study liver development.** Both the frog (*Xenopus*) and zebrafish are being used to assess liver embryogenesis and have already delineated roles for several pathways in liver development, including FGF and secreted frizzled-related protein 5, HNF1 β , 4 and 6, and the Wnt and Notch signaling pathways (Lemaigre F. *Curr Op Genet Dev* 2005;14:582). (20%)
- B1a. Develop methods to select transplanted donor cells and induce homing and engraftment of transplanted cells to the liver.** Gene therapy of liver disease would be benefited by the development of means to target cells to the liver. (0%)
- B1b. Identify how deregulation of genes and pathways involved in normal regeneration contributes to carcinogenesis.** The forkhead transcription factor Foxm1b is induced during hepatocyte proliferation and is necessary for liver

morphogenesis. Overexpression of Foxm1b promotes liver cancer in mice; therefore, modulation of Foxm1b expression may be an approach to prevention or treatment of hepatocellular carcinoma (Costa RH. *Curr Op Genet Dev* 2005;15:42. (10%).

B2a. Validate biomarkers of regeneration in living donor liver donation and acute liver failure. This area of research is encouraged directly in a program announcement on “Development of Disease Biomarkers” (PA-05-098) and is the focus of ancillary studies in the A2ALL cohort study of living donor liver transplantation. (0%)

B2b. Identify pathways that stop proliferation of hepatocytes as liver returns to normal mass. Factors that control or stop hepatocyte proliferation in animal and cell culture models include TGF β , p53, and the cyclin regulatory genes (Romero-Gallo J. *Oncogene* 2005;24:3028). The role of these factors in human liver regeneration awaits study. (10%)

B3. Delineate sequence of molecular and cellular events that lead embryonic stem cells to differentiate into mature hepatocytes. Delineation of the molecular events that lead to differentiation of stem cells to mature hepatocytes is the focus of much basic research in animal models and the Foxm1b transcription factor appears to be a major component. Delineation of steps in this transition in human hepatocytes has not yet been accomplished. (10%)

C1. Develop *ex vivo* and *in vivo* vectors for liver-directed gene therapy. Research on vectors for gene therapy is encouraged through the NIH Molecular Therapy Centers programs. (0%)

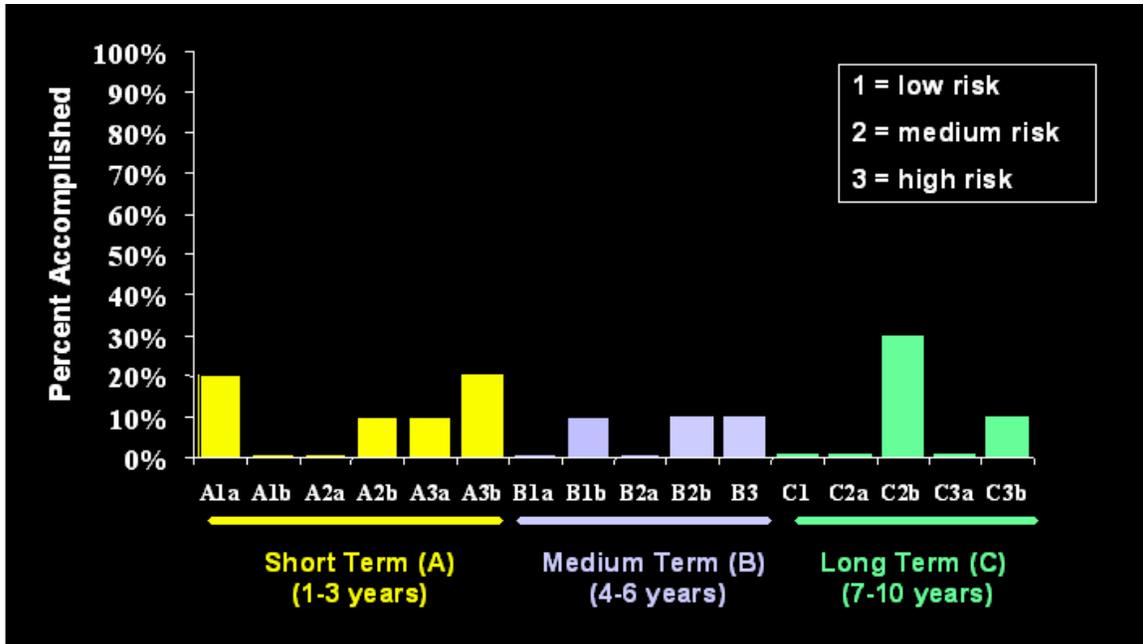
C2a. Develop safe means of promoting normal liver regeneration for acute liver failure, liver resection, and transplantation. Progress in this area will require the initial delineation of the cellular pathways that lead to normal liver regeneration and application of cytokines or small molecules that promote normal regeneration. Candidates include IL-6, TNF α , HGF, EGF, and TGF α . Several of these molecules have been used in animal models of regeneration but none have been applied to humans. (0%)

C2b. Delineate molecular and cellular events that lead from endodermal liver primordium to mature liver in fetal development. The movement of the hepatic endoderm towards the site of liver bud formation and the position of the endoderm as it is exposed to different mesodermal signals for liver development have been mapped (Tremblay KD. *Dev Biol* 2005;280:87). However, the molecular signals accompanying each step require further elucidation. The endodermal transcription factors GATA-6, FOXA1, and FOXA2 are crucial for early liver development (Zhao R. *Mol Cell Biol* 2005;25:2622; Lee CS. *Nature* 2005;435:944). (30%)

C3a. Develop practical gene or cell therapy for metabolic liver disease. Pilot studies of innovative gene and cell therapy for several metabolic liver diseases have been conducted, but practical approaches have yet to be developed. (0%)

C3b. Develop *in vitro* model of hepatic organogenesis. Three dimensional systems for growing hepatocytes have been developed that may ultimately be applicable to use in a hepatic assist device (Monga SP. *Am J Pathol* 2005;167:1279). (10%)

Figure 5. Estimated Progress on Developmental Biology and Regeneration Research Goals, 2005 (Year 1)



Chapter 4

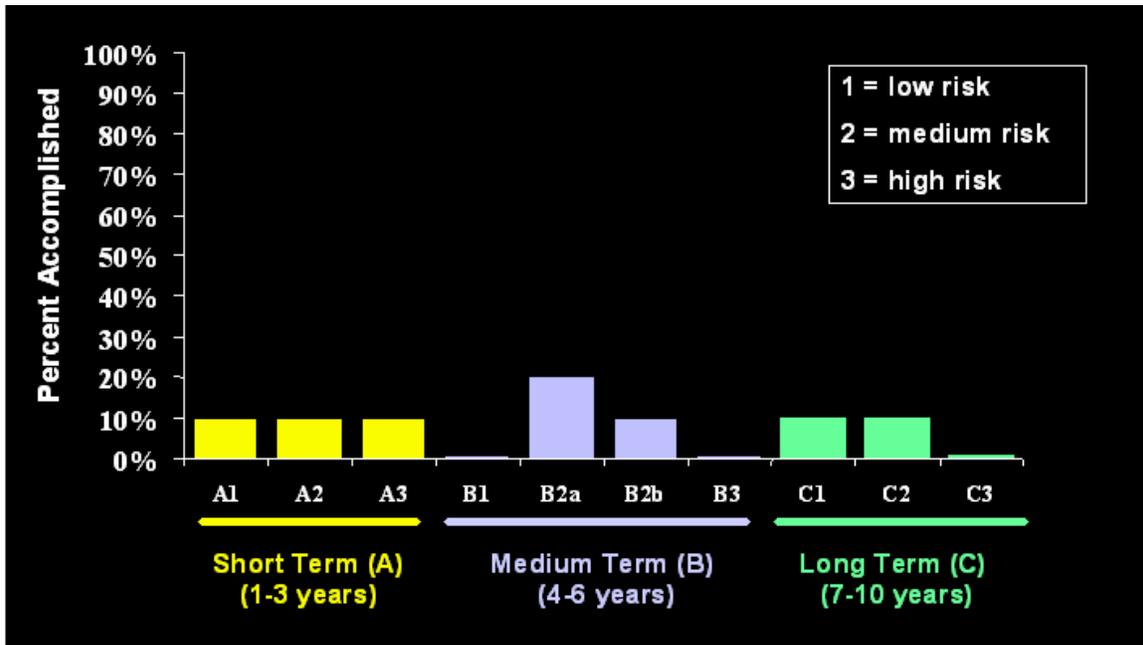
Bile, Bilirubin, and Cholestasis

- A1. Further identify molecular causes of various forms of PFIC.** Recent studies suggest that PFIC-2 may be diagnosed by immunofluorescence, whereas PFIC-3 requires gene-sequencing (Keitel V. *Hepatology*. 2005; 41:1160). The NIH-funded Cholestatic Liver Disease Consortium (CLiC) was established in 2005 and supports research to identify genetic causes of PFIC in cases not explained by defects in the *FIC1*, *BSEP* or *MDR3* genes, which are typically associated with PFIC-1, -2 and -3, respectively. (10%).
- A2. Define structure-function relationships of genes involved in cholestatic liver diseases and identify potential targets for therapy.** Activation of the orphan nuclear receptors CAR and PXR leads to induction of genes that protect the liver against toxic bile acids and xenobiotics; lack of these receptors worsens cholestatic liver disease in mice, making these receptors attractive targets for therapy (Wagner M. *Hepatology*. 2005; 42:420). Research on the orphan nuclear receptors in liver is an extremely active area of investigator-initiated research as well as the NIH-funded Nuclear Receptor Signaling Atlas. (10%)
- A3. More fully define the normal fetal development and maturation of bile salt and bilirubin metabolic pathways.** Studies in rats show that placental mRNA levels of bile acid transporters exceed those of the fetal liver until day 20 of gestation, suggesting that the fetus relies on placental clearance of bile acids (St-Pierre MV. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R1505). (10%)
- B1. Define whether polymorphisms of major bile transporters are involved in drug-induced cholestatic liver disease.** The NIH-sponsored DILIN network is assembling a cohort of patients and controls with drug-induced liver disease for genetic studies of susceptibility to this injury. DNA samples from patients will be tested for polymorphisms of drug-metabolizing enzymes and bile regulatory transporters and receptors. (0%)
- B2a. More fully elucidate the normal pathways of bile salt, lipid, and organic solute uptake, synthesis, transport, and secretion in hepatocytes.** The discovery of Ost α -Ost β to be the bile acid transporter through the basolateral membrane in the mouse ileum and its identification in human ileum, kidney and liver has helped to complete our understanding of the enterohepatic circulation of bile salts (Dawson PA. *J Biol Chem*. 2005;280: 6960; Ballatori N. *Hepatology* 2005;42:1270). (20%)
- B2b. Define the pathways and regulation of hepatic cholesterol synthesis and secretion.** This is an area of active investigator-initiated research. In the past year, the scavenger receptor class B type I (SR-BI) has been shown to be a regulator of cholesterol efflux from macrophages for excretion by the liver into bile and feces (Zhang YZ. *J Clin Invest* 2005;115:2870) Furthermore, the ABCG1 and ABCA1 transporters act synergistically in mediating cholesterol efflux from macrophages and incorporation into HDL particles (Gelissen IC. *Arterioscler*

Thromb Vasc Biol 2005; In press). Biliary cholesterol secretion is regulated largely through the ABCG5/G8 transporters, which in turn are regulated by the nuclear receptors LXR, FXR and PXR (Yu L. *J Biol Chem* 2005;280:8742). (10%)

- B3. Develop drug therapy that stimulates bilirubin metabolic pathways or interferes with bilirubin production in the newborn.** Advances in this area await further elucidation of the role of orphan nuclear receptors and the pathways of bilirubin and bile acid metabolism in the newborn and development of agents that target these pathways. (0%)
- C1. Define molecular basis of pruritus and identify targets for potential therapies.** Clinical and molecular studies indicate that rifampin enhances bile acid detoxification and bilirubin export, while ursodeoxycholic acid (UDCA) stimulates bile acid export, suggesting that this combination might be effective in treating cholestasis and pruritus (Marschall HU *Gastroenterology* 2005; 129: 476-85). Small grants of innovative therapies for pruritus are encouraged in the ongoing program announcement on “Small Clinical Grants in Digestive Diseases, Nutrition and Obesity” (PA-04-088). (10%)
- C2. Define the molecular basis and means of screening for or diagnosing acquired or adult forms of cholestatic liver disease such as cholestasis of pregnancy, sepsis, or total parenteral nutrition.** Two recent studies from Europe have shown that UDCA improves the biochemical laboratory tests and pruritus in women with intrahepatic cholestasis of pregnancy (Glantz A *Hepatology*. 2005;42:1399; Kondrackiene J. *Gastroenterology* 2005;129:894). (10%)
- C3. Develop effective gene therapy for at least one form of severe, neonatal cholestasis or hyperbilirubinemia.** Diseases of particular focus include Crigler-Najjar syndrome and Byler disease (PFIC-1); without liver transplantation, both of these diseases are usually fatal during childhood. Progress in gene therapy is encouraged by the NIH in several requests for applications and program announcements and through grants that use gene therapy centers. (0%)

Figure 6. Estimated Progress on Bile, Bilirubin, and Cholestasis Research Goals, 2005 (Year 1)



Chapter 5 Viral Hepatitis

- A1a. Define basis for interferon resistance in humans.** The NIH-funded Virahep-C study has evaluated clinical factors associated with non-response and has identified race, viral level, sex, degree of hepatic fibrosis and amount of medication taken as key clinical factors. The biologic basis remains unclear, but is the subject of ancillary studies of Virahep-C and several other NIH R01 grants. (10%)
- A1b. Define efficacy of interferon and ribavirin in subgroups of HCV patients.** Studies are ongoing regarding response rates and predictors of response to combination therapy of hepatitis C, including studies in children (Peds-C), liver transplant candidates (A2ALL: LADR Study), patients with renal failure (Intramural NIH; Roche), substance abusers (Sylvestre DL. *J Subs Ab Treat* 2005; 29:159), and minorities (Virahep-C; Latino Study, Roche). (20%)
- A2. Fully define the pathways of interferon induction and effector action against HCV and HBV *in vitro* and *in vivo*.** Multiple studies from NIH-funded investigators have demonstrated interactions between HCV and interferon signaling pathways leading to enhanced production of interferon and interferon-induced gene expression (Gale M Jr and Foy EM. *Nature* 2005;436:939). The interferon-beta promoter stimulator 1 (IPS-1) appears to be a key protein and a potential point of attack by HCV for immune evasion (Meylan E. *Nature* 2005;437:1167). Further research is needed to assess the clinical relevance of these findings in humans. (30%)
- A3. Develop a cell culture system that is fully permissive for HCV replication.** Three groups of NIH-funded investigators have reported a cell culture system for HCV using a full-length cDNA clone of genotype 2 HCV from a Japanese patient with severe hepatitis (Zhong J. *PNAS* 2005;102:9294; Lindenbach BD. *Science* 2005;309:623; Wakita T. *Nature Med* 2005;11:791). Further refinement of cell culture systems is needed to include all HCV genotypes and optimize culture conditions for studies of viral life cycle, neutralization and infectivity. (50%)
- B1a. Fully define early events during HCV and HBV infection.** Early events during HCV and HBV infection have been further characterized by NIH-funded investigations in chimpanzees (Wieland SF and Chisari FV. *J Virol* 2005;79:9369). The relevance of these findings in humans is being evaluated. The early immune responses during acute hepatitis C is a specific focus of the recently NIH-funded Hepatitis C Cooperative Research Centers Program. (20%)
- B1b. Define whether long-term interferon therapy is beneficial in non-responders with HCV.** Long-term peginterferon therapy is being evaluated in both NIH- (HALT-C) and industry-funded (EPIC3, Schering) studies. Results will be available in 2-3 years. (0%)
- B2a. Identify new targets in viral replication and the host for development of small molecule therapeutics (HCV, HBV, HDV).** NIH investigators have identified new targets in viral replication for HBV (receptor blockers, fatty acid biosynthetic pathways), HCV (inhibitors of membrane association, calcineurin

inhibitors, mediators of lipid metabolism), and HDV (prenylation inhibitors). None of these molecules have been tested in humans. (0%)

B2b. Define the molecular basis for antiviral resistance of HBV. Antiviral resistance of HBV has been studied largely by industry-sponsored scientists focusing upon proprietary nucleoside analogs. Prospective studies of humans that would include analysis of antiviral resistance have been proposed. (0%)

B3a. Develop small animal models of HCV replication and liver disease. Current animal models are limited in availability and applicability, but this need is likely to diminish if recently described cell culture systems fulfill their initial promise. NIH initiatives include a contract for *in vitro* and *in vivo* models of HCV infection (AI-25488), and an RFP (AI-05-012) and PA for animal models (DK-05-049). (10%)

B3b. Better characterize the HBV life cycle, virus-host interactions, basis for generation and stability of cccDNA and viral state of HBV in humans. HBV life cycle is the focus of several investigator-initiated NIH grants. An NIH workshop on HBV is scheduled for April 2006. (0%)

C1a. Evaluate new approaches to therapy in all five forms of viral hepatitis. NIH-funded investigators and several biotechnology companies are investigating the potential of RNA silencing (RNAi) as antiviral therapy for hepatitis C and B (Uprichard SL. *PNAS* 2005;102:773; Morrissey D. *Nat Biotech* 2005;23:1002). New approaches have yet to be initiated in humans. (10%)

C1b. Evaluate long-term benefits and risks of combination therapy of HBV. The long-term benefits and risks of combination therapy of HBV are being evaluated in NIH intramural studies, and prospective studies have been proposed. In the short-term, combination therapy (adefovir and lamivudine; peginterferon and lamivudine) does not appear to be better than monotherapy. An NIH workshop on management of HBV is scheduled for April 2006. (10%)

C2a. Develop ways to prevent re-infection after liver transplant for HCV (e.g. HCIG, anti-virals). A trial of therapy of hepatitis C before transplantation has been initiated (A2ALL, LADR Study: NIH and Schering). Pilot studies of HCIG by NIH-funded investigators and a Canadian group have failed to show significant benefit. Monoclonal antibodies to E2 glycoprotein have been shown to have neutralizing activity and may have potential in the transplant situation (Schofield DJ. *Hepatology* 2005;42:1055). (10%)

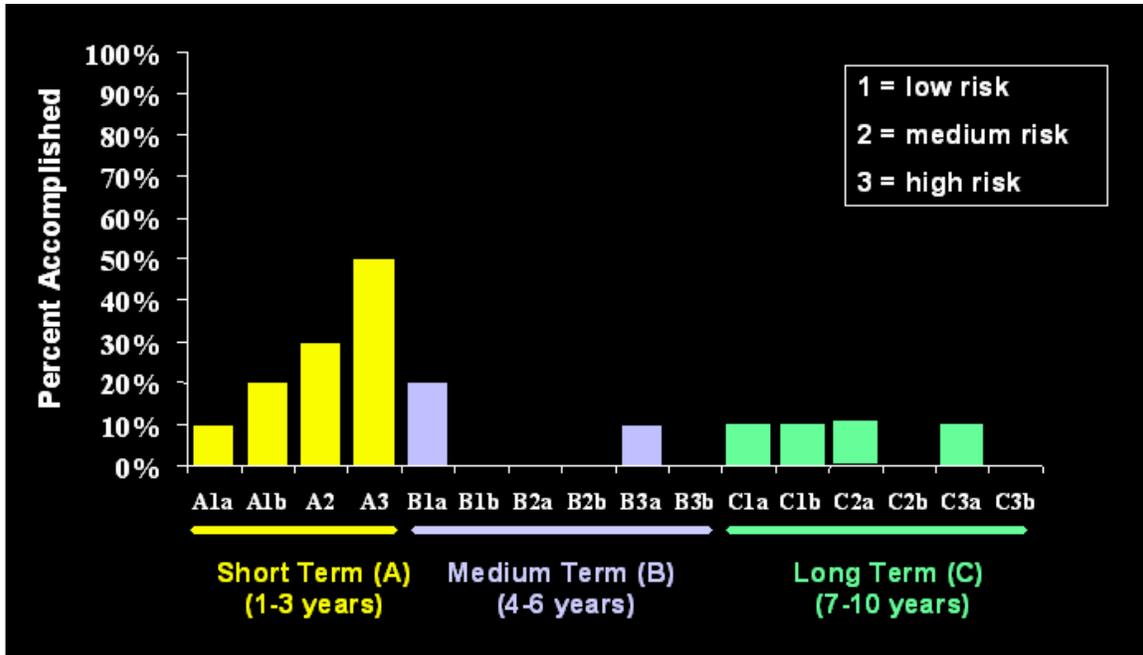
C2b. Achieve sustained response rate of over 90 percent in chronic hepatitis C. The sustained virological response rate to current therapy (peginterferon plus ribavirin) is 50-60 percent, and is higher in patients with genotypes 2 and 3 (approximately 80 percent) than genotype 1 (approximately 45 percent). Recently, two approaches have promised higher response rates: (1) high doses of ribavirin with peginterferon (Lindahl K. *Hepatology* 2005;41:275) and (2) novel protease and polymerase inhibitors with and without peginterferon in several phase II, industry-funded trials. (0%)

C3a. Develop HCV vaccine. An HCV vaccine has been developed by Chiron Corporation and evaluated in phase I trials largely in NIH-sponsored Vaccine and Treatment Evaluation Units. Phase II-III trials are being designed. HCV vaccine

development is an active area of individual investigator, NIH-funded research (10%)

C3b. Develop therapeutic HBV vaccine. NIH-funded basic research on innate immunity and T cell responses to vaccines promises to provide impetus to further work on a therapeutic HBV vaccine. The development of a therapeutic vaccine is being pursued using the transgenic mouse model of HBV infection. (0%)

Figure 7. Estimated Progress on Viral Hepatitis Research Goals, 2005 (Year 1)



Chapter 6 HIV and Liver Disease

A1a. Develop improved regimens of HAV and HBV vaccination. In a recent NIH-sponsored Pediatric AIDS Clinical Trials Group study, administration of a third dose of HAV vaccine gave higher levels of protective antibodies in children than with fewer doses (Weinberg A. *J Infect Dis* 2006;193:302). A study of immunologic boosting of HBV vaccine responses using GM-CSF in seronegative HIV-infected persons is in progress in an NIH-sponsored Adult AIDS Clinical Trials Group (AACTG) study. (10%)

A1b. Define short- and long-term safety and efficacy of peginterferon and ribavirin in different subpopulations of patients with HIV-HCV co-infection. Four large pivotal studies of peginterferon and ribavirin use in HIV/HCV co-infected persons were published in 2004. Additional studies are needed to define response rates in subpopulations of HIV/HCV co-infected patients. An ongoing AACTG study is evaluating whether concurrent HAART therapy improves response rates to peginterferon and ribavirin. (0%)

A2. Define safety and efficacy of peginterferon therapy for acute hepatitis C in HIV co-infection. A retrospective analysis of 11 HIV-infected patients with acute hepatitis C who were treated with interferon, with or without ribavirin, reported that 10 had a sustained virologic response (Vogel M. *J Viral Hepat* 2005;12:207). Better definition of the optimal time of starting, dose of peginterferon and ribavirin, and duration of therapy is needed. (20%)

A3. Define effects of HIV infection on the liver, including on different populations of liver cells. Little direct evidence exists of how HIV affects the liver; however, research is active in this area. A recent study reported no differences in CD8+ and CD4+ lymphocyte responses in the liver of co-infected vs HCV-mono-infected persons (Alatrakchi N. *J Infect Dis* 2005;191:702). HIV/HCV co-infected patients had more intrahepatic Fas expression than mono-infected persons (Macias J. *J Infect Dis* 2005;192:1566). (10%)

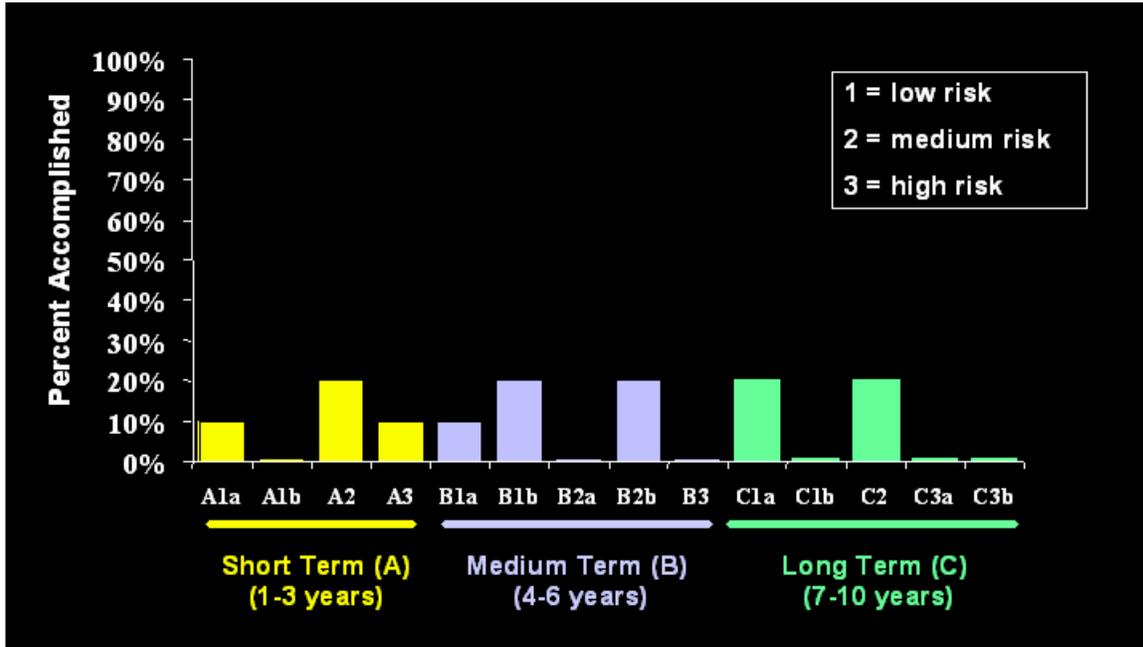
B1a. Define whether long-term peginterferon slows progression of disease in chronic hepatitis C with HIV co-infection. The AACTG is sponsoring a clinical trial entitled "Suppressive Long-term Antiviral Management of Hepatitis C Virus (HCV) in HIV-1 Co-infected Subjects" to evaluate the safety and efficacy of long-term antiviral treatment. (10%)

B1b. Define prevalence, etiology, and severity of different liver diseases in different cohorts of HIV-infected patients. Liver disease was the second leading cause of death in a large cohort of HIV-infected persons from Australia, Europe, and the US. Hepatic steatosis was present in 40 to 56 percent of HCV/HIV-co-infected persons and was associated with Caucasian race, increased body weight, lipodystrophy and stavudine use (Sulkowski MS. *AIDS* 2005;19:585; Marks KM. *J Infect Dis* 2005;192:1943). The role of antiretroviral

- therapy and alcohol intake in the progression of liver disease in HIV-infected persons remains controversial. (20%)
- B2a. Elucidate mechanisms by which HIV infection accelerates fibrosis and disease progression in HBV and HCV infection.** The mechanisms by which HIV infection accelerates progression of liver fibrosis remain largely unknown and are the focus of several ongoing studies. (0%)
- B2b. Define factors that lead to reactivation of HBV in HIV co-infection and develop means of prevention.** The causes of sudden worsening or reactivation of hepatitis B in HIV co-infected persons include (1) loss of anti-HBV due to progressive immune deficiency, (2) stopping antiretroviral drugs with anti-HBV activity, and (3) development (or selection) of HBV resistance mutations. Activity against lamivudine-resistant HBV is excellent for tenofovir and moderate for entecavir, but their optimal use needs to be better defined. (20%)
- B3. Develop noninvasive means of detecting early hepatic mitochondrial dysfunction.** New methods of detecting early mitochondrial dysfunction have not been reported. The NIH has encouraged research in this area through its initiatives on “Noninvasive Methods for Diagnosis and Progression” (PA-04-088) and “Development of Disease Biomarkers” (PA-05-098). (0%)
- C1a. Develop optimal therapeutic regimens for chronic hepatitis B in different stages and patterns of disease in HIV-co-infected patients.** In 2005, a European Consensus Panel recommended tenofovir-emtricitabine as the optimal treatment for hepatitis B in HIV-infected persons (Soriano V. *AIDS* 2005;19: 221). The long-term efficacy of this approach needs further definition and studies of newer agents (telbivudine, clevudine, entecavir) are in order. (20%)
- C1b. Define safety and efficacy of new agents for therapy of hepatitis C in HIV co-infection.** Combination therapy using peginterferon and ribavirin received FDA approval for use in HIV/HCV co-infected persons in 2005 (3 years after approval for HCV mono-infected patients). Although several new compounds with activity against HCV have been developed and are moving through clinical trial phases, HIV/HCV cohorts have not been included in early testing. (0%)
- C2. Develop noninvasive means of assessing liver disease stage and activity in HIV-infected persons.** During 2005, noninvasive methods for assessing the stage of liver disease were reported in HIV/HCV co-infected persons, including algorithms based on the serum testing and liver elastography, which yield reasonable correlations separating minimal from severe fibrosis. (20%)
- C3a. Develop *in vitro* or *in vivo* models of HIV-HCV and HIV-HBV co-infection.** *In vitro* systems of HCV replication were recently developed. Additional research is needed to build upon these systems in order to develop *in vitro* models of HIV/HCV or HIV/HBV co-infection. (0%)
- C3b. Develop means to reliably attribute causality of drug-induced liver disease in HIV-infected persons.** Collaborations between the Drug-Induced Liver Injury Network (DILIN) and the AACTG have been established to develop common instruments for assessing drug-induced liver disease. The complexity of liver

disease and the use of multiple drugs for treatment of HIV-infected persons makes assignment of causality of liver injury a challenge. (0%)

Figure 8. Estimated Progress on HIV and Liver Disease Research Goals, 2005 (Year 1)



Chapter 7 Fatty Liver Disease

- A1. Establish cohort study to prospectively analyze the natural history of the full spectrum of nonalcoholic fatty liver disease.** The NIH-funded NASH Clinical Research Network has begun enrollment of both adults and children with all stages of NAFLD into a database and registry. A total cohort of 1,200 individuals will be enrolled at 8 sites and will be followed for 5 years, thereby providing resources for studies of natural history, genetics, metabolomics and biomarkers. (40%)
- A2. Conduct phase I and II clinical trials of candidate therapies for NASH, TPN-associated liver disease, and alcoholic liver disease (e.g., silymarin, cytokines, anti-cytokines, anti-fibrotic agents).** An RFA to create a Silymarin Clinical Research Consortium was published in 2005 (RFA-AT-05-006) and four clinical sites and a data coordinating center will be funded in 2006 to initiate phase I/II trials of silymarin in treating NASH and chronic hepatitis C. (0%)
- A3. Develop more accurate animal models of nonalcoholic fatty liver disease (including secondary forms) and define molecular characteristics.** Established animal models of alcoholic liver disease include a continuous intragastric (Tsukamoto-French) and a voluntary alcohol ingestion (Lieber-De Carli) model in rats, neither of which reliably produces substantial fibrosis. New models of NASH include genetically-altered mice with abnormalities in nuclear hormone receptors (PPARs, FXR, LXR), insulin and IGF signaling (IRS-1, IRS-2, IGF-I), satiety factors (ob, alpha MSH, melanocortin receptor, NPY), cytokines and their receptors (TNF α , TNFR1, IL6, IFN γ , TGF β), and antioxidant factors (MAT-1 α , NADPH oxidase). These animal models might also be used to assess whether alcohol further exacerbates the injury. A recent PA has encouraged applications in this area: "Animal Models of NIDDK-Relevant Diseases" (PA-05-049). (10%)
- B1a. Elucidate the clinical, metabolic, proteomic, and gene expression patterns associated with various stages of nonalcoholic and alcoholic fatty liver disease.** The NASH Clinical Research Network and several NIH-funded single center clinical groups are preparing cohorts for proteomic and gene expression studies. (10%)
- B1b. Evaluate role and effects of bariatric surgery on NASH.** Several cross-sectional studies have shown that 26 to 44 percent of persons have NASH and 2 to 5 percent have cirrhosis at the time of bariatric surgery. The Longitudinal Assessment of Bariatric Surgery (LABS) was funded as a cooperative agreement with a data coordinating center and 6 clinical sites and has initiated prospective studies of bariatric surgery, including studies of its effects on NASH. (10%)

- B2a. Delineate the hepatic pathways of lipid metabolism and how they are altered in alcoholic and nonalcoholic liver disease.** Stable isotope studies in humans suggest that, in NAFLD, there is increased hepatic lipogenesis and possibly reduced ability to mobilize hepatic lipids into VLDL. Further definition of fatty acid metabolic pathways in humans with fatty liver disease is needed and has been encouraged in the NIH PA on “Mechanisms of Alcoholic and Nonalcoholic Fatty Liver” (PA-05-119). (10%)
- B2b. Develop noninvasive means of distinguishing steatosis from steatohepatitis and for grading and staging disease.** Use of biomarkers and imaging studies including elastography are being evaluated in cohorts of patients with NAFLD, but clinically relevant markers are not available. Development of biomarkers in liver disease has been encouraged in the recent NIH PA on “Development of Disease Biomarkers” (PA-05-098). (0%)
- B3a. Develop rapid-throughput systems to evaluate potential therapies of fatty liver disease.** Until the metabolic abnormalities that underlie fatty liver disease are better defined, *in vitro* systems for screening small molecules will be limited. The NIH Roadmap for Medical Research encouraged research in this area through the RFA on “Pilot-Scale Libraries for High-Throughput Drug Screening” (RFA-RM-05-014), and a PA for similar grants was published as “Development of Assays for High-Throughput Drug Screening” (PA-05-068). (0%)
- B3b. Develop therapy of acute alcoholic hepatitis that promotes recovery and decreases permanent injury.** Small clinical trials of pentoxifylline and TNF antagonists showed modest promise, while a study of high dose TNF- α antibody and prednisone suggested that this approach may be harmful. Both pilot studies of innovative therapies and larger, more rigorous trials of promising therapies are needed. (0%)
- C1a. Establish the efficacy and safety of therapy with insulin-sensitizing agents and vitamin E in NASH.** The NASH Clinical Research Network recently began enrolling subjects into two prospective, randomized, placebo-controlled trials: one using pioglitazone in adults and another using metformin in children. Results should be available in two years. (20%)
- C1b. Establish the efficacy and safety of therapy with SAME in alcoholic liver disease.** A pilot trial of SAME therapy for alcoholic liver disease has been funded by the NIH. (0%)
- C2a. Establish the prevalence and incidence of NASH in the general population as well as special populations in the United States, such as children, minority groups, and patients with diabetes and other dysmetabolic syndromes.** The lack of noninvasive markers hampers efforts to determine the prevalence of NASH in the general population. Serum ALT elevations have been used as a surrogate marker for NAFLD and NASH, but the normal range is not clearly

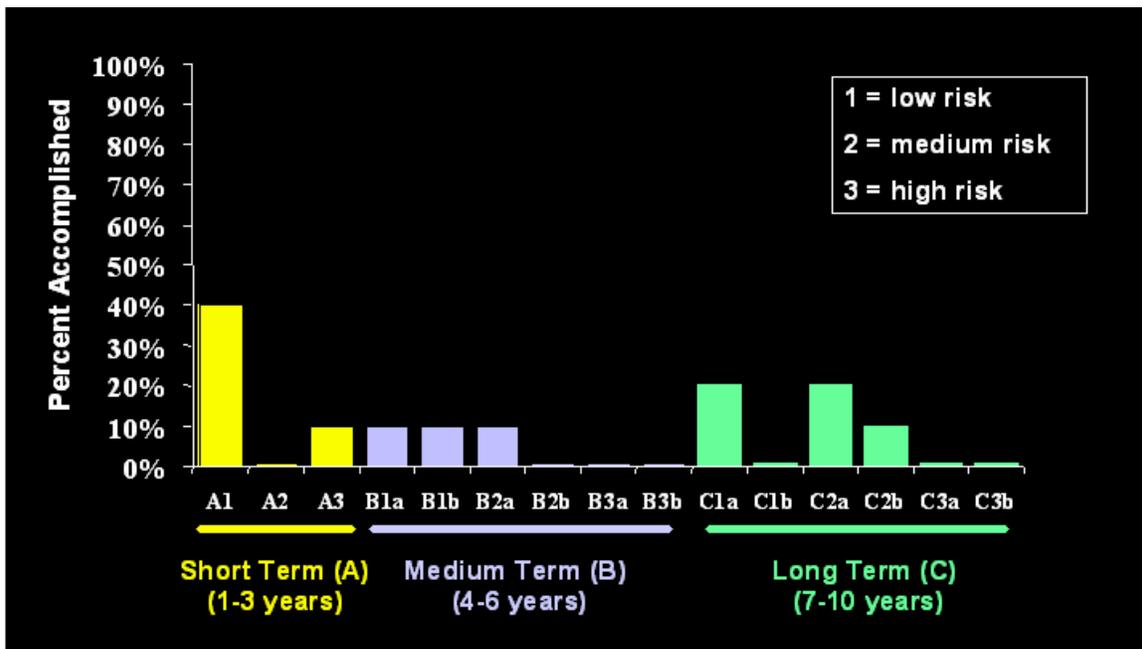
defined (using age, sex, race and weight-based controls), and unexplained ALT elevations are not diagnostic of NAFLD. Imaging studies using NMR spectroscopy suggest that 31 percent of American adults have hepatic steatosis, with the rates being highest in Hispanics (45%), intermediate in whites (33%), and lowest in blacks (24%) (Browning JD, *Hepatology* 2004;40:1387). (20%)

C2b. Better define the safe amounts of alcohol intake in terms of liver disease for different populations. In the NHANES population, moderate alcohol intake is associated with increased ALT levels only in overweight and obese subjects, but not in normal weight persons, suggesting that alcohol exacerbates NAFLD (Ruhl CE. *Clin Gastroenterol Hepatol* 2005;12:1260). Prospective evaluations of alcohol intake and progression of NAFLD and other liver diseases are needed to better define safe alcohol intake in terms of liver disease risk. (10%)

C3a. Identify genetic markers for development of steatohepatitis and its complications. Linkage studies have identified several candidate genes associated with NAFLD, but larger studies using a greater number of patients and analyzing more polymorphisms are needed. (0%)

C3b. Develop screening programs for early detection and intervention with preventative or therapeutic regimens. Until accurate non-invasive markers for NAFLD and better information on means of treatment and prevention are available, screening programs cannot be initiated. (0%)

Figure 9. Estimated Progress on Fatty Liver Disease Research Goals, 2005 (Year 1)



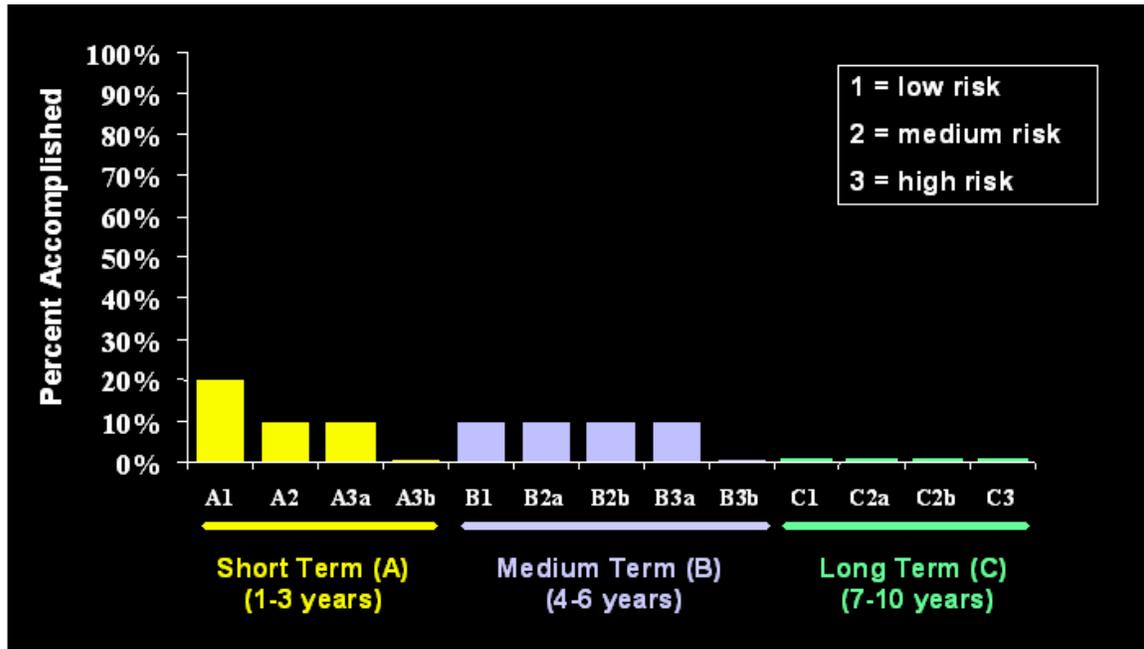
Chapter 8

Drug- and Toxicant-Induced Liver Disease

- A1. Develop definitions and standardization of procedures for diagnosis of hepatotoxicity and assignment of causality.** The NIH-funded Drug-Induced Liver Injury Network (DILIN) has developed a rigorous process for assessing causality and a preliminary instrument that is being tested against other instruments in a prospective manner. The instrument needs further refinement and validation. (20%)
- A2. Develop positive diagnostic assay for acetaminophen toxicity.** An assay for acetaminophen adducts has been developed and is being tested for sensitivity and specificity in detecting acetaminophen-induced liver injury. (10%)
- A3a. Develop *in vitro* or *in vivo* systems for study of allergic and non-allergic idiosyncratic hepatotoxicity.** Mice given low doses of lipopolysaccharide have increased susceptibility to liver injury from several drugs known to cause idiosyncratic drug injury in humans, suggesting that low levels of hepatic inflammation predispose to this injury (Waring JF. *J Pharmacol Exp Ther* 2005; 316:1080). A Program Announcement on “Animal Models of NIDDK-Relevant Diseases” (PA-05-049) encourages research to develop animal models of hepatotoxicity. (10%)
- A3b. Identify chemical substructures that are protoxicant and could be avoided in design of new drugs.** Investigator-initiated work on an atlas of chemical substructures that are protoxicant has started. (0%)
- B1. Develop a cohort of patients with well-characterized hepatotoxicity and controls with availability of serum, tissue, RNA, and DNA for genomic, transcriptomic, proteomic, and metabolomic studies.** The DILIN network has enrolled more than 150 patients with idiosyncratic drug-induced liver injury into a database with careful collection of clinical information, serum, DNA and tissue. (10%)
- B2a. Elucidate molecular mechanisms of common forms of hepatotoxicity.** Efforts to define the molecular mechanisms of acetaminophen toxicity have dominated the research progress in this area with elucidation of the potential roles of stress kinases, DNA damage, ATP depletion, and the Bcl-2 family of apoptosis regulators. (10%)
- B2b. Define incidence of drug-induced liver injury and the contribution of hepatotoxicity to the burden of acute and chronic liver disease in the United States.** Funding for the Acute Liver Failure Study Group has been extended, and a pediatric component has been separately funded. Drug-induced liver disease continues to be the major cause of acute liver failure and secular trends suggest that it is increasing. Population-based studies have also been conducted in Spain, Switzerland, France and Sweden. (10%)

- B3a. Define the role of the innate immune system in both allergic and non-allergic forms of hepatotoxicity.** The role of the innate immune system (NK and NKT cell function, interferon gamma and the Fas/FasL system) has been assessed in the acetaminophen injury model in mice. These studies need to be extended to other types of hepatotoxicity and to humans. (10%)
- B3b. Develop an animal model of adaptation to hepatotoxicity to help define the genes necessary for the adaptive response.** A Program Announcement has been published soliciting investigator-initiated research grants in this specific area (“Animal Models of NIDDK-Relevant Diseases,” PA-05-049). (0%)
- C1. Identify genetic factors that contribute to hepatotoxicity of several major forms of drug-induced liver disease.** This area is the focus of extensive studies by industry and by the NIH-funded DILIN and Pharmacogenetics Research Networks, which are developing genetic screens for susceptibility to drug-induced liver injury. Several NIH- and industry-supported efforts are focusing upon transcriptomics and metabolomics evaluation of drug-induced toxicity in animal models. (0%)
- C2a. Determine the efficacy of nonspecific therapy of hepatotoxicity with antioxidants or hepatoprotective medications.** Both the adult and the pediatric Acute Liver Failure Study Groups are evaluating N-acetylcysteine as therapy for drug-induced and other forms of acute liver failure, the results of which should be available in 2 to 3 years. Trials of other agents have not been conducted. (0%)
- C2b. Develop and assess biomarkers or metabolites to predict the development of hepatotoxicity, and to distinguish between established hepatotoxicity and transient, adaptive enzyme elevations.** The DILIN Network as well as the Pharmacogenetics Research Networks are focused on these issues. This is also an area of interest to industry. Biomarkers are likely to be developed once cellular pathways of drug-induced liver injury are more fully defined. (0%)
- C3. Develop molecular signatures that are diagnostic for major forms of hepatotoxicity.** Investigator-initiated research studies as well as the Pharmacogenetics Research and DILIN Networks are focusing on developing resources and using transcriptomics, proteomics and metabolomics to provide insights into how drugs cause liver injury. An FDA Critical Path Initiative (Liver Toxicology Biomarker Study) has also begun, in order to encourage research in this area. (0%)

Figure 10. Estimated Progress on Drug- and Toxicant-Induced Liver Disease Research Goals, 2005 (Year 1)

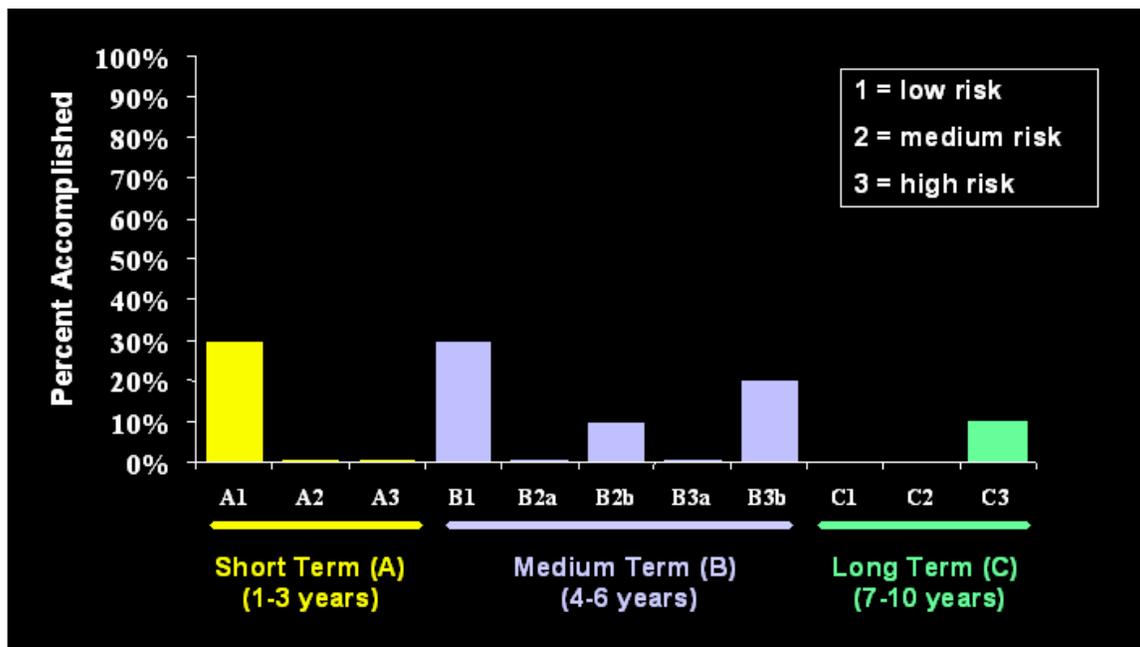


Chapter 9 Autoimmune Liver Disease

- A1. Organize and convene an international, interdisciplinary research workshop on development of animal models of autoimmune liver diseases.** The NIH Workshop on Primary Sclerosing Cholangitis (PSC) in September 2005 included a comprehensive presentation on animal models in this disease. (30%)
- A2. Develop multicenter networks of investigators to study natural history, pathogenesis, etiology, and therapy of autoimmune liver diseases.** Discussion among investigators is ongoing about the organizational and operational structure of such multicenter networks. (0%)
- A3. Define the roles of CD4+ and CD8+ T cells, other effector immunocytes, dendritic cells, and the innate immune system in liver injury in humans (and animal models) with autoimmune liver disease.** This is being addressed currently in the context of NIH-supported research projects. Human studies would be facilitated by autoimmune liver disease networks. (0%)
- B1. Demonstrate whether high-dose ursodiol therapy is effective in retarding the progression of PSC and identify risk factors for progression and for response to treatment.** The NIH sponsors a multicenter controlled trial of high-dose ursodiol in PSC, which completed its enrollment in late 2005. A similar study in Europe has demonstrated the lack of effect of high-dose ursodiol on survival or outcome (Olson R. *Gastroenterology* 2005; 129:1464). (30%)
- B2a. Develop sensitive and specific biomarkers for disease activity and stage in PBC and PSC.** The NIH has encouraged research in this area through its initiative on “Development of Disease Biomarkers” (PA-05-098). (0%)
- B2b. Develop diagnostic criteria and standard definitions for endpoints of therapy.** Discussions on developing standardized terminology and diagnostic criteria for liver and biliary diseases were started at an NIH/AASLD workshop on “Nomenclature, Diagnostic, and Outcome Criteria in Liver and Biliary Diseases” in November 2005 and at the September 2005 NIH Workshop on PSC. (10%)
- B3a. Identify genetic linkages in PBC and refine the HLA associations in autoimmune hepatitis and PSC.** This goal would be facilitated by the establishment of autoimmune liver disease networks. The NIH encourages research to refine understanding of the association of HLA with autoimmune liver diseases through its initiative on “HLA Region Genetics in Immune-mediated Diseases” (RFA-AI-04-039). (0%)
- B3b. Develop animal models for each of the autoimmune liver diseases.** Promising models have been developed for PSC (Mdr2^{-/-} mouse: Popov Y. *J Hepatol* 2005;43:1045) and autoimmune hepatitis (TGFβ1 knockout mouse: Lin JT. *Lab Invest* 2005;85:550). A recent NIH initiative on “Animal Models of NIDDK-relevant Diseases” (PA-05-049) specifically encourages research to develop animal models of autoimmune liver diseases. (20%)

- C1. Develop alternatives to prednisone/azathioprine as maintenance therapy of autoimmune hepatitis and define markers for when and how therapy can be safely stopped.** The NIH sponsors an initiative on “Innovative Grants in Immune Tolerance” (RFA-AI-05-023), which encourages research on alternative maintenance therapy for autoimmune hepatitis. (0%)
- C2. Develop sensitive serum markers for early detection of cholangiocarcinoma in PSC.** The NIH sponsors a multicenter controlled trial of high-dose ursodiol in PSC in which serum and tissue samples are collected and stored for potential investigation of markers for early detection of cholangiocarcinoma. Additionally, the NIH encourages research in this area through its initiative on “Development of Disease Biomarkers” (PA-05-098). (0%)
- C3. Identify modifiable environmental (with or without genetic) triggers for induction of autoimmune hepatitis (from human studies or murine models).** In an NIH-funded multicenter study, risk factors for PBC were sought among a large collection of patients and controls; evidence for infectious and toxic exposures as triggers for PBC were found (Gershwin ME. *Hepatology* 2005; 42:1194). The NIH sponsors an initiative on “Innovative Grants in Immune Tolerance” (RFA-AI-05-023), which encourages research on triggers of autoimmune disease. (10%)

Figure 11. Estimated Progress on Autoimmune Liver Disease Research Goals, 2005 (Year 1)

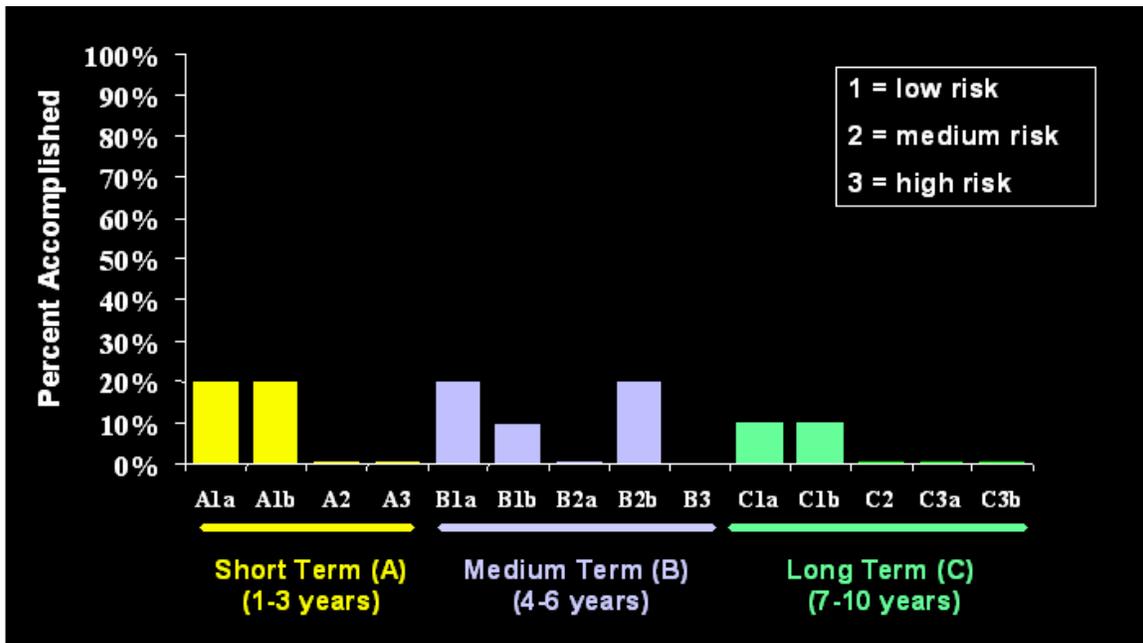


Chapter 10 Pediatric Liver Disease

- A1a. Characterize clinical syndrome, natural history, etiology, cofactors, and complications of pediatric NASH.** The histologic features of NASH are different in children than in adults (Schwimmer JH. *Hepatology* 2005;42:641). A scoring system for NAFLD for use in clinical investigation, natural history studies and therapeutic trials has been developed by the NIH-funded NASH Clinical Research Network (Kleiner DE. *Hepatology* 2005;41:1313). (10%)
- A1b. Develop definitions and diagnostic criteria for the major neonatal cholestatic syndromes.** Investigators in the NIH-funded Biliary Atresia Research Consortium (BARC) and Cholestatic Liver Disease Consortium (CLiC) have developed clinical definitions and diagnostic criteria for the major neonatal cholestatic syndromes for use in an observational longitudinal study. (10%)
- A2. Develop systems to better characterize the frequency, medical burden, and epidemiology of pediatric liver disease.** Epidemiologic research is a component of several NIH-supported studies, including the NASH Clinical Research Network, BARC, CLiC, the Pediatric Acute Liver Failure Study Group (P-ALFSG), the Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C (Peds-C) trial and the SPLIT pediatric liver transplant registry. (0%)
- A3. Elucidate the major cause of idiopathic acute liver failure in children.** The ongoing ALFSG has shown that 52% of cases of acute liver failure in children are of unknown etiology. Investigation of abnormal bile acid metabolism, viruses, toxins, cytokines, abnormal immunological responses and inborn errors of metabolism such as fatty acid oxidation defects are a part of ancillary studies of the recently funded Pediatric ALFSG. (0%)
- B1a. Define structural and functional development of the liver and biliary system.** The transcription factors Foxa and GATA6 play critical roles in the embryogenesis of the liver (Lee CS. *Nature* 2005;435:944; Zhao R. *Mol Cell Biol* 2005;25:2622). Using novel approaches to label embryonic cells, the anatomical disposition of cells during patterning of the embryonic endoderm has been described (Temblay KD. *Dev Biol* 2005;280:87). Studies in zebrafish have shown the critical role of networks of transcription factors and signaling pathways in development of the biliary tree (Sadler KC. *Development* 2005;132:3561). (20%)
- B1b. Evaluate long-term outcomes, complications, and tolerance-inducing regimens in children undergoing liver transplantation.** Analysis of data on children who receive liver transplants from the United Network for Organ Sharing (UNOS) has shown that smaller reduced liver grafts, life support at transplantation, and younger age are associated with increased post-transplant mortality (Barsches NR. *Liver Transpl* 2005;11:1193). The NIH-funded SPLIT registry has identified nutrition as an important and potentially modifiable risk factor for post-transplant outcomes (Utterson EC. *J Pediatr* 2005;147:180). (10%)

- B2a. Delineate the molecular pathogenesis of at least 3 of the neonatal cholestatic syndromes.** A specific focus of the NIH-funded CLiC consortium is to define the molecular pathogenesis of all of the neonatal cholestatic syndromes. (0%)
- B2b. Develop better animal models for neonatal cholestatic syndromes.** Genetic and immunologic studies in a mouse model of rotavirus-induced neonatal biliary injury shows that it recapitulates key liver and biliary features found in children with biliary atresia (Carvalho E. *Gastroenterology* 2005;129:713; Mack CL. *Clin Immunol* 2005;115:200). Two mouse models have been further characterized with inactivation of the following genes associated with neonatal cholestatic syndromes: *Abc11* (Henkel A. *Mamm Genome* 2005;16:903) and *Abcb4/Mdr2* (Popov Y. *J Hepatol* 2005;43:1045). The NIH encourages research in this area through PA-05-049: “Animal Models of NIDDK-Relevant Diseases.” (20%)
- B3. Identify biomarkers for diagnosis, staging, and grading of neonatal cholestatic syndromes.** NIH-funded investigators participating in CLiC are developing study protocols to diagnose, stage, and grade cholestatic syndromes and will be testing a novel customized re-sequencing gene chip and proteomics technology for use in identifying biomarkers of pediatric cholestasis. (0%)
- C1a. Conduct clinical trials to optimize medical and surgical management of biliary atresia.** NIH-funded BARC investigators have initiated a prospective randomized, placebo-controlled trial of corticosteroids after portoenterostomy in infants with biliary atresia. (10%)
- C1b. Evaluate therapies for acute liver failure in children.** Investigators in the newly created NIH-funded Pediatric ALFSG have initiated a prospective, randomized controlled trial of N-acetyl-cysteine as a medical treatment for children with acute liver failure. (10%)
- C2. Based upon molecular pathogenesis, identify small molecule therapies that might alleviate neonatal cholestatic syndromes.** Targets for small molecule therapies include the nuclear hormone receptors that regulate bile acid and anion transport and secretion. *In vitro*, high-throughput screening of small molecules with possible use in neonatal cholestatic syndromes is encouraged through the Roadmap trans-NIH RFA “Assay Development for High Throughput Molecular Screening” (RM-05-011). (0%)
- C3a. Define the etiology of biliary atresia.** This goal is the major focus of the BARC Consortium, which is enrolling patients and collecting clinical data, serum DNA, and liver and biliary tissue for investigation of the etiology of this disease. Ancillary studies to use the resources generated by the BARC Consortium are encouraged in a program announcement on “Ancillary Studies to Major Ongoing NIDDK Clinical Research Studies” (PAR-04-082); several of these studies have been funded. (0%)
- C3b. Develop gene, siRNA, cell transfer, or stem cell therapy for pediatric metabolic disease.** Both NIH- and industry-funded research investigators are extremely active in this area. (0%)

Figure 12. Estimated Progress on Pediatric Liver Disease Research Goals, 2005 (Year 1)



Chapter 11 Genetic Liver Disease

- A1a. More fully define the frequency of disease expression associated with *HFE* C282Y and define major modifying factors.** In the Hemochromatosis and Iron Overload Screening (HEIRS) Study of approximately 100,000 North Americans, C282Y homozygosity was identified in 0.44 percent of whites but only 0.12 percent of African-Americans and less than 0.1 percent of Asians. Most C282Y homozygotes (88% of men; 59% of women) had high serum ferritin levels; no genetic modifiers have been identified as yet (Adams PC. *N Engl J Med* 2005;352:1769). (20%)
- A1b. Identify a cohort of patients with congenital hepatic fibrosis to study its natural history and optimal management.** An intramural NIH protocol has been initiated and enrolled 38 patients with autosomal recessive polycystic kidney disease (ARPKD), many of whom have congenital hepatic fibrosis (CHF). Cross-sectional and longitudinal data, genetic testing, and genotype-phenotype studies are underway. (20%)
- A2a. Establish DNA evaluation centers of excellence for Wilson disease, the porphyrias, and hemochromatosis.** No such centers have been established.(0%)
- A2b. Develop a reliable animal model for the liver disease of cystic fibrosis.** A mouse model with deletion of the *CFTR* gene has been developed that develops progressive liver disease with steatosis, cholangitis, and inspissated secretions (Durie PR. *Am J Pathol* 2004;164: 1481). This model has potential for evaluation of means of prevention or treatment of the liver disease of CF, as well as analysis of modifier genes for development of liver injury in CF. (20%)
- A3. Fully elucidate the molecular mechanisms of intestinal absorption, hepatic metabolism, and biliary excretion of copper.** New steps in the pathway of hepatic metabolism of copper have been elucidated, including the role of several transporters and chaperones (Burstein E. *J Biol Chem* 2005;23:2222). (10%)
- B1a. Develop and apply practical and accurate screening methods for identifying hemochromatosis before significant tissue injury has occurred.** Screening tests for hemochromatosis include transferrin saturation and ferritin. The applicability of these tests in the general population is now being assessed with the HEIRS cohort. (0%)
- B1b. Define the role of heterozygosity for Wilson ATPase and *HFE* mutations in other liver diseases.** The role of *HFE* mutations in worsening other liver diseases is controversial, particularly in its role in progression of fibrosis in hepatitis C and nonalcoholic steatohepatitis. *HFE* mutations and responses to therapy are currently under investigation. Because of their heterogeneity, the Wilson ATPase mutations have not been analyzed in large cohorts of patients with other liver diseases. (10%)

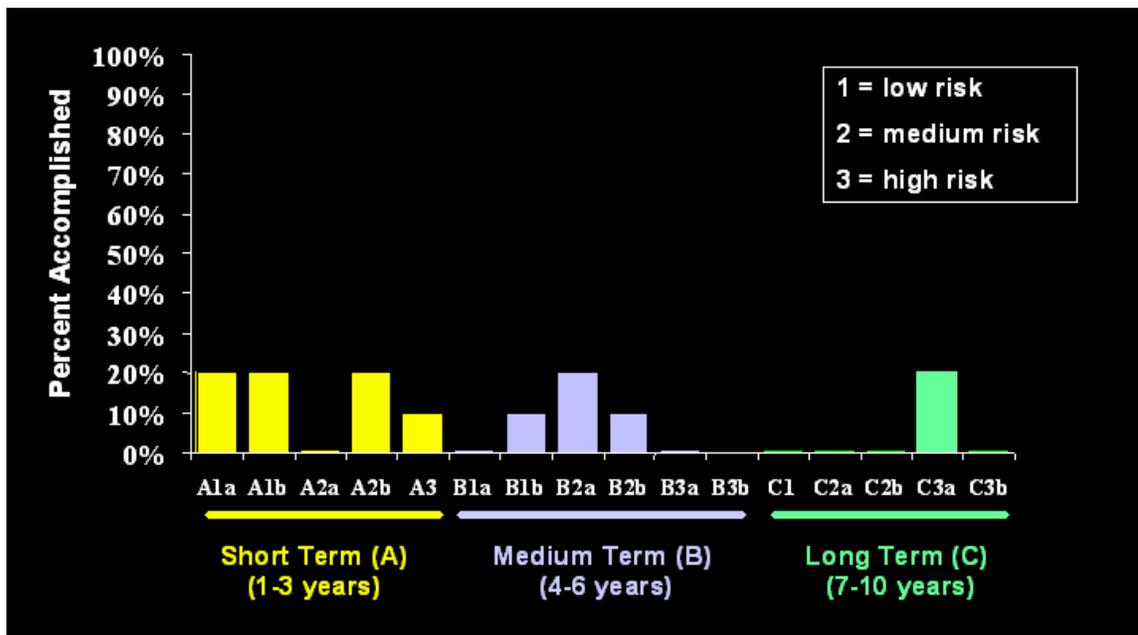
- B2a. Fully define the normal molecular pathways of iron metabolism in humans with specific definition of the roles of HFE and hepcidin.** Major advances have been made in the elucidation of the role of hepcidin and other molecules in iron metabolism. Hepcidin acts as a negative regulator of iron absorption by inducing internalization and degradation of the ferroportin transporter in enterocytes and macrophages (Nemeth E. *Science* 2004;306:2090; Donovan. *Cell Metabol* 2005;1:191). The molecular basis for ferroportin-linked hemochromatosis has been further elucidated (De Domenico I. *PNAS* 2005; 102:8955). The divalent metal ion transporter 1 (DMT1, also known as SLC11A2) has been shown to be the major transmembrane iron transporter of the intestine and erythroid precursors, but hepatocytes and other cells have an alternative, as-yet-unknown, iron uptake mechanism (Gunshin H. *J Clin Invest* 2005; 115:1258). (20%)
- B2b. Define the role of liver iron levels in the course of NASH, alcoholic liver disease, chronic hepatitis C, and porphyria cutanea tarda.** Both serum and hepatic iron levels are often high in patients with chronic liver disease and they often correlate with more advanced fibrosis and poor response to therapy. The pathogenesis of this poor response and possible means of improving responses by iron depletion await further elucidation. (10%)
- B3a. Identify the major genetic causes of inherited iron overload among African Americans, Asian Americans, and Hispanics.** The classical C282Y *HFE* mutation accounts for few cases of iron overload in African and Asian Americans and Hispanics (Barton JC. *Genetic Testing* 2005; 9:231). Other causes possibly related to mutations in other iron transporters or signaling molecules have yet to be identified. (0%)
- B3b. Define the molecular basis of the increase in HCC risk among persons with the porphyrias.** Links between the molecular abnormalities of porphyrin metabolism in the inherited porphyrias and pathways of carcinogenesis have not been identified. Iron overload typical of porphyria cutanea tarda and elevated 5-aminolevulinate typical of the acute porphyrias are known to cause oxidative stress, a potential factor in liver carcinogenesis. (0%)
- C1. Develop rapid metabolic screening test for Wilson disease that could also be applied to newborns or infants and assess test for efficacy and risk-benefit ratio.** Until there is a more complete understanding of copper metabolism and its control, there is unlikely to be a rapid metabolic screening test for Wilson disease. Testing for the most common Wilson ATPase mutations might identify 30 to 40 percent of cases, but this approach is currently not practical for screening purposes. (0%)
- C2a. Define specific genetic modifiers of Wilson disease and porphyrias using animal models and clinical cohorts of patients.** In large patient cohorts from Europe and the United States, no genetic modifiers of Wilson disease have been identified. (0%)
- C2b. Develop an improved therapy for amelioration of acute crises in porphyria.** Intravenous hemin therapy remains the recommended therapy of severe acute

crises (Anderson K. *Ann Intern Med* 2005;142:439), but a possible new approach using recombinant porphobilinogen deaminase is now in phase I/II clinical trials. (0%)

C3a. Develop noninvasive means of accurately defining total body and hepatic iron and copper, either using imaging studies or mathematical models and serum levels of related molecules. MR techniques are capable of measuring iron levels associated with severe iron overload and are being refined to provide more accurate and sensitive quantitative assessments (St Pierre TG. *Ann NY Acad Sci* 2005; 1054:379). Special MRI algorithms have been approved for clinical use in the United States. (20%)

C3b. Develop practical gene or stem cell therapy for AIP and EPP. No gene therapies for AIP or EPP have been developed, although liver transplantation may be effective for intractable cases. Gene therapy research is promoted by NIH-funded Molecular Therapy Centers. (0%)

Figure 13. Estimated Progress on Genetic Liver Disease Research Goals, 2005 (Year 1)



Chapter 12

Liver Transplantation

- A1. Develop further refinements in the MELD and PELD systems that optimize allocation of livers for transplantation.** The MELD and PELD systems are under continuing assessment by the Scientific Registry of Transplant Recipients (SRTR) and the NIH-funded registry entitled Studies of Pediatric Liver Transplantation (SPLIT). The inclusion of outcome or “transplant benefit” in allocation is being studied in depth. Attempts are being made to reduce geographic variation in allocation (Rodriguea-Luna H. *Amer J Transplant* 2005;5:2244). In adults, addition of serum sodium to the MELD score may improve accuracy (Biggins SW. *Hepatology* 2005;41:32). (20%)
- A2. Identify biomarkers for acute cellular rejection and adequacy of immune suppression.** Identification and diagnosis of early rejection remains a clinical challenge and often requires liver biopsy. The NIH encourages this area of research through the Immune Tolerance Network and the program announcement on “Development of Disease Biomarkers” (PA-05-098), which specifically mentions non-invasive means of detection of acute rejection, immune suppression and tolerance. (0%)
- A3. Elucidate pathways of liver regeneration and identify targets for drug or cytokine/anticytokine therapy.** Liver regeneration is an area of active support in multiple investigator-initiated studies. Underpinning the process of liver regeneration is an immense network of up- and down-regulated cellular signaling pathways. Studies have largely been in murine models (White P. *J Biol Chem* 2005; 280:3715). (10%)
- B1a. Define efficacy of peginterferon and ribavirin in pre- and post-transplant HCV infection.** A single center experience with peginterferon and ribavirin suggests that some patients with advanced hepatitis C can clear virus before transplantation and not suffer recurrence in the graft (Everson GT. *Hepatology* 2005; 42:255). The Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) has initiated a controlled trial of peginterferon and ribavirin versus no treatment in patients with hepatitis C awaiting transplantation. (20%)
- B1b. Improve safety and define role of living donor liver transplantation.** The A2ALL cohort study has documented that living donor liver transplantation can improve patient outcome, but only after a steep learning curve in the first 20 patients (Olthoff KM. *Ann Surg* 2005;242:314). Further assessment, including long-term follow up of donors and recipients, is critical. (20%)
- B2a. Delineate molecular pathways of immune tolerance to allografts in humans.** The Immune Tolerance Network is specifically focused on studies to delineate the mechanisms of immune tolerance after transplantation and means of achieving tolerance in patients. (0%)
- B2b. Develop new therapies for hepatitis C that are effective in the transplant situation.** Several HCV-specific protease and polymerase inhibitors have been

developed and are in phase I/II trials in patients with uncomplicated hepatitis C, but none have been evaluated in transplant patients. (0%)

B3a. Elucidate the pathogenesis of post-transplant lymphoproliferative disease (PTLD) and means of prediction, prevention, and control. The NIH-funded SPLIT study group is currently addressing the issue of post-transplant complications and morbidities, including the issue of PTLT and focusing on potentially modifiable risk factors. Low dose chemotherapy is effective in at least two-thirds of cases (Gross TG. *J Clin Oncol* 2005;23:6481). (10%)

B3b. Develop means of improving regeneration after living donor liver transplantation. Regeneration of the liver after live donor liver transplantation is important for both recipient and donor. In animal models, infusions of IL-6 and pretreatment with thyroid hormone improve regeneration; studies in humans have not been done. (0%)

C1a. Define factors important in long-term success of liver transplantation in children as defined by quality of life and social/psychological development. The SPLIT study group is currently accumulating data on factors associated with long-term successful liver transplantation in children, including functional outcomes in terms of quality of life and academic achievement. (0%).

C1b. Determine efficacy of chemotherapy and local ablative treatment of HCC done in the peri-transplant period. The recently released program announcement entitled, "Etiology, Prevention, and Treatment of Hepatocellular Carcinoma" (PA-05-137) encourages research in local ablative therapy. (0%)

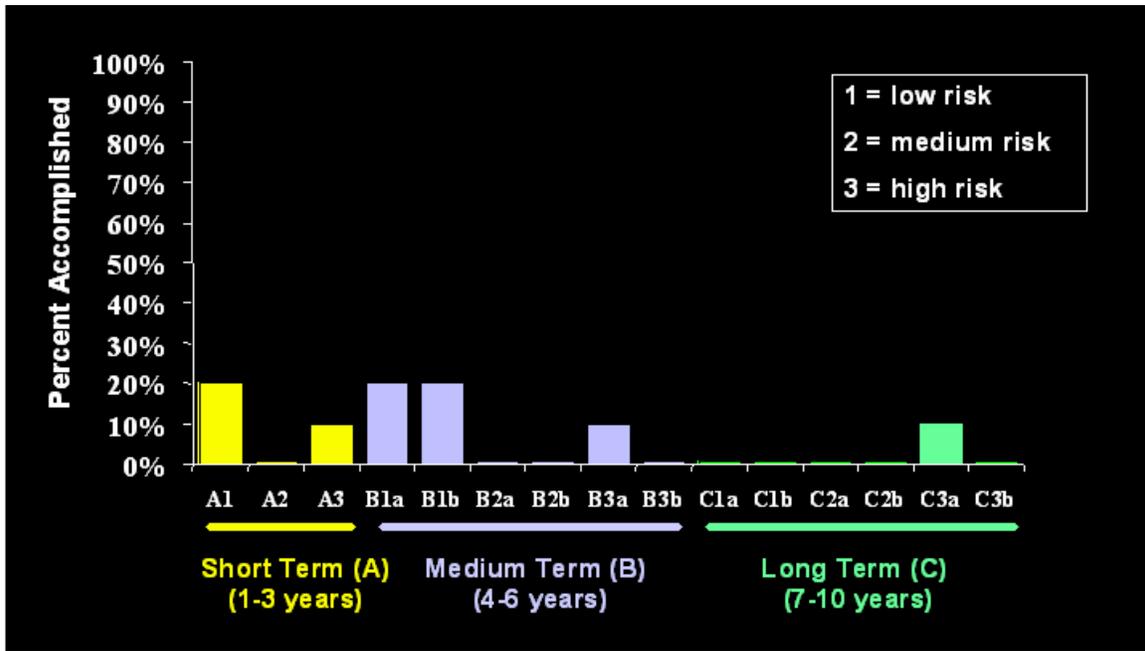
C2a. Based upon molecular mechanisms, develop and assess tolerance-inducing regimens, including studies in children. Trials of new agents or combinations of agents that might accelerate tolerance have been proposed in the SPLIT study group. (0%)

C2b. Identify biomarkers that predict tolerance and the ability to discontinue immunosuppression after liver transplantation. The NIH-sponsored Immune Tolerance Network serves as the umbrella organization of a trans-NIH effort to clinically characterize immunological tolerance in transplantation. (0%)

C3a. Develop means to prevent recurrence of hepatitis C after liver transplantation. Trials of peginterferon and ribavirin therapy in patients before transplantation aimed at prevention of recurrence are underway in the A2ALL cohort study. Small pilot studies of HCIG from Canada and from the NIH-funded Antiviral Cooperative Study Group (ACSG) demonstrated no effect of this product in preventing recurrence. (10%)

C3b. Develop gene or cell therapy for at least one metabolic liver disease that delays or replaces liver transplantation. The NIH research portfolio supports several R01 grants focused on gene and cell therapy of liver diseases that are currently treated with transplantation, including alpha-1-antitrypsin deficiency, PFIC, and Crigler-Najjar syndrome. Gene therapies are being evaluated in animal models, but not in human subjects with these diseases. (0%)

Figure 14. Estimated Progress on Liver Transplantation Research Goals, 2005 (Year 1)



Chapter 13

Complications of Liver Disease

A1a. Hold a research workshop on improvement and standardization of clinical measurements of cirrhosis and portal hypertension. The NIH is organizing a workshop entitled “Measurement of Hepatic Vein Pressure Gradient: Role in Management of Portal Hypertension” to be held on June 16-17, 2006 with 17 invited speakers. (0%)

A1b. Define whether N-acetylcysteine is beneficial in acute liver failure. Two prospective randomized controlled trials of N-acetylcysteine (NAC) for non-acetaminophen induced acute liver failure have received NIH funding. A trial in adults has accrued 140 patients; a trial in children has just begun. (20%)

A2. Better define the natural history of hepatopulmonary syndrome and whether early detection is beneficial. An NIH funded National Network on Hepatopulmonary Syndrome has been initiated. The presence of HPS does not appear to have a detrimental effect on the outcome of liver transplantation (Kim HY. *Trans Proc* 2004;36:2762-3). (10%)

A3a. More fully elucidate the pathophysiology of portal hypertension. Portal hypertension appears to be mediated in part by deficiency in nitric oxide activity the cause of which has been partially elucidated in rodent models (Laleman W. *Hepatology* 2005;42:1382). Hydrogen sulfide (H₂S) may independently help regulate portal pressure and be deficient in cirrhosis (Fiorucci S. *Hepatology* 2005; 42:539). The hyperdynamic splanchnic circulation of cirrhosis is mediated in part by vascular endothelial growth factor (VEGF), a potent angiogenesis signaling molecule (Fernandez M. *J Hepatol* 2005;43:6). These findings provide potential targets for more physiologically based therapies for portal hypertension. (10%)

A3b. Better characterize the cause of increased susceptibility to bacterial infections in cirrhosis. Elucidation of the basic mechanisms by which patients with cirrhosis are at increased risk for infections might lead to means of prevention. Infections remain a major cause of morbidity and mortality in patients with cirrhosis. (0%)

B1. Define optimal nonspecific approaches to management of hepatic encephalopathy, hepatorenal syndrome, refractory ascites, prevention of bacterial infection, and coagulopathy in patients with cirrhosis. A clinical trial of terlipressin vs placebo for treating hepatorenal syndrome in cirrhotic patients is nearing completion. (0%)

B2a. Define whether hypothermia is beneficial in acute liver failure for management of cerebral edema. An investigator-initiated trial of hypothermia for acute liver failure has been planned, but has yet to be funded. (0%)

B2b. Define natural history and identify predictors of development and growth of varices. A recent NIH-funded study has shown that the level of portal pressure is predictive of variceal growth and bleeding and that a fall in portal pressure

indicates a better prognosis (Groszmann R. *N Engl J Med* 2005; 353:2254-61). (20%)

B3a. Identify small molecule targets that would lead to better control of portal hypertension at different stages of disease. No new agents for portal hypertension have been approved for use. *In vitro*, high-throughput screening of small molecules is encouraged by the NIH Roadmap through the trans-NIH RFA on “Assay Development for High Throughput Molecular Screening” (RM-05-011). (0%)

B3b. Develop a noninvasive means of measuring portal pressure. The NIH-sponsored research workshop on “Measurement of Hepatic Vein Pressure Gradients: Role in Management of Portal Hypertension” planned for June 2006 will include presentations on noninvasive approaches. Efforts to develop noninvasive means to assess portal pressure are encouraged by the program announcement on “Noninvasive Methods for Diagnosis and Progression” (PA-04-088). (0%)

C1a. Elucidate the optimal approach to manage patients with varices that have not bled (primary prevention). A recent study on prevention of varices in patients with cirrhosis showed minimal effects of beta blocker therapy (Groszmann R. *N Engl J Med* 2005;353:2254), while a second study showed slowing of growth of small varices (Merkel C. *Gastroenterology* 2004;127:476). Further studies are warranted to compare band ligation, beta blocker therapy, and more innovative approaches. (10%)

C1b. Define whether monitoring portal pressure (HVPG) improves management of patients with chronic liver disease. The workshop on “Measurement of Hepatic Vein Pressure Gradient: Role in Management of Portal Hypertension” to be held on June 16-17, 2006 will deal directly with this issue. (0%)

C2a. Develop a noninvasive means to assess hepatic regeneration and reserve in liver failure. Efforts to assess regeneration and reserve function in cases of liver failure are encouraged by NIH-funded initiatives on “Development of Disease Biomarkers” (PA-05-098) and “Noninvasive Methods for Diagnosis and Progression” (PA-04-088). (0%)

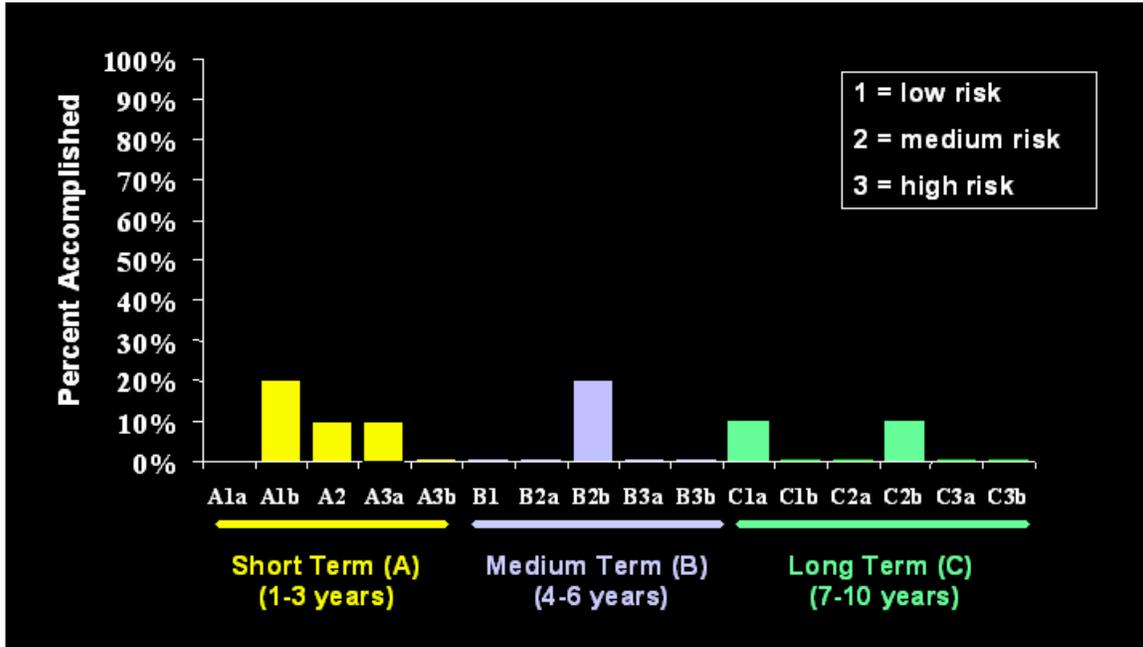
C2b. Develop and evaluate better drugs for portal hypertension. Recent animal studies have been conducted and human studies are planned to evaluate new drugs that are nitric oxide-releasing derivatives of ursodeoxycholic acid for treating portal hypertension. (10%)

C3a. Develop an artificial or bioartificial hepatic support and demonstrate that it prolongs survival in acute liver failure. Several industry groups are currently pursuing the development of artificial or bioartificial liver support devices. An NIH-sponsored meeting on acute liver failure that will focus on progress in liver support devices is being planned for December 2006. (0%)

C3b. Develop noninvasive means to screen for large varices. Studies are ongoing in this area, focusing on developing noninvasive indicators of varices size, such as

platelets and splenic size. Development of noninvasive screening tests for varices are encouraged in the program announcement on “Noninvasive Methods for Diagnosis and Progression” (PA-04-088). (0%)

Figure 15. Estimated Progress on Complications of Liver Disease Research Goals, 2005 (Year 1)

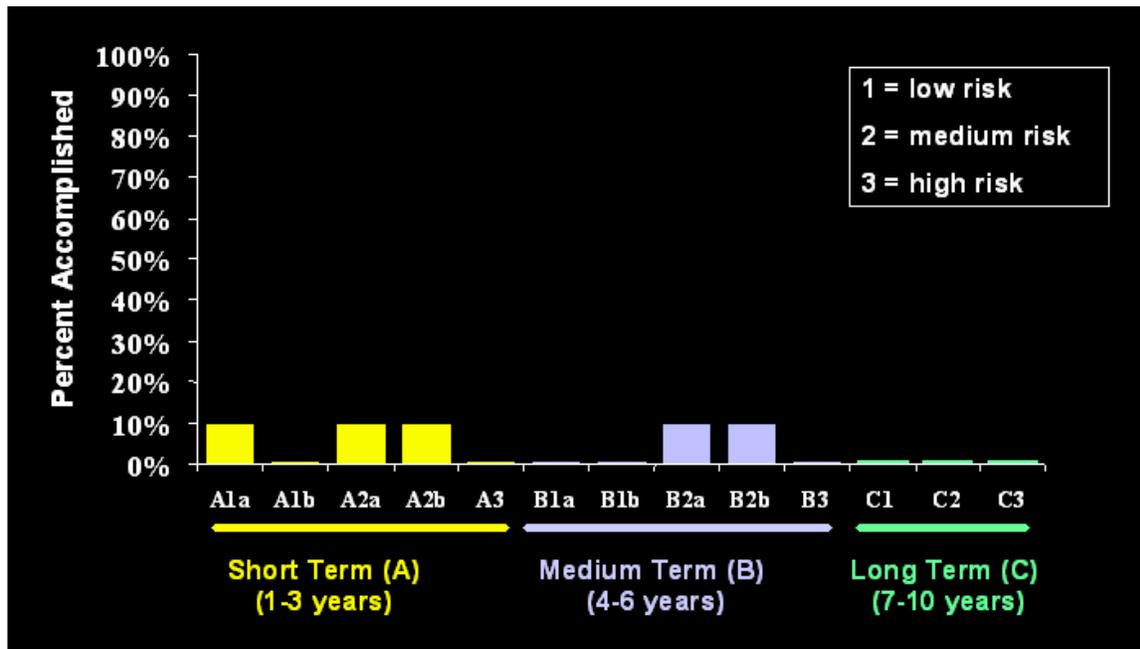


Chapter 14 Liver Cancer

- A1a. Establish liver cancer serum and tissue bank.** Serum banks of patients with early hepatocellular carcinoma (HCC) and liver disease controls are being established through the NCI-supported Early Disease Recognition Network (EDRN) and through the HALT-C trial. (10%)
- A1b. Establish means of active surveillance of HCC in the United States.** Discussions of creating a prospective database on HCC cases have been held between the NCI and NIDDK. Databases have been initiated by the American Society for Clinical Oncology and other academic hepatology groups. (0%)
- A2a. Identify potential biomarkers for early HCC.** Targeted proteomics has identified Gp73 as a promising marker of HCC, the role of which is being evaluated (Block TM. *PNAS* 2005;102:779). (10%)
- A2b. Define the molecular signatures and heterogeneity of HCC and determine how they correlate with clinical features.** Intramural NCI investigators have described signature gene expression microarray patterns associated with HCC that correlate with survival (Lee JS *Nat Genetics* 2004;36:1306), while other groups have found associations between gene expression patterns and HCC stage (Nam SW. *Hepatology* 2005;42:809). (10%)
- A3. Develop functional imaging techniques that can distinguish HCC from benign lesions.** The NCI, NIDDK, NIBIB and NIAAA have jointly published a PA on “Etiology, Prevention, and Treatment of Hepatocellular Carcinoma” (PA-05-137/138) that encourages research on HCC, a major focus of which is functional imaging of tumors. (0%)
- B1a. Demonstrate the relative efficacy, safety, and benefits of local ablative therapies for HCC.** The PA listed above (PA-05-137/138) encourages studies of therapy of HCC. Impressive results have been obtained with percutaneous image-guided radiofrequency ablation (Lencioni R. *Radiology* 2005;234:961). Prospective controlled trials are warranted. (0%)
- B1b. Develop standardized terms and nomenclature for diagnosis, staging, and grading of HCC.** The AASLD, in collaboration with the NIH, is organizing a research workshop on development of standardization of terminology and staging systems for HCC which is scheduled for December 2006. Comparisons of current staging systems have been published (Marrero JA. *Hepatology* 2005;41:707). (0%)
- B2a. Validate reliability of biomarkers for early detection of HCC.** Analyses of several biomarkers for HCC (e.g., DCP and AFP-L3) are underway as a part of the HALT-C trial, and the EDRN is sponsoring a validation study of DCP. Serum samples from patients in the HALT-C trial are being stored in a repository and will provide an outstanding resource to evaluate new markers for detection of HCC before it is clinically apparent. (10%)

- B2b. Identify risk factors for HCC associated with NASH.** Epidemiologic studies have clearly linked obesity and diabetes with increased risk of HCC (El Serag HB. *Gastroenterology* 2005;126:460), and prospective studies of NASH are incorporating screening tests for HCC in the NIH-funded NASH Clinical Research Network. (10%)
- B3. Identify target for potential therapy of HCC from molecular studies on human tissue and/or animal models.** Several potential cellular pathways have been identified in HCC that might serve as targets for non-cytolytic therapy. Such studies are encouraged in PA-05-137. (0%)
- C1. Demonstrate an effective strategy for prevention of HCC in high-risk populations.** The HALT-C trial and a similar study supported by industry (Schering, Epic-3) are evaluating the role of long-term, low dose peginterferon as a means of decreasing disease progression and development of HCC in patients with chronic hepatitis C and advanced fibrosis or cirrhosis. Studies of chemoprevention of HCC in aflatoxin-endemic areas are underway and focus upon oltipaz and chlorophyllin. (0%)
- C2. Define the cellular and molecular pathways that lead to hepatocarcinogenesis.** This is the topic of many current investigator-initiated research program grants and is a research area highlighted in PA-05-137. Pathways recently identified in association with HCC include those of frizzled-7/beta catenin (Merle P. *J Hepatol* 2005;43:854), platelet-derived growth factor C (Campbell JS. *PNAS* 2005; 102:3389), and hedgehog (Sicklick JK. *Carcinogenesis* 2005; In press). (0%)
- C3. Based upon molecular analyses, develop effective, noncytotoxic therapy for HCC.** Noncytotoxic therapies of HCC targeted at cellular and molecular pathways await demonstration of the importance of specific pathways in hepatic carcinogenesis. (0%)

Figure 16. Estimated Progress on Liver Cancer Research Goals, 2005 (Year 1)



Chapter 15

Gallbladder and Biliary Disease

- A1. Fully characterize at least 10 murine *Lith* genes related to cholesterol gallstones.** Continuous momentum is being generated to dissect out murine genes that are causally correlated to cholesterol gallstone formation, including intestinal *Apob48* (Wang HH. *Hepatology* 2005;42:894), sterol transporters *Abcg5*, *Abcg8*, and *Npc111* (Wittenburg H. *Mamm Genome* 2005;16:495), and estrogen receptor alpha (Wang HH. *J Lipid Res* 2005; Epub) (20%)
- A2. Develop small animal model for cholangiopathies that would allow analysis of effects of chronic necroinflammation on biliary epithelium.** Bile duct ligation in the rat is the standard animal model for cholangiocyte injury, which has been used to assess the role of cell signaling molecules. The *Mdr2 (Abcb4)* knock-out mouse has been shown to develop toxic bile and liver disease that resembles PSC and can be used to assess novel therapeutics (Popov Y. *J Hepatol* 2005;43:1045). A recent program announcement (“Animal Models of NIDDK- Relevant Diseases,” PA-05-049) encourages applications to develop animal models of cholangiopathies. (20%)
- A3. Develop molecular imaging techniques for visualization of the biliary tract that would provide accurate assessment of size, shape, position, motility, and inflammation, as well as a means of early detection and staging of neoplasia.** Positron emission tomography, using metabolic mapping of cellular function with fluorodeoxyglucose, may afford better imaging of cholangiocarcinoma and earlier diagnosis (Reinhardt MJ. *J Nucl Med* 2005;46:1158). Development of novel, non-invasive imaging techniques for the detection of cholangiocarcinoma is encouraged in the program announcement on “Non-Invasive Methods for Diagnosis and Progression” (PA-04-088). (10%)
- B1. Develop a cohort study of calculous and acalculous biliary pain to allow for analysis of risk factors and roles of genetic factors, microlithiasis, gallbladder motility, sphincter of Oddi dysfunction, and nucleation factors.** A small grant has been funded to develop a clinical network on sphincter of Oddi dysfunction, an area specifically highlighted in the program announcement “Endoscopic Clinical Research in Pancreatic and Biliary Diseases” (PAR-03-033). (10%)
- B2. Characterize the role of enterohepatic species of *Helicobacter* and other candidate bacteria in development of cholesterol gallstones in both mice and humans.** Different *Helicobacter* species but not *Helicobacter pylori* have different abilities to induce gallstones in mice fed lithogenic diets (Maurer KJ. *Amer J Physiol* 2005;290:G175). Using molecular techniques, DNA sequences of *Helicobacter* species have been found in cholecystic bile in humans (Neri V. *Aliment Pharmacol Ther* 2005; 22:715). (10%)
- B3. Identify plasma or urine markers for lithogenicity of bile using proteomics or metabolomics.** Grant applications using proteomics and metabolomics to characterize liver and biliary diseases have been encouraged in program

announcements, including PA-04-081 (“Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases”). (0%)

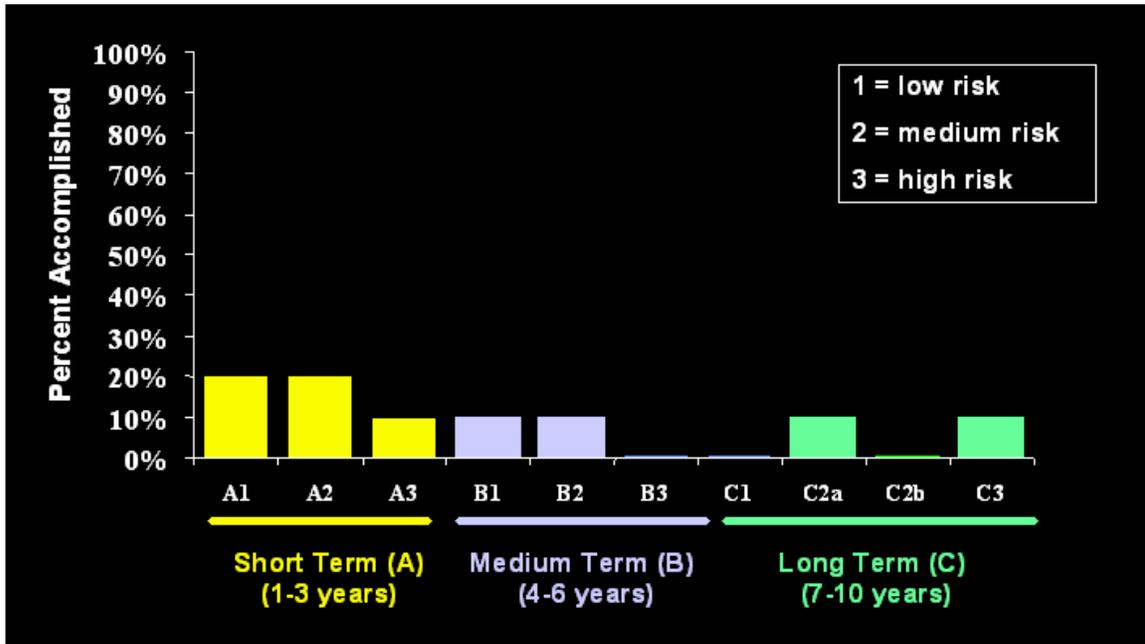
C1. Establish prospective database on cohort of patients with high risk of gallbladder cancer (e.g., American Indians) to allow development and assessment of means of early diagnosis and management. Epidemiological analysis on the risk factors of gallbladder cancer have confirmed a higher incidence (4.1 times in males; 2.6 times in females) of gallbladder cancer amongst American Indians and Alaska natives when compared with age- and sex-matched white populations (Paltoo DN. *Public Health Rep* 2004;119:443). No prospective studies have been initiated. (0%)

C2a. Identify at least 5 human *LITH* genes associated with increased risk of gallstones, based upon homology with murine genes and family studies. Using intercrosses between mouse strains, several new loci for cholesterol gallstone formation have been identified and one (*Lith9*) has been linked to the cholesterol transporter genes *Abcg5/Abcg8*, which have human homologues that are believed to be important in cholesterol gallstone formation (Wittenburg H. *Mamm Genome* 2005;16:495). (10%)

C2b. Develop noninvasive biomarker for cholangiocarcinoma. Prospective studies on primary sclerosing cholangitis have included collection of serial serum samples in patients who are at high risk for developing cholangiocarcinoma. Research grants on development of biomarkers have been encouraged by program announcements (PA-05-098: “Development of Disease Biomarkers”). (0%)

C3. Develop practical and effective approach to or means of prevention of cholesterol gallstones in high-risk populations. Until the etiology and specific risk factors for development of gallstones has been fully elucidated, practical and effective approaches to prevention will not be possible. In a long-term study in a large cohort of women, high-carbohydrate, high-glycemic response diets were associated with higher subsequent risks of cholecystectomy (Tsai CJ. *Gastroenterology* 2005;129:105). (10%)

Figure 17. Estimated Progress on Gallbladder and Biliary Disease Research Goals, 2005 (Year 1)



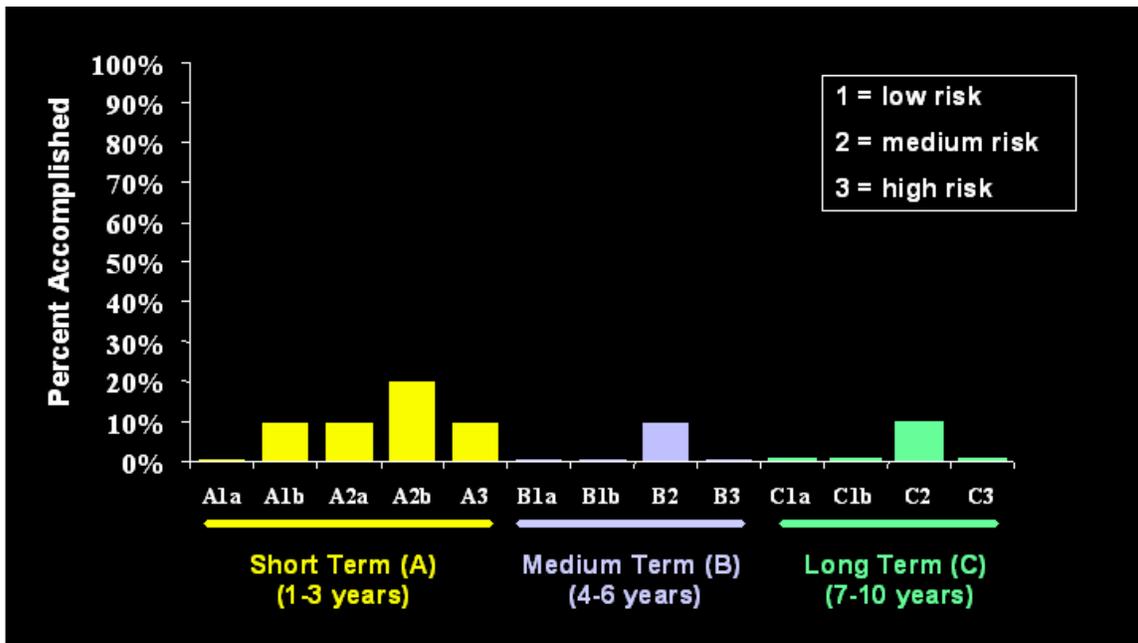
Chapter 16

Liver Imaging and Biotechnology

- A1a. Develop standardized definitions, diagnostic criteria, and methodology for liver imaging.** A workshop was sponsored by the NIH and the American Association for the Study of Liver Diseases (AASLD) at its annual meeting in November 2005 focusing on “Standardization of Nomenclature, Diagnostic, and Outcome Criteria in Liver and Biliary Diseases.” (0%)
- A1b. Better define the role, efficacy, and safety of image-guided local therapies for HCC, such as radiofrequency and thermal ablation.** A multi-center feasibility study of percutaneous radiofrequency ablation of hepatocellular carcinoma in cirrhotic patients was funded by the NIH through the American College of Radiology Imaging Network, and patient enrollment will begin in the near future. (10%)
- A2a. Create a liver tissue bank with correlative imaging data to facilitate clinical research.** Liver tissue is collected in several NIH-funded tissue bank efforts, including an effort dedicated to proteomics and biomarkers for hepatocellular carcinoma. Further integration of tissue with imaging results is needed. (10%)
- A2b. Develop improved techniques for established imaging methods for liver disease, such as optical, MRI, or PET/CT scanning.** Many NIH-funded investigator-initiated research (R01) grants are supported in this area, including efforts in contrast enhanced ultrasound, novel MRI contrast agent for monitoring thermal ablation, novel radial MRI techniques, and multifunctional low-density lipoprotein nanoplateforms. (20%)
- A3. Evaluate molecular imaging techniques in animal models of liver disease.** Continued funding of multiple *In vivo* Cellular and Molecular Imaging Centers (ICMICs) and Small Animal Imaging Resource Programs (SAIRPs) by the NIH has contributed to this goal. The NIH has also funded individual R01 grants in this area, including a study of quantitative FDG-PET for imaging woodchuck HCC at Case Western Reserve University. (10%)
- B1a. Validate standardized definitions, diagnostic criteria, and methodology for liver imaging in prospectively studied patients with liver disease.** This goal will follow the development of definitions and diagnostic criteria (goal A1a). (0%)
- B1b. Extend studies on validation to international populations.** This goal will follow the development of definitions and diagnostic criteria (goal A1a). (0%)
- B2. Develop bioinformatics such that computer-aided diagnostics are useful in evaluation of liver disease.** Many bioinformatics efforts were funded by the NIH in 2005 including the NIH Roadmap Bioinformatics and Computational Biology initiative. Evaluation of liver disease can benefit from these non-disease specific initiatives. (10%)

- B3. Apply promising molecular imaging techniques to human liver diseases or processes using antibody, receptor ligand, metabolically active, or substrate-defining probes.** Molecular imaging techniques is the focus of several NIH-funded, investigator-initiated R01 grants. (0%)
- C1a. Apply definitions, criteria, and methodology for liver imaging as surrogate endpoints to therapy of liver diseases.** This goal will follow development of definitions and diagnostic criteria (goal A1a). (0%)
- C1b. Develop practical means of assessing liver (fat content, fibrosis, inflammation, functionality) for population-based studies.** The NIH sponsored a workshop on imaging of renal and liver fibrosis in 2005 in an effort to prompt research in this area. (0%)
- C2. Develop imaging techniques that are fully integrated into therapy of liver disease.** This area is a focus of several R01 grants, including one on the development of a 3D time-resolved tomographic interventional platform at the University of Wisconsin-Madison. (10%)
- C3. Develop molecular imaging methods that provide individualized information for monitoring and therapy of liver disease, including pharmacokinetics and pharmacodynamics of targeted therapies.** Molecular imaging techniques is the focus of several NIH-funded, investigator-initiated R01 grants. (0%)

Figure 18. Estimated Progress on Liver Imaging and Biotechnology Research Goals, 2005 (Year 1)



Benchmark Goals

The Trans-NIH Action Plan for Liver Disease Research concluded with the description of ten “benchmark” goals. These were cited as goals that were cross-cutting, representative and important. Ultimately, these benchmark goals could be used as a measure of the success of the Action Plan. All ten goals are long-term and not likely to be fully achieved within the first few years after release of the Action Plan. The ten benchmark goals are listed below with a brief statement about progress and prospects for their achievement.

1. Improve success rate of therapy of hepatitis C. The current optimal regimen of therapy for hepatitis C (24-48 weeks on peginterferon plus ribavirin) yields a sustained virological response (long-term eradication of the virus) in 75 to 80 percent of persons with hepatitis C virus (HCV) genotypes 2 and 3 but in only 45 to 50 percent of persons with HCV genotype 1, the most common genotype in the United States. Response rates are lower for other cohorts, including the elderly, African Americans, and persons with HIV infection, renal disease or other co-morbidities. These rates have not improved in the last year. However, several promising HCV-specific protease and polymerase inhibitors have been described recently that are likely to result in improved response rates in the future. Thus, there is general optimism that response rates in hepatitis C will advance appreciably in the next few years as new agents become available that can be given alone or in combination with peginterferon.

2. Develop effective therapies for fatty liver disease, both alcoholic and non-alcoholic. Several large-scale clinical trials of therapy of nonalcoholic steatohepatitis are being designed or are underway that will evaluate the role of weight loss (through such means as behavioral therapy, bariatric surgery, anti-obesity medications), insulin sensitizing agents (e.g., metformin, thiazolidinediones), antioxidants (e.g., vitamin E, betaine), and hepatoprotective agents (silymarin, S-adenosylmethionine). Several of these approaches may also be applicable to alcoholic liver disease. Clearly, new therapies for nonalcoholic steatohepatitis will be developed in the next few years; their degree of efficacy and general applicability remain to be defined.

3. Develop regimens of antiviral therapy that are effective in long-term management of hepatitis B. A total of five medications have been approved for use in chronic hepatitis B in the United States, two within the last year. Licensed therapies include standard interferon alfa, peginterferon, lamivudine, adefovir dipivoxil and entecavir. Preliminary findings using several of the oral nucleoside analogues demonstrate that they can be given long-term and provide sustained benefit. These results require further long-term follow-up and verification. The relative benefits and risks of monotherapy versus combination therapy also warrant careful prospective study. Nevertheless, there have been major advances in the therapy of hepatitis B, and achievement of this goal is in sight.

4. Develop sensitive, specific, and non-invasive means of assessing disease stage (i.e. extent of fibrosis) in chronic liver disease. Multiple publications have assessed the use

of routine laboratory tests to predict the presence of advanced fibrosis in patients with hepatitis C, hepatitis B, and nonalcoholic steatohepatitis. No combination of tests is totally accurate and these approaches do not reliably detect early stages of fibrosis. Imaging tests for fibrosis are improving; one promising method is elastography, which measures the degree of stiffness of the liver. Prospective studies of elastography are now being developed, which should demonstrate the sensitivity and specificity of this technique. Meanwhile, more basic research on use of magnetic resonance and molecular imaging to detect fibrosis is being encouraged and actively pursued.

5. Develop sensitive and specific means of screening individuals at high risk for early hepatocellular carcinoma. Preliminary studies using gene expression arrays and proteomics have provided several possible targets for early detection of HCC, but none have been subjected to critical clinical evaluation. Meanwhile, standard assays for screening such as alpha-fetoprotein, alpha-fetoprotein L3, and des-gamma-carboxy prothrombin are now being evaluated critically for their sensitivity and specificity.

6. Develop means to prevent gallstones. While genetic markers for gallstone development have been identified in mice and are being applied to human populations, none have revealed targets for possible therapy or prevention of gallstone formation.

7. Elucidate the cause of biliary atresia. This goal is the specific focus of the Biliary Atresia Research Consortium (BARC), first funded in 2003, which now consists of 10 clinical centers and a data coordinating center. BARC has initiated both retrospective and prospective studies of children with biliary atresia, as well as a controlled trial of corticosteroid therapy during the post-operative period of hepatopertoenterostomy. The Consortium has also received funding for three ancillary studies directed at the etiology of biliary atresia, focusing on either genetics, proteomics, or gene expression arrays. A meeting with sessions on etiology of biliary atresia has been organized by the NIH for September 11-12, 2006.

8. Improve the safety and define optimal use of living donor liver transplantation. Living donor organs are currently used in approximately 9 percent of pediatric and 5 percent of adult liver transplants in the United States. In the last year, the results from the "Adult-to-Adult Living Donor Liver Transplantation Cohort Study" (A2ALL) showed the importance of transplant center experience in recipient outcome. Further studies from this study and in pediatric liver transplantation are likely to further define optimal use and safety of this life-saving procedure.

9. Develop standardized and objective diagnostic criteria of major liver diseases and their grading and staging. Preliminary meetings have been held on this topic by the NIH in collaboration with the American Association for the Study of Liver Diseases, and agreements in co-supporting this initiative have been developed. Early attempts have been made at standardizing diagnosis of primary sclerosing cholangitis and hepatitis B, but none have yet been published or accepted.

10. Decrease the mortality rate from liver disease. The ultimate goal of the Action Plan for Liver Disease Research is to decrease morbidity and mortality from liver and biliary disease in the United States. One difficulty in assessing this goal is the lack of consistently reliable data on the prevalence, incidence, and death rates from liver disease. A major source of information is the National Center for Health Statistics (CDC) and their yearly publication of Vital Statistics for the United States. Mortality rates in these reports are based upon death records, which can be unreliable, but are consistent enough to measure trends. Another problem is that publication of Vital Statistics are 2 to 3 years delayed, so that the most recent results are currently from 2003. For the purpose of this goal, overall numbers of deaths, death rates, and age-adjusted death rates are obtained from the Vital Statistics report on death rates for 113 Selected Causes. The overall rates of liver and biliary disease deaths are assessed using the totals of relevant ICD codes in this listing, including B15-B19 (Viral Hepatitis), C22 (Malignant Neoplasms of the Liver and Intrahepatic Bile Ducts), K70, K73-K74 (Chronic Liver Disease and Cirrhosis), and K80-82 (Cholelithiasis and Other Disorders of the Gallbladder). The age-adjusted death rates for 2000-2003 are shown in Table 3 below.

Table 3. Age-Adjusted Death Rates per 100,000 population

Diagnosis	2000	2001	2002	2003
Chronic Liver Disease and Cirrhosis	9.5	9.4	9.4	9.2
Viral Hepatitis	1.9	1.8	2.0	1.8
Malignant Neoplasms of the Liver and Intrahepatic Bile Ducts	4.7	4.7	5.0	4.9
Cholelithiasis and Disorders of the Gallbladder	1.0	1.0	1.0	1.0
Total: Liver and Biliary Disease	17.1	16.9	17.4	16.9
Total Number of Deaths	47,635	48,068	50,076	50,052

While the total numbers of deaths from liver and biliary disease increased slightly over the four years, the age-adjusted death rates were stable. Secular trends in death rates from liver and biliary diseases are likely to lag several years behind advances in research and development of improved means of diagnosis, monitoring, treatment, and prevention of liver disease.

Conclusions and Future Plans

Many important advances in liver disease research were made in 2005, the first year after publication of the Trans-NIH Action Plan for Liver Disease Research. Progress was observed in each of the 16 topic areas and toward many of the 214 research goals. While none of the listed research goals was completely achieved, many have had important advances and are likely to be fully accomplished in the next few years.

This progress review will be used by the Institutes and Centers of the NIH, as well as other Federal Agencies involved in liver disease research, to plan initiatives for the year 2006 and beyond. An annual review of the Action Plan is conducted yearly by the Liver Disease Subcommittee of the Digestive Diseases Interagency Coordinating Committee. This document will be used at the time of this meeting. At five and ten years following release of the Action Plan, it will be reviewed more formally by a larger group, consisting of outside experts, concerned lay individuals, NIH staff, and representatives from the 16 Working Groups on the Action Plan, in order to assess progress and the need for further efforts in promoting specific areas of liver disease research.

Appendix 1. Acronyms Used in this Progress Review

A2ALL	Adult-to-Adult Living Donor Liver Transplantation Cohort Study
AACTG	Adult AIDS Clinical Trials Group
AASLD	American Association for the Study of Liver Diseases
ABC	ATP-binding cassette
ACSG	Antiviral Cooperative Study Group
AFP	alpha-fetoprotein
AIP	acute intermittent porphyria
ALFSG	Adult Acute Liver Failure Study Group
ALT	alanine aminotransferase
Apob48	apolipoprotein B-48
ARPKD	autosomal recessive polycystic kidney disease
ATP	adenosine triphosphate
BARC	Biliary Atresia Research Consortium
BSEP	bile salt export pump
CAR	constitutive androstane receptor
cccDNA	covalently closed circular DNA
CDC	Centers for Disease Control and Prevention
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane regulator
CHF	congenital hepatic fibrosis
CLiC	Cholestatic Liver Disease Consortium
CT	computed tomography
DCP	des-gamma-carboxy prothrombin
DDICC	Digestive Diseases Interagency Coordinating Committee
DILIN	Drug-Induced Liver Injury Network
DMT1	divalent metal ion transporter 1
EDRN	Early Disease Recognition Network
EGF	epidermal growth factor
EPP	erythropoietic protoporphyria
FDA	Food and Drug Administration
FDG-PET	fluorodeoxyglucose-positron emission tomography
FGF	fibroblast growth factor
Fox	Forkhead Box
FXR	farnesoid X receptor
H ₂ S	hydrogen sulfide
HAART	highly active antiretroviral therapy
HALT-C	Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial
HAV	hepatitis A virus
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCIG	hepatitis C immune globulin
HCV	hepatitis C virus
HDL	high-density lipoprotein
HDV	hepatitis D virus

HEIRS	Hemochromatosis and Iron Overload Screening study
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HLPP	Human Liver Proteome Project
HNF	hepatocyte nuclear factor
HPS	hepatopulmonary syndrome
HRSA	Health Resources and Services Administration
Hrs	hepatocyte growth factor-regulated tyrosine kinase substrate
HURSO	High Dose Ursodiol for Primary Sclerosing Cholangitis study
HVPG	hepatic venous pressure gradient
ICMIC	<i>In vivo</i> Cellular and Molecular Imaging Center
IFN	interferon
IGF-I	insulin-like growth factor-I
IKK β	I κ B kinase
IL	interleukin
IPS-1	interferon-beta stimulator 1
IRG	initial review group
IRS	insulin receptor substrate
LABS	Longitudinal Assessment of Bariatric Surgery
LADR	Low Dose Accelerating Regimen study (of A2ALL)
LXR	liver X receptor
MDR	multi-drug resistance
MELD	Model for End-stage Liver Disease
MRI	magnetic resonance imaging
MSH	melanocyte-stimulating hormone
NAC	N-acetylcysteine
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
NCCAM	National Center for Complementary and Alternative Medicine
NCI	National Cancer Institute
NCMHD	National Center on Minority Health and Health Disparities
NCRR	National Center for Research Resources
NF- κ B	necrosis factor- κ B
NHANES	National Health and Nutrition Examination Survey
NHGRI	National Human Genome Research Institute
NHLBI	National Heart, Lung, and Blood Institute
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIAID	National Institute of Allergy and Infectious Diseases
NIBIB	National Institute of Biomedical Imaging and Bioengineering
NICHD	National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIGMS	National Institute of General Medical Sciences

NIH	National Institutes of Health
NK	natural killer cell
NKT	natural killer T cell
NMR	nuclear magnetic resonance
Npc111	Niemann-Pick C1 Like 1
NPY	neuropeptide Y
NTCP	Na ⁺ /taurocholate co-transporting polypeptide
OATP	organic anion transporter
OCT	organic cation transporter
ODS	Office of Dietary Supplements
OLTs HBV	Orthotopic Liver Transplantation for Hepatitis B Study
ORD	Office of Rare Diseases
PA	program announcement
PACTG	Pediatric AIDS Clinical Trials Group
PALFSG	Pediatric Acute Liver Failure Study Group
PBC	primary biliary cirrhosis
PDGF-C	platelet-derived growth factor-C
PELD	Model for Pediatric End-stage Liver Disease
PET	positron-emission tomography
PFIC	progressive familial intrahepatic cholestasis
Peds-C	Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C
PGRN	Pharmacogenetics Research Network
PIVENS	Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis study
PPAR	peroxisome proliferator-activated receptor
PSC	primary sclerosing cholangitis
PSR	phosphatidylserine receptor
PTLD	post-transplant lymphoproliferative disease
PXR	pregnane X receptor
RFA	request for applications
SAMe	S-adenosyl methionine
siRNA	small interfering RNA
SPLIT	Study of Pediatric Liver Transplantation
SR-BI	scavenger receptor class B type I
TONIC	Treatment of Nonalcoholic Fatty Liver Disease in Children trial
TGF	transforming growth factor
TNF- α	tumor necrosis factor- α
TNFR1	tumor necrosis factor receptor 1
UDCA	ursodeoxycholic acid
UNOS	United Network for Organ Sharing
VEGF	vascular endothelial growth factor
Virahep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C
VLDL	very low density lipoprotein