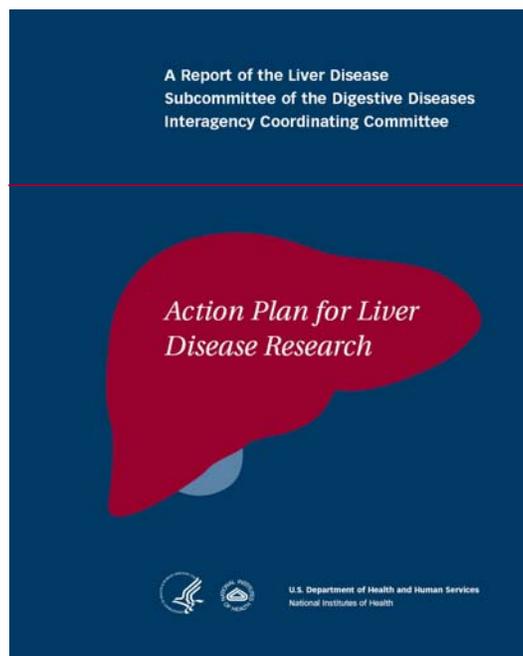


**Progress Review for 2006 (Year Two Analysis):**  
***Action Plan for Liver Disease Research***



## Table of Contents

Objective.....	3
Introduction.....	3
Action Plan: 16 Topic Areas of Liver Disease-Related Research.....	5
Active Initiatives in Liver Disease Research.....	6
NIH Funding of Liver Disease Research.....	9
Progress Review (Year Two Analysis: 2006).....	10
Chapter 1. Cell and Molecular Biology of the Liver.....	11
Chapter 2. Liver Injury, Inflammation, Repair, and Fibrosis.....	14
Chapter 3. Developmental Biology and Regeneration.....	17
Chapter 4. Bile, Bilirubin, and Cholestasis.....	21
Chapter 5. Viral Hepatitis.....	24
Chapter 6. HIV and Liver Disease.....	28
Chapter 7. Fatty Liver Disease.....	31
Chapter 8. Drug- and Toxicant-Induced Liver Disease.....	35
Chapter 9. Autoimmune Liver Disease.....	38
Chapter 10. Pediatric Liver Disease.....	41
Chapter 11. Genetic Liver Disease.....	44
Chapter 12. Liver Transplantation.....	48
Chapter 13. Complications of Liver Disease.....	51
Chapter 14. Liver Cancer.....	54
Chapter 15. Gallbladder and Biliary Disease.....	57
Chapter 16. Liver Imaging and Biotechnology.....	60
Conclusions and Future Plans.....	63
Benchmark Goals.....	63
Appendix 1. Acronyms Used in this Progress Review.....	A-1

## 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 2006, the second year following its release. It builds upon the first-year Progress Review, which surveyed progress during 2005 and is available on the Action Plan's web site (<http://liverplan.niddk.nih.gov>). 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 Action Plan is currently being used to inform a parallel planning process for the broader field of digestive diseases research—the National Commission on Digestive Diseases’ development of a long-range research plan for digestive diseases. The Commission was established in 2005 by the NIH Director, with a primary charge to develop the research plan, which will include a chapter dedicated to diseases of the liver and biliary system. Additional information on the Commission’s activities is available on its website (<http://NCDD.niddk.nih.gov>). Experts from the NIH and external research community involved in both planning processes are helping to coordinate these efforts.

The current document is a Progress Review of the Action Plan for the year 2006, approximately 2 years after its release. It follows the 2005 Progress Review, which was released during the first year following publication of the Action Plan. This Progress Review was prepared by the Liver Disease Research Branch with the assistance of the chairpersons and current 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 and other members of the research community. 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 2006 that are apropos to each research goal. Finally, the degree of progress made toward each research goal during the 2 years since the Action Plan’s release is estimated on a scale of 0 percent (no progress) to 100 percent (full attainment of the goal) in increments of 10 percent. Estimates are broken out by the degree of progress during 2006, as well as cumulative progress made in the past 2 years since the Action Plan’s release. These percentages are purely estimates but are made on the basis of agreement among the experts who provided input. 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, 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, Catherine McKeon, 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, and Sam Zakhari
- **Drug- and Toxicant-Induced Liver Disease:** Drs. Neil Kaplowitz, 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, Paul Adams, and Alan Guttmacher
- **Liver Transplantation:** Drs. Jean Emond, Michael Lucey, John Roberts, Hugo Rosen, and James Everhart
- **Complications of Liver Disease:** Drs. Thomas Boyer, Michael Fallon, Roberto Groszmann, William Lee, and Leonard Seeff
- **Liver Cancer:** Drs. Adrian Di Bisceglie, Greg Gores, Snorri Thorgeirsson, Josep M. Llovet, Jordi Bruix, and Jaye Viner
- **Gallbladder and Biliary Disease:** Drs. Sum Lee, Martin Carey, Nicholas LaRusso, Henry Pitt, and James Everhart
- **Liver Imaging and Biotechnology:** Drs. King Li, Glenn Krinsky, Jonathan Kruskal, Claude Sirlin, 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 2006 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 that were active in 2006**

<b>Initiative Number</b>	<b>Title</b>	<b>Sponsoring ICs</b>
PAR-06-171	Endoscopic Clinical Research in Pancreatic and Biliary Diseases	NIDDK, NCI
PA-06-177 (now PA-07-015)	Research Grants for Studies of Hepatitis C in the Setting of Renal Disease	NIDDK
PA-07-320	Development of Assays for High Throughput Drug Screening	NIDDK, NCI, NIAID
PA-06-185 (now PA-07-016)	Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases	NIDDK
PA-06-143 (now PA-07-025)	Non-Invasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological and Digestive Diseases	NIDDK
PA-07-012	Animal Models of NIDDK-Relevant Diseases	NIDDK, NIAID
PA-06-147 (now PA-07-052)	Development of Disease Biomarkers	NIDDK, NIBIB, NIAAA, ODS
PA-07-026	Developmental Biology and Regeneration of the Liver	NIDDK, NIAAA, NCI, NICHD
PA-07-068	Mechanisms of Alcoholic and Nonalcoholic Fatty Liver	NIAAA, NIDDK, ODS
PA-06-295 (now PA-07-258)	Etiology, Prevention and Treatment of Hepatocellular Carcinoma	NCI, NIDDK, NIBIB, NIAAA

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.

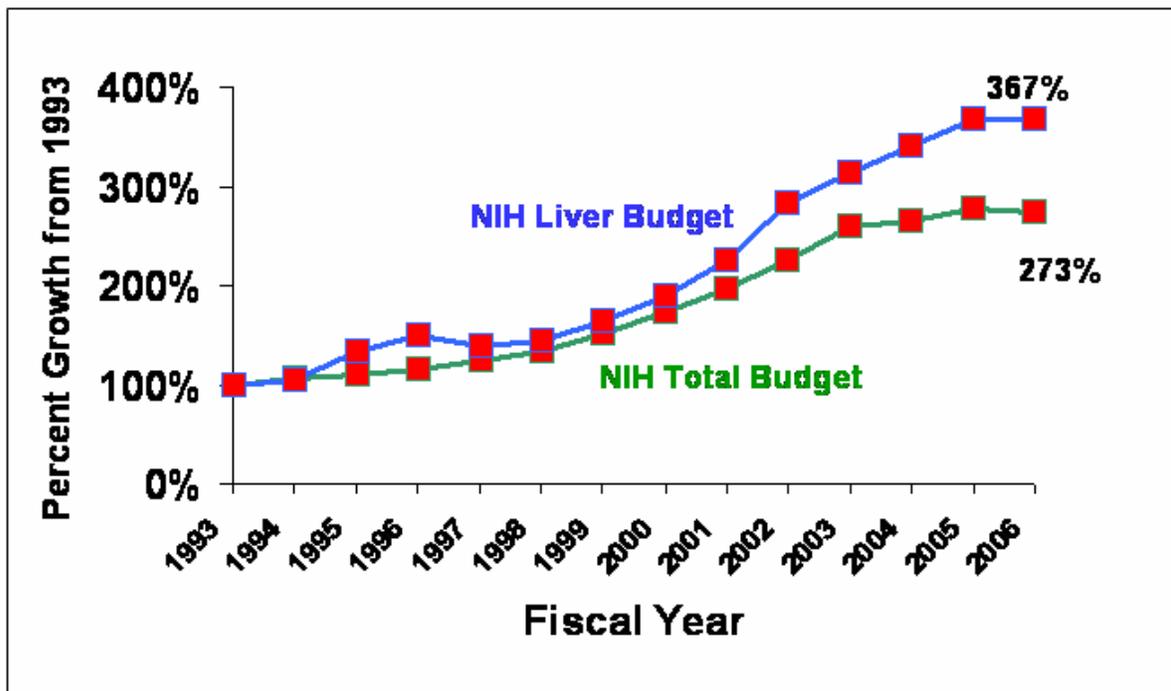
**Table 2. Clinical and Epidemiologic Studies and Relevant Announcements**

<b>Short Title/ Initiative #</b>	<b>Full Title</b>	<b>Sponsoring ICs/Agencies</b>
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")	NIDDK, NICHD
<i>PIVENS</i>	Pioglitazone vs Vitamin E for Nondiabetic Patients with Nonalcoholic Steatohepatitis	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
SyNCH	Silymarin in NASH and Chronic Hepatitis C	NCCAM, NIDDK
Virahep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C	NIDDK
PAR-06-216 (now PAR-07-024)	Ancillary Studies to Major Ongoing NIDDK and NHLBI Clinical Research Studies	NIDDK, NHLBI
PAR-06-301	Pilot and Feasibility Clinical Research Studies in Digestive Diseases and Nutrition	NIDDK

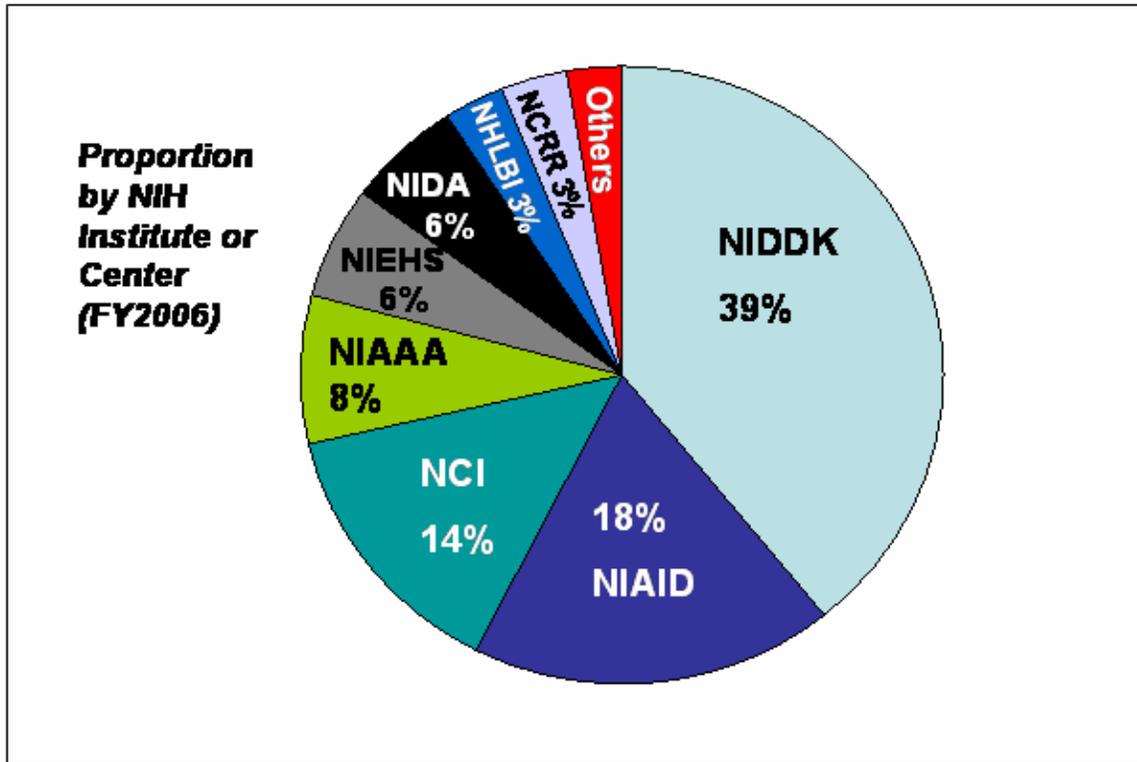
### NIH Funding of Liver Disease Research

The trans-NIH Action Plan for Liver Disease Research provided 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 2006 was approximately \$454 million, which represented no increase from FY 2005. For comparison, the overall NIH budget decreased by 0.5 percent between FY 2005 and FY 2006. 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 2006 is shown in Figure 2. The majority of funding in liver disease research continues to derive from 8 Institutes: NIDDK (39%), NIAID (18%), NCI (14%), NIAAA (8%), NIEHS (6%), NIDA (6%), NHLBI (3%), and the NCRR (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 1993-2006



**Figure 2. NIH Support of Liver Disease Related Research**



### Progress Review: Year Two Analysis (2006)

The following 16 sections describe the second 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.

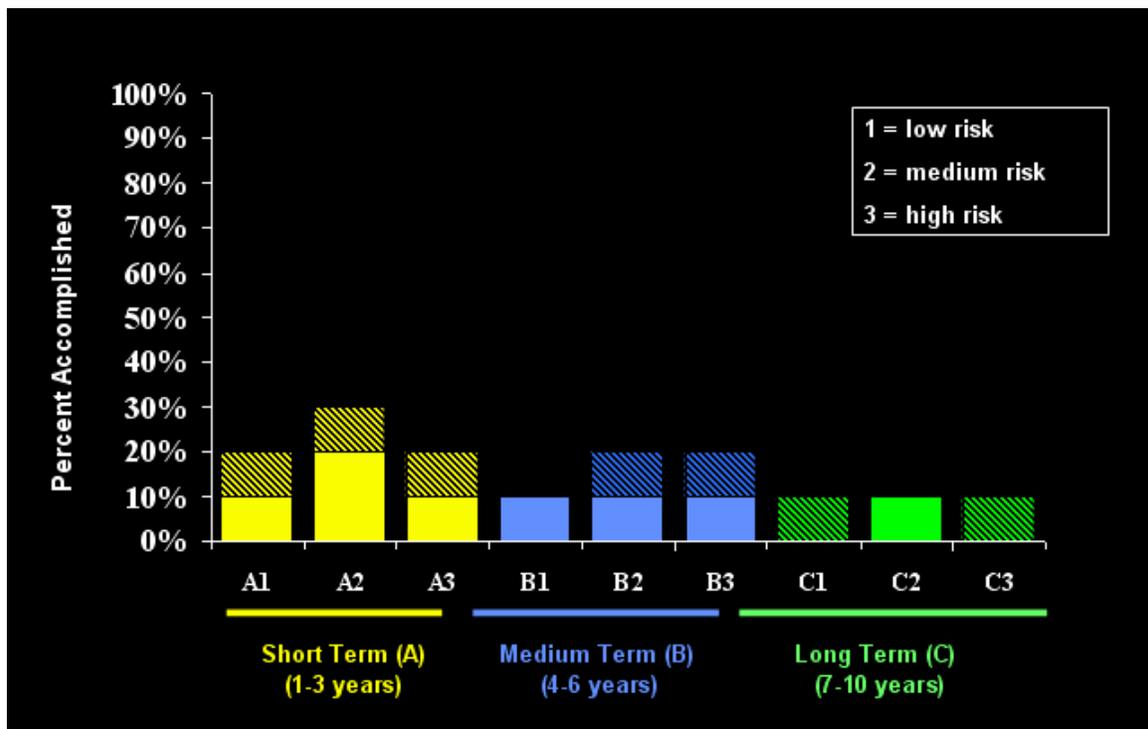
## Chapter 1

### Cell and Molecular Biology of the Liver

- A1. Define major pathways and molecular participants in signal transduction in liver cells.** Cilia on cholangiocytes have been shown to respond to bile flow and induce increases in intracellular calcium and decreases in cyclic AMP mediated through flow receptors (polycystin-1), calcium channels (polycystin-2), and adenylyl cyclase on the cilia, providing a new model for regulation of ductal bile secretion (Masyuk AI. *Gastroenterology* 2006;131:911). The ryanodine receptor is a well-known calcium-release channel in muscle; now a truncated form of this receptor has been shown to modulate cytosolic free calcium oscillations in liver, demonstrating a new level of complexity in regulation of calcium signaling in hepatocytes (Pierobon N. *J Biol Chem* 2006;281:34086). (2006 10%; Total 20%)
- A2. Elucidate the mechanisms of lipid metabolism and transport in liver as it relates to whole body lipid homeostasis.** Studies in cell culture have further strengthened the “convergent mechanism” for feedback control of cholesterol synthesis and uptake by hepatocytes; this control is mediated by sterol-regulatory element binding proteins (SREBPs) in the ER. In the presence of low levels of cholesterol, SREBPs move to the Golgi and release a transcription factor portion that upregulates cholesterol synthetic and transporter pathways, but in the presence of high levels of cholesterol, SREBPs are bound to Scap and Insig-1 and retained in the ER, resulting in decreased transcription of target genes (Gong Y. *Cell Metab* 2006;3:15). (2006 10%; Total 30%)
- A3. Determine how intra- and inter-cellular signals are integrated *in vivo* to regulate liver function.** Fibroblast growth factor (FGF)-15 has been identified as playing an important role in gallbladder filling (Choi M. *Nat Med* 2006;12:1253). FGF-15 is induced in the terminal ileum as a result of bile acid signaling through FXR and circulates in a hormonal fashion to the liver and gallbladder where it leads to gallbladder filling by acting on cAMP-linked receptors on biliary smooth muscle cells. Thus, intra- and inter-cellular signals coordinate to control gallbladder filling and emptying. (2006 10%; Total 20%)
- B1. Elucidate physiological importance of liver plasma membrane transporters and mechanisms of action.** Further purification of the organic anion transporting protein 1a1 (Oatp1a1) demonstrated sites of phosphorylation and potential means of modulation (Xiao Y. *Biochemistry* 2006;45:3357). (2006 0%; Total 10%)
- B2. Develop cell culture model that reflects different liver cell interactions (e.g., hepatocyte with Kupffer cell, cholangiocyte, stellate cell, or endothelial cell).** Progress has been made in creating 3-D microenvironments to grow hepatocytes and investigate the interactions between hepatocytes and non-parenchymal cells (Albrecht DR. *Nat Methods* 2006;3:369). Hepatocyte patterning and function in culture is improved by co-culture with Kupffer or endothelial cells (Zinchenko YS. *Tissue Eng* 2006;12:2241). (2006 10%; Total 20%)

- B3. Elucidate intra- and extra-cellular events that determine hepatocyte polarity.** Radixin is a major hepatocyte protein that has been shown to tether proteins such as MRP2 to the canalicular membrane; siRNA suppression of radixin results in loss of polarity, structure, and function of apical hepatocyte membranes (Wang W. *Gastroenterology* 2006; 131:878). (2006 10%; Total 20%)
- C1. Elucidate major elements in process of transcellular vesicle trafficking in the hepatocyte.** Upregulation of cell surface transporters by signaling molecules requires their interaction with activating proteins (often kinases), motor proteins, intracellular organelles, plasma membranes and microtubules. The stimulation of choleresis in cholangiocytes, as well as bile acid transport in hepatocytes, has been shown to be mediated by cyclic AMP stimulation of microtubular transport of molecules (aquaporin and sodium taurocholate cotransporter protein [ntcp], respectively) by kinesin and dynein motor proteins along microtubules (Tietz PS. *Biol Cell* 2006;98:43; Sarkar S. *Traffic* 2006; 7:1078). Activation of the atypical protein kinase C (zeta) is required for ntcp trafficking. (2006 10%; Total 10%)
- C2. Elucidate how cells interact with each other (e.g., via gap junctions, ECM, paracrine, and endocrine signaling).** Little new information is available on cellular crosstalk in the liver. (2006 0%; Total 10%)
- C3. Develop knowledge base of normal liver proteome, including analysis of individual cell types, subcellular compartments, and changes along hepatic acinus.** International collaborations in liver proteomics are coordinated through the Human Liver Proteome Project (Zheng J. *Proteomics* 2006;6:1716). Technologies to comprehensively and quantitatively characterize the normal liver proteome, including subcellular compartments, have been evaluated on reference samples obtained from human fetal livers, adult human livers, as well as rat and mouse livers (Gilchrist A. *Cell* 2006;127:1265; Foster LJ. *Cell* 2006;125:187; Ying W. *Mol Cell Proteomics* 2006;5:1703; Song Y. *Proteomics* 2006;6:5269). (2006 10%; Total 10%)

**Figure 3. Estimated Progress on Cell and Molecular Biology Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



## Chapter 2

### Liver Injury, Inflammation, Repair, and Fibrosis

- A1a. Identify individual liver cell type-specific responses to inflammatory mediators.** Liver cell injury is accompanied by an increase in integrin-linked kinase, which may play a role in activation of stellate cells through IP<sub>3</sub> signaling pathways and thus represent a target for anti-fibrotic therapy (Shafiei MS. *J Biol Chem* 2006;281:24863). (2006 0%; Total 20%)
- A1b. Elucidate whether and how nutritional factors affect liver cytotoxic and fibrotic pathways.** Free fatty acids can trigger inflammation and hepatocyte injury via activation of toll like receptor (TLR) 4 signaling; mice without TLR4 are resistant to the cytotoxic effects of free fatty acids (Shi H. *J Clin Invest* 2006;116:3015). Inflammatory pathways involving c-Jun N-terminal kinase (JNK) and caspase activation mediate the apoptotic effects of free fatty acids, and blocking either pathway can decrease fatty acid-induced apoptosis of hepatocytes (Malhi H. *J Biol Chem* 2006;281:12093). Free fatty acids also induce ER stress, this effect being specific to saturated fatty acids (Wei Y. *Am J Physiol Endocrinol Metab* 2006;291:E275). (2006 10%; Total 20%)
- A2a. Define the role of anti-apoptotic therapy in liver injury, fibrosis, and regeneration.** The pan-caspase inhibitor orthovanadate was shown to prevent liver sinusoidal endothelial cell apoptosis in a rat model of ischemia/reperfusion injury (Ohi N. *Am J Pathol* 2006;168:1097). Human trials using this agent to improve organs for liver transplantation are underway. (2006 10%; Total 20%)
- A2b. Identify the impact of individual leukocyte sub-populations and their mediators on liver injury, fibrosis, and regeneration.** NKT cells may play a role in ischemia/reperfusion injury and are activated through CD1d-dependent pathways (Lappas CM. *J Exp Med* 2006;203:2639). CD8<sup>+</sup> cytotoxic lymphocytes mediate many forms of liver injury; their direct contact with hepatocytes through sinusoidal cell fenestrations has been shown by electron microscopy (Warren A. *Hepatology* 2006; 44:1182). Even liver-specific, activated CD8 cells do not induce liver injury without a second signal mediated through TLR3 activation, perhaps by stimulating TNF- $\alpha$  and interferon- $\alpha$  production (Lang KS. *J Clin Invest* 2006; 116:2456). Neutrophils are important in several forms of drug- or toxin-induced liver injury and may, therefore, represent a therapeutic target (Liu ZX. *Hepatology* 2006;43:1220). (2006 10%; Total 20%)
- A3. Develop noninvasive biomarkers for fibrosis.** Ultrasound elastography has been found to accurately reflect hepatic fibrosis (Foucher J. *Gut* 2006;55:403), presence of cirrhosis (Ganne-Carrié N. *Hepatology* 2006;44:1511), increase in portal pressure (Carrion JA. *Liver Transpl* 2006;12:1791), and presence of varices in patients with liver disease (Kazemi F. *J Hepatol* 2006;45:230). This area of research is encouraged through the program announcement for small

business (SBIR/STTR) grants “New Technologies for Liver Disease” (PA-06-396/397). (2006 20%; Total 30%)

**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 their relative role in human liver disease requires further elucidation. The c-Jun N-terminal kinase (JNK) signaling pathway plays a central role in hepatocyte injury in mouse models (Gunawan BK. *Gastroenterology* 2006; 131:165; Wang Y. *J Biol Chem* 2006;281:15258). (2006 10%; Total 20%)

**B2a. Identify the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemic-reperfusion injury and the role of sinusoidal cells.**

Kupffer cell-dependent TNF signaling mediates injury in small-for-size liver transplantation in the mouse model (Tian Y. *Proc Natl Acad Sci USA* 2006;103:4598). Matrix metalloproteinase-9 is important in inducing cytokine responses and recruitment of leukocytes to the liver in ischemia/reperfusion injury (Khandoga A. *J Leukoc Biol* 2006;79:1295). (2006 10%; Total 10%)

**B2b. Identify the proteomic response of the liver and liver-derived serum proteins as intermediate biomarkers for liver disease progression and response to therapy.** Results from the Human Liver Proteome Project have shown that the proteomic profile of normal human liver is relatively stable, allowing for reliable detection of changes with liver injury (Zhang X. *Proteomics* 2006;6:5260). Proteomic analyses have been used to develop biomarkers for hepatocellular carcinoma (Feng JT. *Oncogene* 2006;25:3810; Takashima M. *Proteomics* 2006;6:3894). (2006 10%; Total 10%)

**B3. Develop gene-, cell-, or pharmacology-based therapies for hepatic injury.** Modulators of the activity of several types of nuclear receptors (e.g., FXR, LXR, PXR, PSR, CAR, PPAR $\alpha$ , and PPAR $\gamma$ ) are being developed and evaluated as therapies for liver disease. A small randomized controlled trial showed that thiazolidinediones improve metabolic, biochemical, and histological features of nonalcoholic steatohepatitis (Belfort R. *N Engl J Med* 2006; 355:2297). (2006 10%; Total 20%)

**C1. Develop relevant and robust animal models of hepatic injury, inflammation, and fibrosis progression and resolution.** In the past year, three animal models for primary biliary cirrhosis have been described, including a Nod.c3c4 congenic mouse (Irie J. *J Exp Med* 2006;203:1209), TGF $\beta$  receptor II dominant-negative mouse (Oertelt S. *J Immunol* 2006;177:1655), and IL-2 receptor  $\alpha$  knockout mouse (Wakabayashi K. *Hepatology* 2006;44:1240). All three models develop biliary inflammation and injury, as well as serum autoantibodies, that are characteristic of the human disease. (2006 20%; Total 30%)

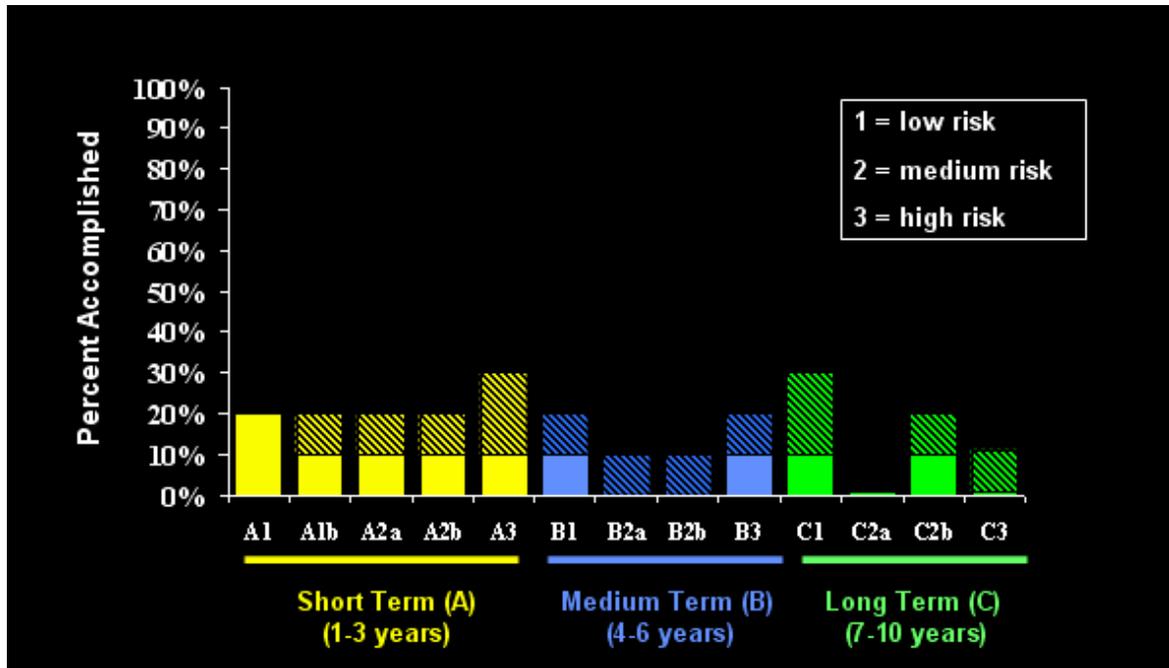
**C2a. Using high-throughput screens, identify candidate small molecules that modify cytotoxic and fibrotic pathways in liver cells.** Progress in this area will require development of methods for screening small molecules that augment or impede cell signaling pathways related to apoptosis and fibrosis. This area is the

focus of NIH Roadmap initiatives, including the RFA “Pilot-Scale Libraries for High-Throughput Drug Screening” (RFA-RM-06-003). (2006 0%; Total 0%)

**C2b. Define genetic determinants of disease risk and progression in acute/ chronic liver injury, fibrosis, and regeneration.** Genetic determinants of hepatic fibrosis were studied in a cohort of 916 patients with chronic hepatitis C, and several single nucleotide polymorphisms (SNPs) were identified that were associated with more rapid fibrosis progression (Huang H. Gastroenterology 2006; 130:1679). The biological implications of these polymorphisms are being pursued. (2006 10%; Total 20%)

**C3. Develop mechanism-based drug therapy in fibrotic disease, targeting pro-fibrogenic and fibrosis resolution pathways.** Industry-sponsored trials of a PPAR- $\gamma$  agonist and an angiotensin receptor blocker for prevention of fibrosis progression are currently underway. Cannabinoid receptor 1 (CB1) antagonists were found to inhibit the development of liver fibrosis in mouse models and may represent an approach to treatment of human liver diseases (Teixeira-Clerc F. Nat Med 2006;12:671). (2006 10%; Total 10%)

**Figure 4. Estimated Progress on Liver Injury, Inflammation, Repair, and Fibrosis Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year’s progress.]**



## Chapter 3 Developmental Biology and Regeneration

### **A1a. Identify and characterize hepatic stem cells in fetal and adult liver.**

Multipotent progenitor cells have been isolated from human fetal liver that can differentiate into hepatocytes and cholangiocytes (Dan YY. Proc Natl Acad Sci USA 2006;103: 9912; Simper-Ronan R. Development 2006;133:4269). In mice, embryonic stem cells can be induced to differentiate into hepatic lineages and can contribute to hepatic repair when transplanted into immune-deficient mice (Heo J. Hepatology 2006;44:1478). (2006 10%; Total 30%)

**A1b. Profile transcriptional network during endodermal specification, liver growth, and regeneration.** Microarray analyses and genomic location analysis of different stages of liver development and regeneration are being evaluated by investigator-initiated research program grants (Odom DT. Mol Syst Biol 2006; 2:2006.0017), but there has yet to be a synthesis of results. (2006 0%; Total 0%)

**A2a. Identify noninvasive biomarker or imaging methods 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 several program announcements (PA-07-026: “Developmental Biology and Regeneration of the Liver”; PA-07-052, “Development of Disease Biomarkers”). (2006 0%; Total 0%)

**A2b. Define role of inflammation, fibrosis, and cell injury in regeneration.** Defective regeneration occurs in the situation of “small-for-size” liver grafts after partial liver transplantation. In a rat model, induction of c-Jun N-terminal kinase and cyclin D1 were critical in the normal regenerative process and were deficient in the blunted regeneration associated with a small-for-size graft. This defect may be partially reversed by providing energy sources and decreasing oxidative stress (Zhong Z. Transplantation 2006;82:241). (2006 10%; Total 20%)

**A3a. Define role of nonparenchymal cells in liver regeneration and liver development.** This and other areas of research on liver regeneration are actively encouraged in a specific program announcement, “Developmental Biology and Regeneration of the Liver” (PA-07-026). (2006 0%; Total 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 studies in these models have already delineated roles for several pathways in liver development. (2006 0%; Total 20%)

**B1a. Develop methods to select transplanted donor cells and induce homing and engraftment of transplanted cells to the liver.** Somatostatin is a regulatory peptide that appears to act as a chemoattractant for hepatic precursor cells and may be involved in migration of hepatic oval cells to sites of injury and regeneration (Jung Y. Lab Invest 2006;86:477). Proliferation of stem cell-

derived hepatocytes in the liver is modulated by cell-cell competition and the opposing processes of proliferation vs apoptosis (Oertel M. Gastroenterology 2006; 130:507). Gene therapy of liver disease would be benefited by the development of a means of targeting cells to the liver and providing growth advantages. (2006 10%; Total 10%)

**B1b. Identify how deregulation of genes and pathways involved in normal regeneration contributes to carcinogenesis.** Gene array studies from human cases of hepatocellular carcinoma (HCC) demonstrate upregulation of several genes coding for factors known to be important in regeneration, including Wnt and downstream mediators of the transcription factor AP-1 and the MAPK/ERK pathway (Llovet JM. Gastroenterology 2006;131:1758). Activation of Ras and JAK/STAT signaling is enhanced in virtually all cases of human HCC, and inhibition of these pathways may cause tumor cell apoptosis (Calvisi DF. Gastroenterology 2006;130:1117). (2006 10%; Total 20%)

**B2a. Validate biomarkers of regeneration in living donor liver donation and acute liver failure.** This area of research is encouraged directly in the program announcement PA-07-052 (“Development of Disease Biomarkers”) and is the focus of ancillary studies in the A2ALL cohort study of living donor liver transplantation. (2006 0% Total 0%)

**B2b. Identify pathways that stop proliferation of hepatocytes as liver returns to normal mass.** Factors that control or stop hepatocyte proliferation in human liver regeneration have not yet been defined. (2006 0%; Total 10%)

**B3. Delineate sequence of molecular and cellular events that lead embryonic stem cells to differentiate into mature hepatocytes.** Factors that lead to differentiation of stem cells to mature hepatocytes are likely to be multiple, interrelated, and redundant. In the presence of activin A, mouse embryonic stem cells differentiate into endodermal progenitors, and with addition of bone morphogenetic protein-4 (BMP-4) and fibroblast growth factor (FGF), they differentiate further into hepatic cells that produce albumin and alpha-fetoprotein (Gouon-Evans V. Nat Biotech 2006; 24:1402). Delineation of steps in the transition from human stem cells to hepatocytes has not yet been accomplished. (2006 10%; Total 20%)

**C1. Develop *ex vivo* and *in vivo* vectors for liver-directed gene therapy.** Research on vectors for gene therapy is encouraged through the NIH-funded Molecular Therapy Centers programs. Second generation adeno-associated virus vectors (AAV7, 8 and 9) have been developed for gene therapy and shown to provide high-level transgene expression in mice and non-human primates, but have yet to be tested for safety and efficacy in humans (Gao GP. J Virol 2006; 80: 6192). (2006 0%; Total 0%)

**C2a. Develop safe means of promoting normal liver regeneration for acute liver failure, liver resection, and transplantation.** Several small molecules and growth factors have promise as a means of promoting regeneration in humans. Platelet-derived serotonin promotes liver regeneration in the mouse, apparently through serotonin receptors on hepatocytes that might be a target to stimulate

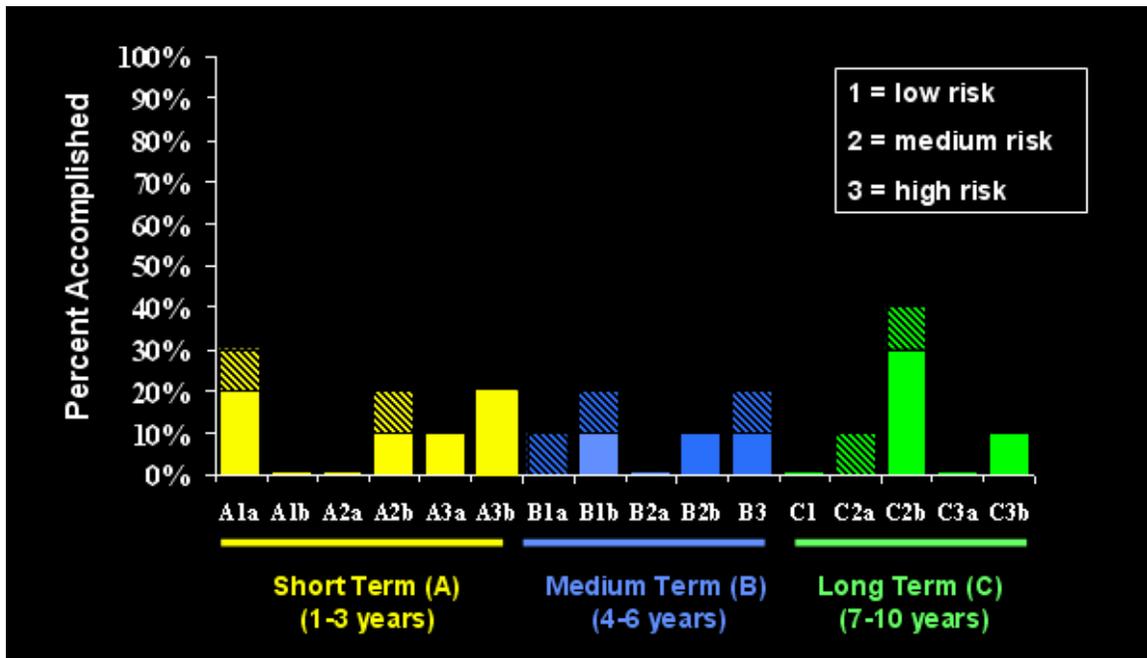
regeneration (Lesurtel M. Science 2006;312:104). Also in mice, nuclear receptor (farnesoid-X-receptor [FXR])-dependent bile acid signaling is required for normal regeneration suggesting that administration of bile acids or FXR ligands might be used to promote regeneration (Huang W. Science 2006;312:233). (2006 10%; Total 10%)

**C2b. Delineate molecular and cellular events that lead from endodermal liver primordium to mature liver in fetal development.** Development of the liver from the embryonal foregut is mediated by FGF, which is produced by surrounding mesenchymal tissue and acts on endodermal epithelium. Recent studies have elucidated the intracellular pathways for FGF effects, including the MAP kinase pathway that induces differentiation of endoderm into hepatocytes, the PI<sub>3</sub> kinase pathway that leads to cell proliferation; coordination of these signals leads to normal hepatogenesis (Calmont A. Dev Cell 2006;11:339). Mesodermal Wnt2 signaling has been shown to be essential in liver specification in the zebrafish model system (Ober EA. Nature 2006;442: 688); parallel studies in mice have also demonstrated the importance of the Wnt/beta-catenin pathway (Tan X. Gastroenterology 2006;131:1561). Cell junction pathways are also important in the epithelial transformation of the developing liver (Battle MA. Proc Natl Acad Sci USA 2006; 103:8419). (2006 10%; Total 40%)

**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. (2006 0%; Total 0%)

**C3b. Develop *in vitro* model of hepatic organogenesis.** Studies on the sequence of cell signaling events that determine hepatic specification in animal models from *Xenopus* to mice are setting the stage for the ability to reproduce hepatic organogenesis *in vitro*. (2006 0%; Total 10%)

Figure 5. Estimated Progress on Developmental Biology and Regeneration Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]



## Chapter 4

### Bile, Bilirubin, and Cholestasis

- A1. Further identify molecular causes of various forms of PFIC.** In a multicenter study, 11 children with PFIC-3 were identified who developed hepatocellular carcinoma during childhood; in all cases that could be analyzed, mutations in the bile salt export pump gene *ABCB11* were identified (Knisely AS. *Hepatology* 2006;44:478). (2006 10%; Total 20%)
- A2. Define structure-function relationships of genes involved in cholestatic liver diseases and identify potential targets for therapy.** The mechanism is unknown by which mutations in the gene encoding ATP8B1, a canalicular phospholipid flippase, results in the liver injury of Byler disease (PFIC-1). In a mouse model for PFIC-1 (*Atp8b1* knockout), hepatocanalicular membrane lipid composition was altered, rendering membranes more vulnerable to the toxic effects of bile acids and less able to transport hydrophobic bile acids into bile, and thus promoting toxic bile retention, a possible mechanism of the cholestatic liver injury (Paulusma CC. *Hepatology* 2006; 44:195). (2006 0%; Total 10%)
- A3. More fully define the normal fetal development and maturation of bile salt and bilirubin metabolic pathways.** No new advances have been made in this area. (2006 0%; Total 10%)
- B1. Define whether polymorphisms of major bile transporters are involved in drug-induced cholestatic liver disease.** Variability in expression of the major hepatic bile salt and anion transporters has been investigated in normal populations. In a study of DNA from healthy populations, 76 single nucleotide polymorphisms (SNPs) were identified in the multidrug resistance protein 3 (MDR3) gene (*ABCB4*) and 86 in the bile salt export pump (BSEP) gene (*ABCB11*), among which four were predicted to have functional consequences (Lang T. *Drug Metab Dispos* 2006;34:1582). In studies on liver tissue, high expression of hepatic transporters was found in 30% and low expression in 32% of specimens, with several SNPs being associated with decreased gene expression, including one in *ABCB11* and two in *ABCC2* (Meier Y. *Hepatology* 2006;44:62). These studies provide candidate genes for analysis in cases of drug-induced liver disease. (2006 10%; Total 10%)
- B2a. More fully elucidate the normal pathways of bile salt, lipid, and organic solute uptake, synthesis, transport, and secretion in hepatocytes.** The bile acid transporters OST $\alpha$  and OST $\beta$  are expressed on the sinusoidal membranes of human hepatocytes and mediate efflux of bile acids into the plasma. Recent studies indicate that these transporters are regulated by FXR and increase in expression and activity with cholestasis, both in animal models and in humans with liver disease (Landrier JF. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G476; Boyer JL. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G1124). The role of multidrug resistance associated protein 4 (MRP4) in bile production and cholestatic liver disease is not well defined. In an *Mrp4*

knockout (-/-) mouse model, this protein was found to play a protective role in the adaptive response to cholestatic liver injury (Mennone A. *Hepatology* 2006; 43:1013). Bile acids have functions beyond cholesterol and lipid homeostasis. In rodents, bile acids increase energy expenditure and decrease weight gain, effects shown to be due to increases in intracellular cyclic AMP and enzymes that activate thyroid hormone (Watanabe M. *Nature* 2006;439:484). Furthermore, bile acids that reach the terminal ileum induce expression and release of fibroblast growth factor 19, which acts in a hormonal fashion to promote gallbladder filling through cyclic AMP-stimulated relaxation of smooth muscle cells, thus acting to autoregulate the hepatoenteric circulation of bile acids (Choi M. *Nat Med* 2006;12:1253). The effects and significance of bile acids in regulation of energy expenditure and gallbladder filling in humans await further study. (2006 20%; Total 40%)

**B2b. Define the pathways and regulation of hepatic cholesterol synthesis and secretion.** This is an area of active investigator-initiated research. The mechanism by which the transporter complex ABCG5/ABCG8 mediates cholesterol transport into bile is not known. In studies of *Abcg8* knockout (-/-) mice, the cholesterol content of the outer leaf of liver canalicular membranes was reduced, suggesting that ABCG5/ABCG8 acts by flopping cholesterol from the inner to the outer leaflet of the canalicular membrane (Kosters A. *J Lipid Res* 2006;47:1959). (2006 10%; Total 20%)

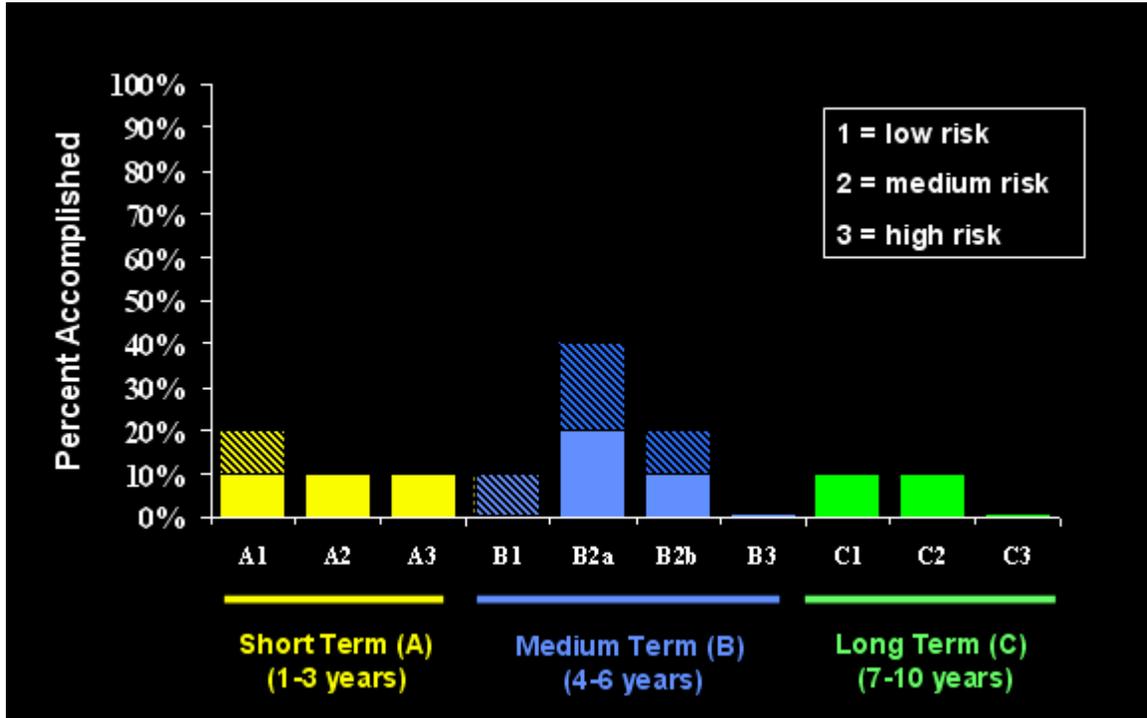
**B3. Develop drug therapy that stimulates bilirubin metabolic pathways or interferes with bilirubin production in the newborn.** Better information is needed on the metabolic pathways and their status at birth before interventions can be designed. In a prospective, long-term follow up study of severely jaundiced newborns (bilirubin levels  $\geq 25$  mg/dL), growth, development, and cognitive function were similar among jaundiced compared to control children, suggesting that current practices of phototherapy and exchange transfusion are usually successful in preventing neurological damage (Newman TB. *N Engl J Med* 2006;354:1889). (2006 0%; Total 0%)

**C1. Define molecular basis of pruritus and identify targets for potential therapies.** Small grants of innovative therapies for pruritus are encouraged in the ongoing program announcement PA-06-301, "Pilot and Feasibility Clinical Research Studies in Digestive Diseases and Nutrition." (2006 0%; Total 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.** The association of these diseases with specific defects in hepatocyte transporters is under study. (2006 0%; Total 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), both of which are usually fatal to children without liver transplantation. (2006 0%; Total 0%)

Figure 6. Estimated Progress on Bile, Bilirubin, and Cholestasis Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]



## Chapter 5 Viral Hepatitis

- A1a. Define basis for interferon resistance of HCV in humans.** Only half of patients with chronic hepatitis C treated with an optimal regimen of peginterferon and ribavirin have a sustained virological response. Clinical factors that are independently associated with response are white race, low viral levels, female sex, lower body weight, absence of insulin resistance, lesser degrees of hepatic fibrosis, and better medication compliance. The biologic basis for non-response to treatment is the focus of considerable NIH-funded research, and preliminary results suggest roles for USP 18 (Randall G. *Gastroenterology* 2006;131:1584), IL-10 (Paladino N. *J Virol* 2006;80:9144), and inhibition of STAT-1 (Lin W. *J Virol* 2006;80:9226). Immune function may also be important. A three-day research workshop on mechanisms of action of interferon and ribavirin in hepatitis C is scheduled for March 3-5, 2007, sponsored by the American Association for the Study of Liver Diseases. (2006 10%; Total 20%)
- 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 in children, liver transplant candidates, patients with renal failure, substance abusers, and minorities. The recently completed NIH-funded Virahep-C study revealed sustained virological response rates of 28% among African Americans treated with peginterferon and ribavirin compared to 52% in Caucasian Americans; predictors of response were the same in the two racial groups, and no clinical factor was found to explain the racial differences (Conjeevaram HS. *Gastroenterology* 2006;131:470). (2006 10%; Total 30%)
- A2. Fully define the pathways of interferon induction and effector action against HCV and HBV *in vitro* and *in vivo*.** Multiple studies have demonstrated interactions between HCV or HBV and interferon signaling pathways leading to altered gene expression (Lanford RE. *Hepatology* 2006;43:961). Further progress has been made in understanding how the HCV NS3/4A protease inhibits RIG-I signaling and interferon-beta induction by cleavage of mitochondrial antiviral signaling protein (MAVS) as first reported in 2005 (Loo YM. *Proc Natl Acad Sci USA* 2006;103:6001; Cheng G. *Proc Natl Acad Sci USA* 2006;103:8499). These findings suggest that the HCV protease inhibitors may be beneficial in inhibiting HCV replication in two ways: (1) directly, by blocking viral protein processing, and (2) indirectly, by rescuing endogenous interferon signaling. (2006 10%; Total 40%)
- A3. Develop a cell culture system that is fully permissive for HCV replication.** Further progress has been made in development of the infectious HCV systems first reported in 2005. A cell culture-infectious genotype 1a virus (Yi M. *Proc Natl Acad Sci USA* 2006;103:2310) and cell culture-infectious HCV chimeric viruses within the background of the genotype 2a JFH1 strain have been developed (Pietschmann T. *Proc Natl Acad Sci USA* 2006;103:7408). Cell culture-grown HCV is infectious in animal models and serum from these infected

animals is directly infectious in cell culture (Lindenbach BD. Proc Natl Acad Sci USA 2006;103:3805). These systems have provided new insights into the cell surface receptors for HCV and the potential for neutralizing antibody to HCV. There are still no means for directly culturing virus from typical clinical specimens. (2006 10%; Total 60%)

**B1a. Fully define early events during HCV and HBV infection.** Investigation of the early immune responses during acute hepatitis C is a specific focus of the recently NIH-funded Hepatitis C Cooperative Research Center Program. A study of patients with acute hepatitis C indicates that both CD4 and CD8 T cell responses are important in clearance of virus and recovery (Urbani S. Hepatology 2006;44:126). (2006 0%; Total 20%)

**B1b. Define whether long-term interferon therapy is beneficial in non-responders with HCV.** Two trials of long-term peginterferon therapy in patients with advanced chronic hepatitis C are ongoing (HALT-C and EPIC3). An announcement of results is expected within the next year. (2006 0%; Total 0%)

**B2a. Identify new targets in viral replication and the host for development of small molecule therapeutics (HCV, HBV, HDV).** Host cell and viral targets for which new information became available in 2006 include the cyclophilins (Ishii N. J Virol 2006;80:4510; Paeshuyse J. Hepatology 2006;43:761) and the NS2-3 protease (Lorenz IC. Nature 2006;442:831). The mechanisms by which these hepatitis viruses persist in the face of immune pressure are being elucidated in animal models using viruses such as LCMV, and roles are emerging for PD1 (Day CL. Nature 2006;443:350), CD27 signaling (Matter M. J Exp Med 2006; 203:2145), and IL10 (Brooks DG. Nat Med 2006;12:1301) in downregulating B or T cell responses. Further evaluation is warranted of the role of these molecules and the effects of inhibiting their function during chronic HBV, HDV, and HCV infection (Urbani S. J Virol 2006;80:11398). (2006 10%; Total 10%)

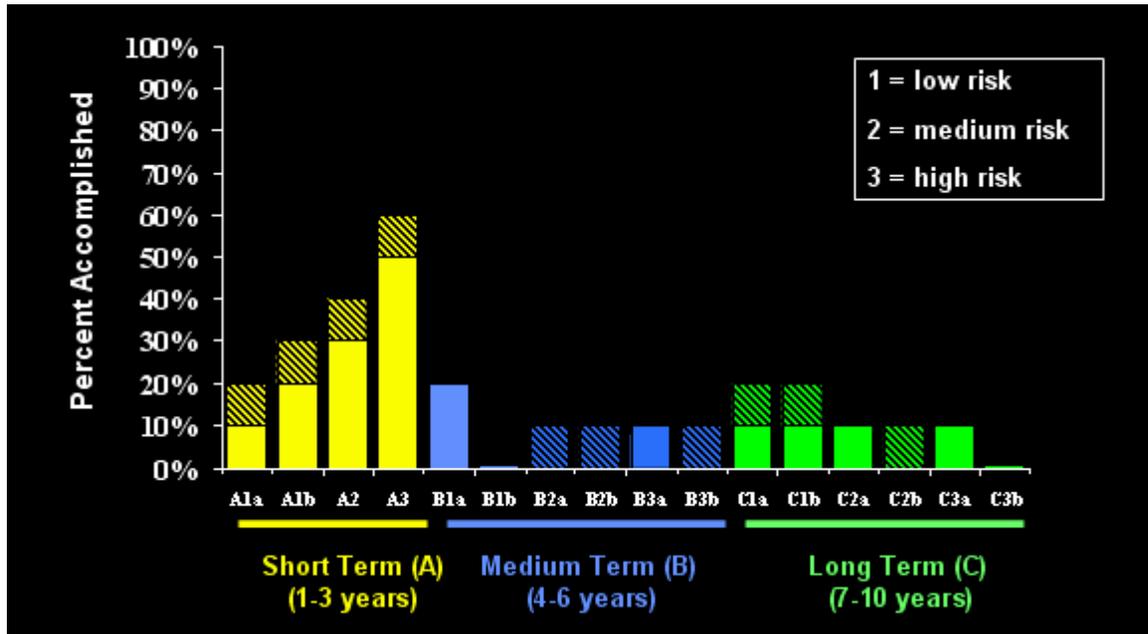
**B2b. Define the molecular basis for antiviral resistance of HBV.** Resistance patterns to adefovir and entecavir have been further defined in patient populations being treated for hepatitis B (Yim HJ. Hepatology 2006;44:703; Colonna RJ. Hepatology 2006;44:1656). The full implications of these resistance mutations are not well known. Tenofovir and entecavir, the two most potent anti-HBV agents approved for use in humans, have low rates of viral resistance even when given for several years as monotherapy. Prospective studies are needed. (2006 10%; Total 10%)

**B3a. Develop small animal models of HCV replication and liver disease.** Current animal models suitable for studying HCV replication and pathogenesis are limited in availability and applicability, but are still needed for basic and pre-clinical studies, particularly for more novel antiviral targets. (2006 0%; Total 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 Management of Chronic Hepatitis B was held in April 2006, at which time the needs for future research in this area were detailed. The early steps of HBV binding, uptake, and trafficking to the nucleus of hepatocytes have been

- partially elucidated *in vitro* (Stoeckl L. Proc Natl Acad Sci USA 2006;103:6730; Funk A. Hepatology 2006;44:685). (2006 10%; Total 10%)
- C1a. Evaluate new approaches to therapy in all five forms of viral hepatitis.** Progress has been made in developing small molecule inhibitors of HCV replication, targeting the RNA polymerase and serine protease. Preliminary results of an HCV protease inhibitor are very promising (Reesink HW. Gastroenterology 2006;131:997). Phase I and II clinical trials of several HCV polymerase and protease inhibitors are underway. (2006 10%; Total 20%)
- C1b. Evaluate long-term benefits and risks of combination therapy of HBV.** The benefits and risks of combination therapy of HBV have been evaluated only in small, short-term prospective studies. In the NIH workshop held in April 2006, the shortcomings of reported studies on combination therapy were detailed. Recently, more potent nucleoside analogues for hepatitis B have been introduced, including entecavir (Lai CL. N Engl J Med 2006;354:1011; Chang TT. N Engl J Med 2006; 354:1001) and tenofovir (Peters MG. Hepatology 2006;44:1110), both of which are associated with a low rate of antiviral resistance, at least after 1-2 years. Long-term, appropriately controlled and powered studies are being designed. (2006 10%; Total 20%)
- C2a. Develop ways to prevent re-infection after liver transplant for HCV (e.g. HCIG, anti-virals).** Clinical trials have been initiated of peginterferon and ribavirin therapy of hepatitis C before transplantation aimed at prevention of re-infection. Lowering HCV RNA to undetectable levels in preparation for transplantation has been followed in some cases by a lack of HCV infection of the graft; proof of the overall efficacy and safety of this approach requires prospective controlled observation. (2006 0%; Total 10%)
- C2b. Achieve sustained response rate of over 90 percent in chronic hepatitis C.** The sustained virological response rate to current therapy is 50-60%, and is higher in patients with genotypes 2 and 3 (~80%) than genotype 1 (~45%). Several promising new HCV protease and polymerase inhibitors with potent activity against HCV have been described. Combination studies of these new agents with peginterferon, with and without ribavirin, have been initiated, but no final results have been reported. (2006 10%; Total 10%)
- C3a. Develop HCV vaccine.** Research on HCV vaccine development continues, and promising results ("proof of principle") have been obtained using T cell HCV vaccines in the chimpanzee model (Folgori A. Nat Med 2006;12:190). In addition, a candidate recombinant HCV E1/E2 antigen vaccine developed by the Chiron Corporation is being evaluated in phase I clinical trials. (2006 0%; Total 10%)
- C3b. Develop therapeutic HBV vaccine.** NIH-funded basic research on innate immunity and means of augmenting T cell responses to vaccines promises to provide the impetus for further work on a therapeutic HBV vaccine. In the duck hepatitis B virus model, DNA vaccines expressing DHBV surface antigen suppress viral replication (Miller DS. Virology 2006; 351:159). (2006 0%; Total 0%)

**Figure 7. Estimated Progress on Viral Hepatitis Research Goals, 2006 (Year 2)**  
[Cross-hatching indicates recent year's progress.]



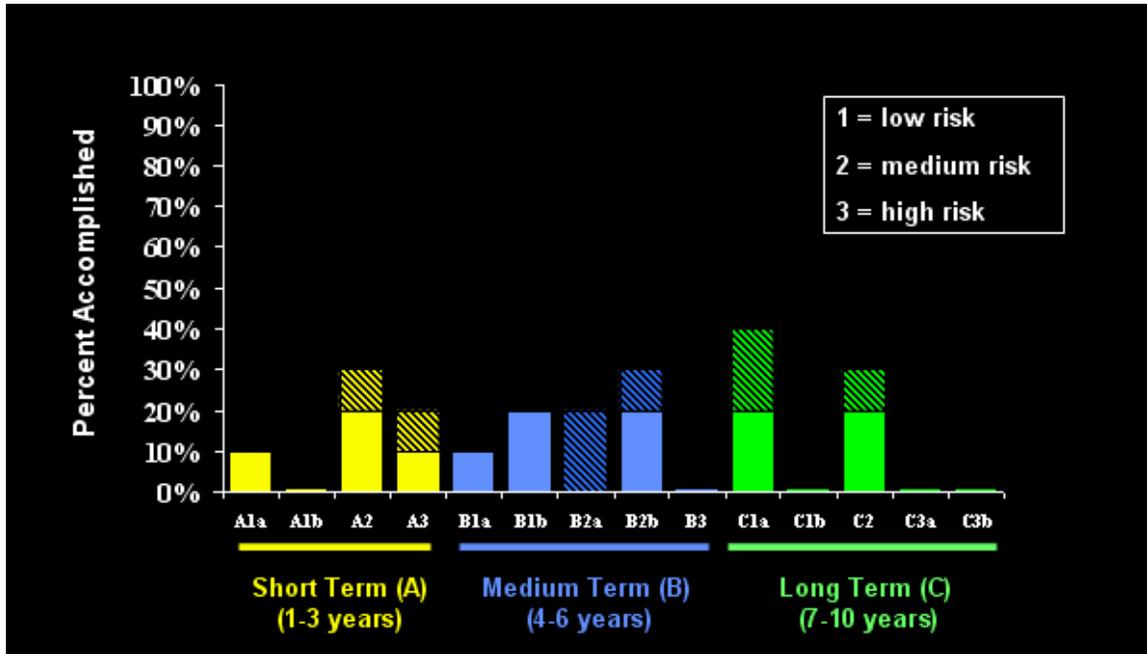
## Chapter 6 HIV and Liver Disease

- A1a. Develop improved regimens of HAV and HBV vaccination.** Both HAV and HBV vaccination is now recommended for all infants in the United States. No new regimens of administration specific for HIV-infected persons have been developed. (2006 0%; Total 10%)
- A1b. Define short- and long-term safety and efficacy of peginterferon and ribavirin in different subpopulations of patients with HIV-HCV co-infection.** No new studies have been published on response rates and safety profiles of standard peginterferon and ribavirin therapy in subsets of patients with HIV-HCV co-infection. (2006 0%; Total 0%)
- A2. Define safety and efficacy of peginterferon therapy for acute hepatitis C in HIV co-infection.** An observational study of 9 patients with HIV infection who developed acute HCV infection has documented a rate of chronicity that is similar to that of patients without HIV infection (~78%) and shown that early therapy with peginterferon and ribavirin is effective in eradicating HCV infection in most, but not all, subjects (~75%) (Luetkemeyer A. *J Acquir Immune Defic Syndr* 2006; 41:31). Better definition is needed of the optimal timing and regimen of therapy during acute hepatitis C. (2006 10%; Total 30%)
- A3. Define effects of HIV infection on the liver, including on different populations of liver cells.** Gene expression arrays have demonstrated that patients with HIV/HCV co-infection have similar intrahepatic gene expression profiles as patients with HCV infection alone (Walters KA. *Virology* 2006;350: 453). Certain profiles predict a lack of response to peginterferon and ribavirin therapy, in that patients with high levels of interferon-induced genes are less likely to have a virological response to therapy (Lempicki RA. *J Infect Dis* 2006; 193:1172). HIV infection may lead to an increase in STAT1 activation, which, in turn, can lead to increased hepatocyte apoptosis and Fas ligand expression (Balasubramanian A. *J Infect Dis* 2006; 194:670). (2006 10%; Total 20%)
- B1a. Define whether long-term peginterferon slows progression of disease in chronic hepatitis C with HIV co-infection.** The Adult AIDS Clinical Trials Group (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. (2006 0%; Total 10%)
- B1b. Define prevalence, etiology, and severity of different liver diseases in different cohorts of HIV-infected patients.** Liver disease continues to be a major cause of morbidity and mortality in HIV-infected patients. The etiologies include hepatitis B and C, nonalcoholic and alcoholic liver disease, and drug-induced liver injury. Nevirapine hepatotoxicity is associated with variants of the *MDR1* gene, but not of common *CYP* polymorphisms (Haas DW. *Clin Infect Dis* 2006; 43: 783). (2006 0%; Total 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 largely remain unknown. Among HIV/HCV co-infected subjects, the severity of fibrosis correlates with lower CD4 T cell counts (Monto A. *Am J Gastroenterol* 2006;101:1509). Furthermore, progression of the liver disease may be slowed by anti-retroviral therapy and successful suppression of HIV (Brau N. *J Hepatol* 2006;44:47; Verma S. *Clin Infect Dis* 2006; 42:262) (2006 20%; Total 20%).
- B2b. Define factors that lead to reactivation of HBV in HIV co-infection and develop means of prevention.** The causes of sudden worsening of hepatitis B in HIV co-infected persons include: (1) loss of anti-HBV due to progressive immune deficiency, (2) stopping antiretroviral drugs with activity against HBV, and (3) development (or selection) of HBV antiviral resistance mutations. Recent data suggest that improved regimens of therapy for hepatitis B have decreased the mortality rate of patients with HBV/HIV co-infection (Martin-Carbonero L. *J Viral Hepat* 2006;13:851). (2006 10%; Total 30%)
- 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 of Diabetes, Kidney, Urological, Hematological and Digestive Diseases” (PA-07-025) and “Development of Disease Biomarkers” (PA-07-052). (2006 0%; Total 0%)
- C1a. Develop optimal therapeutic regimens for chronic hepatitis B in different stages and patterns of disease in HIV-co-infected patients.** Tenofovir has been shown to have more potent antiviral activity against HBV than adefovir in HIV/HBV co-infected persons (Peters MG. *Hepatology* 2006;44:1110). The combination of tenofovir and emtricitabine is now recommended as standard of therapy for HBV/HIV co-infection (Hammer SM. *JAMA* 2006;296:827). (2006 20%; Total 40%)
- C1b. Define safety and efficacy of new agents for therapy of hepatitis C in HIV co-infection.** Several new compounds with activity against HCV have been developed and are moving through clinical trial phases, HIV/HCV co-infected cohorts have not been included in early testing. (2006 0%; Total 0%)
- C2. Develop noninvasive means of assessing liver disease stage and activity in HIV-infected persons.** Both standard laboratory tests and elastography show reliability in predicting fibrosis in patients with HIV/HCV co-infection (Barreiro P. *Clin Infect Dis* 2006; 42:1032; Wilson LE. *Hepatology* 2006;43:788). (2006 10%; Total 30%)
- 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. (2006 0%; Total 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 and to pool resources in an attempt to elucidate pathogenesis.(2006 0%; Total 0%)

**Figure 8. Estimated Progress on HIV and Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year’s progress.]**



## Chapter 7

### Fatty Liver Disease

- A1. Establish cohort study to prospectively analyze the natural history of the full spectrum of nonalcoholic fatty liver disease.** In a cross-sectional study of 218 apparently healthy men, fatty liver disease correlated with physical fitness in addition to body mass index (Church TS. *Gastroenterology* 2006;130:2023). The NIH-funded NASH Clinical Research Network has enrolled a cohort of more than 700 individuals who will be followed for 5 years, providing resources for studies of natural history, genetics, and biomarkers. (2006 10%; Total 50%)
- 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).** A phase I study of silymarin has been initiated and phase II trials planned in both NASH and hepatitis C by the Silymarin in NASH and Hepatitis C (SynCH) Consortium. Studies of S-adenosylmethionine (SAME) have been planned as a part of the NASH Clinical Research Network. In two children with short bowel syndrome and severe total parenteral nutrition (TPN)-associated liver disease, substitution of a fish oil-based lipid intravenous emulsion (with omega-3 fatty acids) for the standard soy-based TPN preparation was followed by resolution of the cholestasis and all biochemical evidence of liver injury (Gura KM. *Pediatrics* 2006;118:e197). Further, controlled studies of omega-3 fatty acid-based lipid preparations are warranted. (2006 10%; Total 10%)
- A3. Develop more accurate animal models of nonalcoholic fatty liver disease (including secondary forms) and define molecular characteristics.** A phenotype of obesity, metabolic syndrome, fatty liver and shortened life span was reproduced in mice by deletion of the DNA repair enzyme *neil1*, suggesting that oxygen –radical-induced DNA damage might predispose to fatty liver (Vartanian V. *Proc Natl Acad Sci USA* 2006;103:1864). The high-fat diet induced model of fatty liver disease in the mouse continues to provide novel insights and potential approaches to therapy. Molecular profiling of mice with genetic predisposition to diet-induced fatty liver suggests disruption of choline metabolism and a contribution of symbiotic gut microflora (Dumas ME. *Proc Natl Acad Sci USA* 2006;103:12511). Several molecules have been found to reverse or attenuate fatty liver in mouse models, including GLP-1 receptor agonists (Ding X. *Hepatology* 2006;43:173), SAME (Oz HS. *J Biochem Mol Toxicol* 2006;20:39), omega-3 polyunsaturated fatty acids (Svegliati-Baroni G. *Am J Pathol* 2006;169:846), metalloproteinase inhibitors (Alwayn IP. *Am J Physiol Gastrointest Liver Physiol* 2006;291: G1011), and resveratrol, an antioxidant component of red grapes (Baur JA. *Nature* 2006;444: 337). (2006 10%; Total 20%)

- 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. Adiponectin has been found to be decreased in serum of patients with NASH, and recent studies demonstrated that its expression is also decreased in adipose tissue from patients with fatty liver disease (Barnova A. *Obes Surg* 2006;16:1118). The intrahepatic gene expression profile in human alcoholic hepatitis has been described and differs from both normal liver and alcohol-induced steatosis, with major increases in claudins, osteopontin, CD209, and selenoprotein (Seth D. *J Hepatol* 2006;45:306). A meeting on nuclear receptors in liver and digestive diseases is planned for November 2007, which will include presentations on the potential roles of the orphan nuclear receptors in NASH. (2006 0%; Total 10%)
- B1b. Evaluate role and effects of bariatric surgery on NASH.** Small single-center studies demonstrate improved serum lipoproteins, insulin resistance, serum aminotransferase levels, and histological features of NASH after gastric bypass surgery for obesity (Barker KB. *Am J Gastroenterol* 2006;101:368). The Longitudinal Assessment of Bariatric Surgery (LABS) was funded as a cooperative agreement with a data coordinating center and six Clinical Sites and has enrolled over 4000 patients, of whom a subset will be evaluated for the effects of surgery on NASH. (2006 10%; Total 20%)
- B2a. Delineate the hepatic pathways of lipid metabolism and how they are altered in alcoholic and nonalcoholic liver disease.** Elevated free fatty acids can induce hepatocyte apoptosis in cell culture, and this injury has now been shown to be mediated through the c-Jun N-terminal kinase pathway by activation of pro-apoptotic mediators of mitochondrial dysfunction (Malhi H. *J Biol Chem* 2006;281:12093; Schattenberg JM. *Hepatology* 2006;43:163). The role of these pathways in patients with NASH awaits further study. Alcohol is known to induce an increase in hepatic lipids, which is mediated in part by upregulation of plasminogen activator inhibitor (PAI-1). In an animal model of alcoholic liver disease, metformin significantly blunted the increase in hepatic lipids and blocked activation of PAI-1 (Bergheim I. *Gastroenterology* 2006;130:2099). Increase in lipids in response to ethanol is also partially due to sterol response element binding proteins (SREBPs), and, therefore, inhibition of their induction may ameliorate alcohol's effects on the liver (Ji C. *J Hepatol* 2006;45:717). (2006 10%; Total 20%)
- 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 diagnosis currently rests on findings by liver biopsy. Serum biomarkers of liver cell apoptosis (caspase-3-generated cytokeratin-18 fragments) show promise

as means of separating simple steatosis from NASH (Wieckowska A. Hepatology 2006;44:27). (2006 10%; Total 10%)

**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 this area in the RFA “Pilot-Scale Libraries for High-Throughput Drug Screening” (RFA-RM-06-003), and a PA for similar grants was published as “Development of Assays for High-Throughput Drug Screening” (PA-07-054). (2006 0%; Total 0%)

**B3b. Develop therapy of acute alcoholic hepatitis that promotes recovery and decreases permanent injury.** A randomized controlled trial from the UK found that a cocktail of antioxidants was not as effective as standard use of corticosteroids as therapy of severe acute alcoholic hepatitis (Phillips M. J Hepatol 2006;44:784). An NIH-sponsored trial of antibody to TNF soluble receptor in alcoholic hepatitis has achieved over 50% enrollment. (2006 10%; Total 10%)

**C1a. Establish the efficacy and safety of therapy with insulin-sensitizing agents and vitamin E in NASH.** A short-term, small randomized controlled trial of pioglitazone in patients with diabetes and NASH demonstrated marked beneficial effects on the metabolic, serum biochemical, and histological features of disease (Belfort R. N Engl J Med 2006;355:2297). A randomized controlled trial in 90 Italian children with NASH showed a beneficial effect of weight loss, but not of vitamin E (Nobili V. Aliment Pharmacol Ther 2006;24:1553). The NASH Clinical Research Network has completed enrollment in a prospective, placebo-controlled trial of two years of pioglitazone vs vitamin E vs placebo in 280 adults. A similar trial of metformin vs vitamin E vs placebo in 180 children with NASH has reached 50% enrollment. Results should be available in two years. (2006 20%; Total 40%)

**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. A Cochrane Review has concluded that there is insufficient evidence supporting or refuting the benefits of use of SAME in alcoholic liver disease, and prospective randomized controlled trials are needed.(2006 0%; Total 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.** An autopsy study in children from San Diego showed that 10% of children had fatty liver, of whom 23% met histological criteria for NASH. Fatty liver was more common in older children, in overweight or obese children and in those of Hispanic ethnicity compared to non-Hispanic whites and blacks (Schwimmer JB. Pediatrics 2006;118:1388). In studies using MRI spectroscopy, fatty liver and

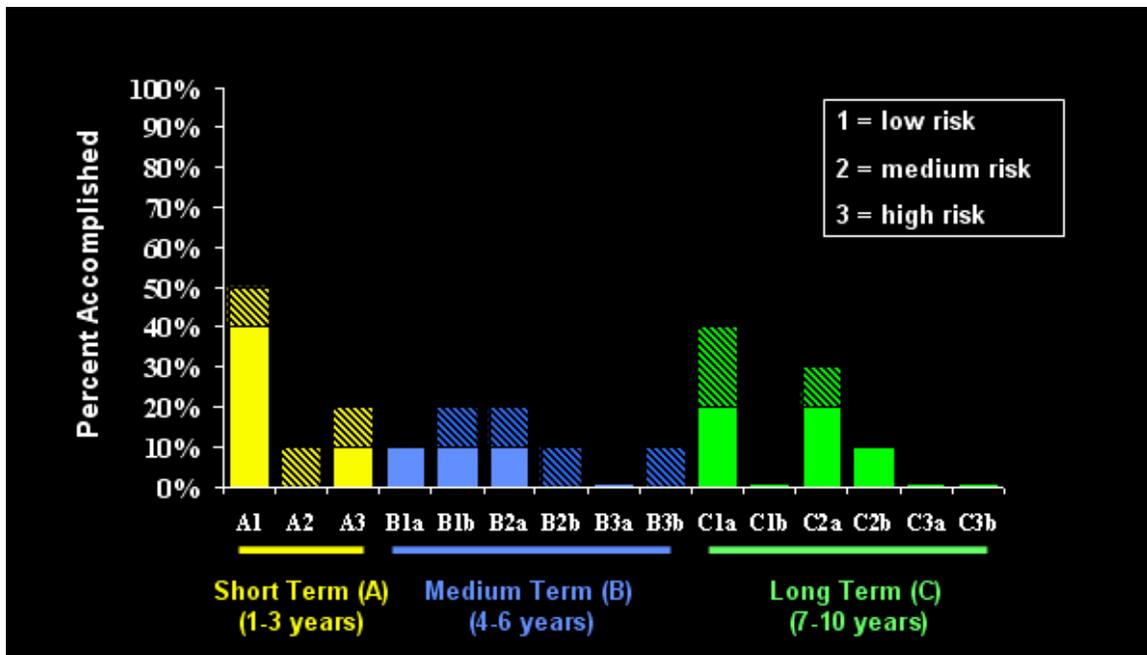
insulin resistance were more frequent in Asian-Indian men than in matched populations of whites and blacks (Petersen KF. Proc Natl Acad Sci USA 2006;103:18273). These findings strongly implicate genetic predisposition to development of NASH in both children and adults. (2006 10%; Total 30%)

**C2b. Better define the safe amounts of alcohol intake in terms of liver disease for different populations.** Prospective evaluations of alcohol intake and progression of NAFLD and other liver diseases are needed to better define safe alcohol intake in liver disease. (2006 0%; Total 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 on a greater number of patients and investigating an expanded set of polymorphisms are needed. (2006 0%; Total 0%)

**C3b. Develop screening programs for early detection and intervention with preventative or therapeutic regimens.** Without accurate noninvasive markers for NAFLD and better information on means of treatment and prevention, screening programs cannot yet be initiated. (2006 0%; Total 0%)

**Figure 9. Estimated Progress on Fatty Liver Disease Research Goals, 2006 (Year 2)**  
 [Cross-hatching indicates recent year's progress.]



## 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 that is being prospectively assessed and compared against other instruments. (2006 0%; Total 20%)
- A2. Develop positive diagnostic assay for acetaminophen toxicity.** An assay for acetaminophen adducts has been developed and shown to identify 90-100% of cases of acetaminophen-induced acute liver failure in adults (Davern TJ. *Gastroenterology* 2006;130:687) and in children (James LP. *Pediatrics* 2006;118:676). These adducts are not present in cases of acute liver failure of other known causes, but are present in 12-19% of cases of unknown cause. Thus, some idiopathic cases of acute liver failure may be caused by unrecognized or unacknowledged acetaminophen overdose—a finding that has major therapeutic implications. The test requires further modification to become clinically useful and its sensitivity for milder forms of acetaminophen injury requires elucidation. (2006 30%; Total 40%)
- A3a. Develop *in vitro* or *in vivo* systems for study of allergic and non-allergic idiosyncratic hepatotoxicity.** Modest degrees of inflammation induced by lipopolysaccharide make rats more sensitive to hepatotoxicity from diclofenac, a nonsteroidal anti-inflammatory agent associated with idiosyncratic liver injury in humans (Deng X. *J Pharmacol Exp Ther* 2006;319:1191). Animal models for idiosyncratic drug injury in humans would be extremely helpful in elucidating the this cause of liver injury and are specifically encouraged in the NIH Program Announcement (PA-07-012) “Animal Models of NIDDK-Relevant Diseases.” (2006 0%; Total 10%)
- A3b. Identify chemical substructures that are protoxicant and could be avoided in design of new drugs.** Investigators have initiated the development of an atlas of chemical substructures that are protoxicant, which were highlighted at an American Chemical Society-sponsored Prospectives Conference entitled “Applying Mechanisms of Chemical Toxicity to Predict Drug Safety” in June 2006. (2006 0%; Total 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 250 patients with idiosyncratic drug-induced liver injury into a database with careful collection of clinical information, serum, DNA, and tissue. Collaborations are being pursued with other clinical and laboratory investigators of drug-induced liver disease. (2006 10%; Total 20%)
- B2a. Elucidate molecular mechanisms of common forms of hepatotoxicity.** Molecular mechanisms of acetaminophen toxicity have been further defined with

identification of c-jun N-terminal kinase, an enzyme involved in the ER stress response, as a major downstream mediator of liver injury (Gunawan BK. *Gastroenterology* 2006;131:165). These findings have potential therapeutic implications. Investigation of mitochondrial nucleoside transport has shown that rodents lack the mitochondrial targeting signal for the equilibrative nucleoside transporter 1 (Lee E-W. *J Biol Chem* 2006;281:16700), which may account for the lack of toxicity of certain nucleoside analogues (fialuridine) in rodents that proved to be hepatotoxic in humans. This finding may lead to better animal models for screening agents with potential mitochondrial toxicity. (2006 10%; Total 20%)

**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.** Drug-induced liver disease continues to be the major cause of acute liver failure, and secular trends indicate that it is increasing. A two-week course of moderately high, but recommended, doses of acetaminophen induces significant aminotransferase elevations in approximately one-third of normal volunteers (Watkins PB. *JAMA* 2006;296:87). The relatively newly licensed macrolide antibiotic telithromycin has been linked to several cases of acute liver failure (Clay KD. *Ann Intern Med* 2006;144:415). (2006 0%; Total 10%)

**B3a. Define the role of the innate immune system in both allergic and non-allergic forms of hepatotoxicity.** Comparison of gene expression arrays from different strains of mice with differing sensitivity to acetaminophen liver toxicity demonstrated that heat shock protein 70 levels were lower and osteopontin levels higher in susceptible mice, suggesting that these mediators of the innate immune system may play a role in acetaminophen toxicity (Welch KD. *Chem Res Toxicol* 2006;19:223). These studies need to be extended to humans. (2006 10%; Total 20%)

**B3b. Develop an animal model of adaptation to hepatotoxicity to help define the genes necessary for the adaptive response.** A Program Announcement (PA-07-052) entitled "Development of Disease Biomarkers" has language specifically encouraging investigator-initiated research grants in this area. (2006 0%; Total 0%)

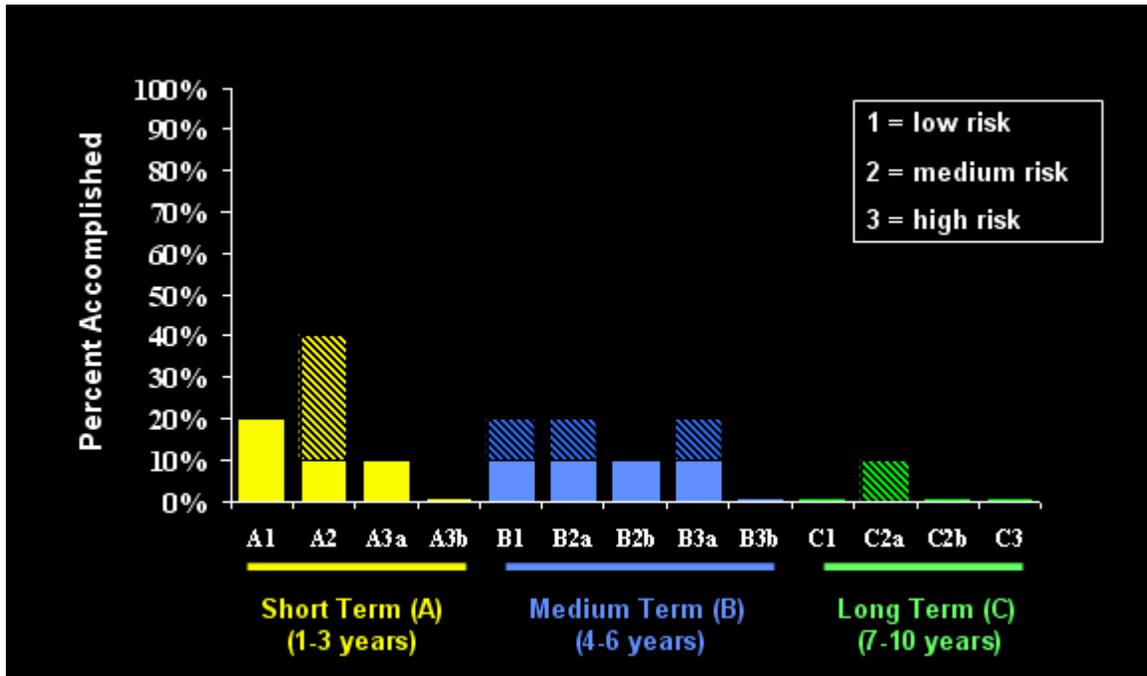
**C1. Identify genetic factors that contribute to hepatotoxicity of several major forms of drug-induced liver disease.** This area is the focus of extensive studies by industry and by the NIH-funded DILIN and Pharmacogenetics Research Networks, which are developing genetic screens for susceptibility to drug-induced liver injury. (2006 0%; Total 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 adult trial ended enrollment in December 2006, and results should be available in the next year. (2006 10%; Total 10%)

**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 Network, are focused on these issues. The FDA is developing guidance on monitoring for hepatotoxicity in pre-marketing trials of new pharmaceutical agents. Biomarkers are likely to be developed once cellular pathways of drug-induced liver injury are more fully defined. (2006 0%; Total 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 Toxicity Biomarker Study) has also begun in this area. (2006 0%; Total 0%)

**Figure 10. Estimated Progress on Drug- and Toxicant-Induced Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



## Chapter 9 Autoimmune Liver Disease

- A1. Organize and convene an international, interdisciplinary research workshop on development of animal models of autoimmune liver diseases.** A recent publication featured a summary and recommendations based on a 2005 research workshop on primary sclerosing cholangitis (PSC), in which animal models were discussed (LaRusso NF. *Hepatology* 2006;44:746). Several meetings on autoimmunity and the liver are being planned by academic societies. (2006 0%; Total 30%)
- A2. Develop multicenter networks of investigators to study natural history, pathogenesis, etiology, and therapy of autoimmune liver diseases.** Members of the research community have been encouraged to develop multicenter clinical research networks in autoimmune liver disease, and NIH grant applications are expected in 2007. Several multicenter groups from the US, UK, Italy and France have published results on natural history and pathogenesis. In a study of 282 patients with primary biliary cirrhosis (PBC) from France, followed for up to 13 years, 12 patients (4.3%) developed an autoimmune hepatitis (AIH)-like syndrome that was associated with a poor prognosis, particularly if corticosteroids were withheld (Poupon R. *Hepatology* 2006;44:85). (2006 10%; Total 10%)
- 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.** Single-center and multicenter cohorts have been used to assess immunological features of PBC, PSC, and AIH. Patients with PBC have increased numbers of natural killer (NK) cells (Chuang YH. *J Autoimmun* 2006;26:232) and autoreactive T cells (Shimoda S. *Gastroenterology* 2006;131:606), and reduced numbers of regulatory T cells (Tregs) in peripheral blood, and similar findings have been reported in animal models (Lan RY. *Hepatology* 2006;43:729). Tregs are also reduced in AIH (Longhi MS. *J Immunol* 2006;176:4484). With clearer definition of the pathogenic auto-antigens, antigen-specific T cell response can be better assessed for its role in liver and biliary injury. (2006 10%; Total 10%)
- 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.** An NIH-funded multicenter controlled trial of high-dose ursodiol in PSC has been fully enrolled and final results are expected in the next few years. (2006 0%; Total 30%)
- B2a. Develop sensitive and specific biomarkers for disease activity and stage in PBC and PSC.** Liver histology is not always reliable in assessing disease activity and stage in PBC and PSC. Promising approaches include elastography to assess liver stiffness or fibrosis and use of antigen-specific antibody levels or T cell responses to assess disease activity. The NIH encourages research in this area

through a specific PA: “Development of Disease Biomarkers” (PA-07-052). (2006 0%; Total 0%)

**B2b. Develop diagnostic criteria and standard definitions for endpoints of therapy.** Developing standardized terminology and diagnostic criteria for autoimmune liver disease would be a central component of an autoimmune clinical research network. (2006 0%; Total 10%)

**B3a. Identify genetic linkages in PBC and refine the HLA associations in autoimmune hepatitis and PSC.** Studies on ~ 500 patients from the UK and Italy indicate that PBC is associated with the class II MHC allele DRB1\*0801 (12% vs 4% in controls), while protection against PBC was associated with DRB1\*13 (Donaldson PT. *Hepatology* 2006; 44:667). The HLA genotype DRB1\*04 has been associated with AIH in studies from Asia, but less frequently in European populations where DRB1\*03 is commonly associated with the disease. In a study of U.S. patients, the combination of class II alleles DRB1\*03-DRB1\*04 was more common in patients with AIH than controls (14% vs 4%) and linked to worse outcomes (Montano-Loza AJ. *Liver Int* 2006;26:1201). (2006 10%; Total 10%)

**B3b. Develop animal models for each of the autoimmune liver diseases.** In the last year, three promising models for PBC have been described, including the *Nod.c3c4* congenic mouse (Irie J. *J Exp Med* 2006;203:1209), the TGF $\beta$  receptor II dominant-negative mouse (Oertelt S. *J Immunol* 2006;177:1655) and an IL-2 receptor  $\alpha$  knockout (-/-) mouse (Wakabayashi K. *Hepatology* 2006;44:1240). Each of these mouse models develops liver disease and AMA reactivity with specificity for PDC-E2, typical of the human autoantibody. Further work in the *Mdr2* knockout (-/-) mouse model for PSC indicates that side-chain modification of ursodiol yields a bile acid with greater therapeutic activity against PSC than standard ursodiol (Fickert P. *Gastroenterology* 2006;130:465). (2006 20%; Total 40%)

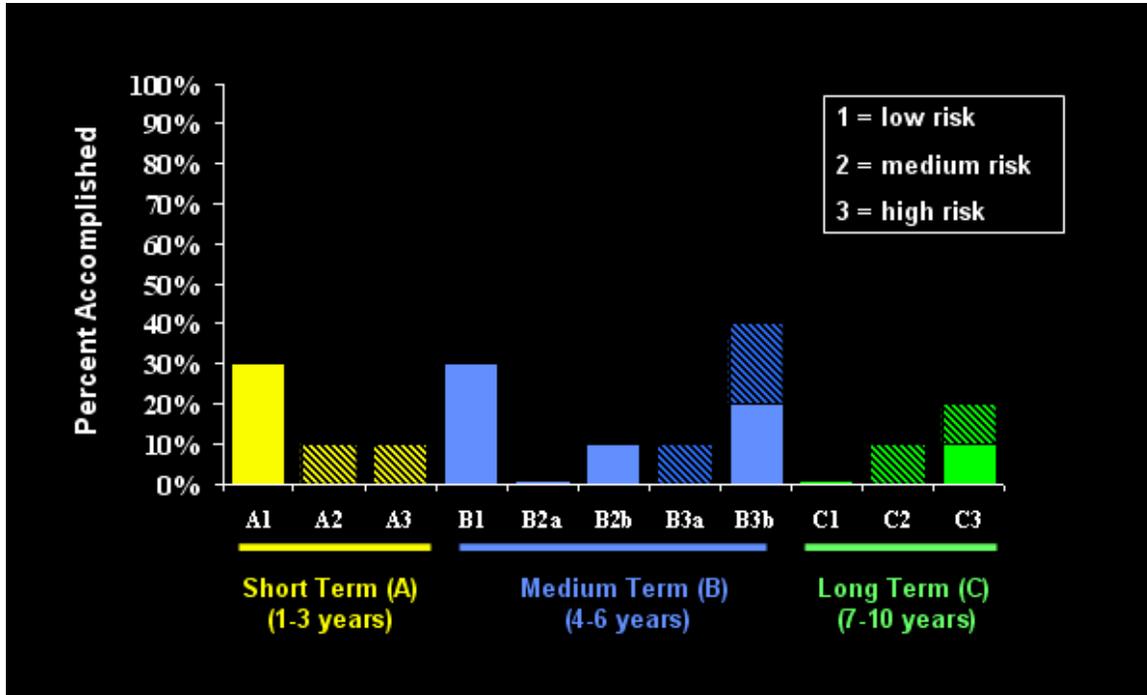
**C1. Develop alternatives to prednisone/azathioprine as maintenance therapy of autoimmune hepatitis and define markers for when and how therapy can be safely stopped.** The NIH sponsors an initiative on “Innovative Grants on Immune Tolerance” (RFA-AI-05-023), which encourages research on alternative maintenance therapy for autoimmune diseases. (2006 0%; Total 0%)

**C2. Develop sensitive serum markers for early detection of cholangiocarcinoma in PSC.** Proteomic profiling of serum and tissue from patients with cholangiocarcinoma has identified several patterns of protein peaks that correlate with the presence of cholangiocarcinoma, but no specific serum protein(s) has been identified that reliably predicts the presence of this cancer (Scarlett CJ. *Hepatology* 2006;44:658). Research grant applications on development of biomarkers are encouraged in program announcement (PA) 07-052, “Development of Disease Biomarkers.” (2006 10%; Total 10%)

**C3. Identify modifiable environmental (with or without genetic) triggers for induction of autoimmune liver disease (from human studies or murine models).** Structure-activity relationships between alkynolic compounds and

mitochondrial antigens showed that the 2-octynamides bound to the specific immunogenic regions of mitochondrial antigens and may therefore provide the trigger for PBC. These compounds are now being frequently found in commercial cosmetic products (Rieger R. J Autoimmun 2006;27:7). (2006 10%; Total 20%).

**Figure 11. Estimated Progress on Autoimmune Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



## Chapter 10 Pediatric Liver Disease

- A1a. Characterize clinical syndrome, natural history, etiology, cofactors, and complications of pediatric NASH.** The frequency of nonalcoholic fatty liver disease is increasing among children and adolescence. Among severely obese adolescents undergoing bariatric surgery, 83% had fatty liver disease and 20% had NASH (Xanthakos S. Clin Gastroenterol Hepatol 2006;4:226). In an autopsy series from the United States, 10% of children had fatty liver; rates increased with age (17% in 15-19 year olds) and were higher in Hispanic (12%) and Asian (10%) children than in whites (8.6%) or blacks (1.5%). Although cirrhosis was uncommon, 23% of children with fatty liver met diagnostic criteria for NASH (Schwimmer JB. Pediatrics 2006;118:1388). (2006 10%; Total 20%)
- 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) are in the process of developing and publishing clinical definitions and diagnostic criteria for the major neonatal cholestatic syndromes of children. (2006 0%; Total 10%)
- A2. Develop systems to better characterize the frequency, medical burden, and epidemiology of pediatric liver disease.** Epidemiology is a component of several NIH-supported studies, including the NASH Clinical Research Network, BARC, CLiC, the Pediatric Acute Liver Failure Study Group (PALFSG), the Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C (Peds-C) trial, and the SPLIT pediatric liver transplant registry. (2006 0%; Total 0%)
- A3. Elucidate the major cause of idiopathic acute liver failure in children.** The causes of acute liver failure (ALF) in children in the United States include acetaminophen overdose (14%), metabolic diseases (10%), autoimmunity (6%), and drug-induced liver injury (5%). In approximately half of cases the etiology is unknown (Squires RH. J Pediatr 2006;148:652). Testing for acetaminophen adducts in children with ALF of indeterminate cause suggests that 12% are due to unacknowledged or unrecognized acetaminophen-overdose (James LP. Pediatrics 2006;118:676). (2006 20%; Total 20%)
- B1a. Define structural and functional development of the liver and biliary system.** Factors that lead to differentiation of the liver are likely to be multiple, interrelated, and redundant. The Wnt/beta-catenin pathway has been found to be important in normal liver development in zebrafish and mice (Ober EA. Nature 2006;442:688; Tan X. Gastroenterology 2006;131:1561). Cell junction pathways are also important (Battle MA. PNAS 2006; 103:8419). Development of the liver from the embryonal foregut is mediated by fibroblast growth factor (FGF), which is produced by surrounding mesenchymal tissue and acts on endodermal epithelium. Recent studies have elucidated the intracellular pathways for FGF actions, which occur via MAP kinase pathways inducing differentiation of endoderm into hepatocytes, while the PI<sub>3</sub> kinase pathway leads to cell

proliferation (Calmont A. *Dev Cell* 2006;11:339). The coordination of these signals is key to normal hepatogenesis. (2006 20%; Total 40%)

**B1b. Evaluate long-term outcomes, complications, and tolerance-inducing regimens in children undergoing liver transplantation.** New approaches to study of long-term outcomes and tolerance-inducing regimens in children are being pursued in the NIH-funded SPLIT registry. The long-term prognosis of children undergoing liver transplantation for hepatic malignancy is reasonably good, with 10-year survivals of 58% for HCC and 66% for hepatoblastoma (Austin MT. *J Pediatr Surg* 2006; 41:182). (2006 0%; Total 10%)

**B2a. Delineate the molecular pathogenesis of at least 3 of the neonatal cholestatic syndromes.** Using a variety of molecular approaches, Jagged1 (*JAG1*) mutations can be identified in 94% of children with Alagille syndrome, and a proportion of the remaining children have mutations in the gene encoding NOTCH2, the receptor for Jagged 1 (Warthen DM. *Hum Mutat* 2006;27:436; McDaniel R. *Am J Hum Genet* 2006; 79:169). Mutations in *VPS33B* have been linked to arthrogyrosis-renal dysfunction-cholestasis (ARC) syndrome, as well as other forms of intrahepatic cholestasis lacking extrahepatic features of the ARC syndrome (Bull LN. *J Pediatr* 2006; 148:269). (2006 20%; Total 20%)

**B2b. Develop better animal models for neonatal cholestatic syndromes.** Progressive familial intrahepatic cholestasis type 1 (PFIC-1) is linked to abnormalities of the *FIC1* gene, now referred to as *ATP8B1*. The function of this gene is not known, but a mouse *Atp8b1* knockout (-/-) model has been used to demonstrate the role of this gene in phospholipid membrane asymmetry, its deficiency leading to susceptibility of hepatocyte canalicular membranes to hydrophobic bile acid injury (Paulusma CC. *Hepatology* 2006;44:195). (2006 10%; Total 30%)

**B3. Identify biomarkers for diagnosis, staging, and grading of neonatal cholestatic syndromes.** NIH-funded CLiC investigators are developing study protocols to diagnose, stage, and grade cholestatic syndromes, and ancillary studies have now been funded that are directed at testing a novel customized re-sequencing gene chip and for use of proteomics technology to identify biomarkers for pediatric cholestasis. (2006 0%; Total 0%)

**C1a. Conduct clinical trials to optimize medical and surgical management of biliary atresia.** Enrollment has started in the NIH-funded randomized, placebo-controlled trial of corticosteroids after hepatopertoenterostomy in infants with biliary atresia (START). Recent retrospective analyses suggest that perioperative corticosteroids are beneficial (Escobar MA. *J Pediatr Surg* 2006; 41:99). (2006 0%; Total 10%)

**C1b. Evaluate therapies for acute liver failure in children.** A trial of N-acetylcysteine in children with acute liver failure has reached 50% of its planned enrollment and should be completed in two years. (2006 0%; Total 10%)

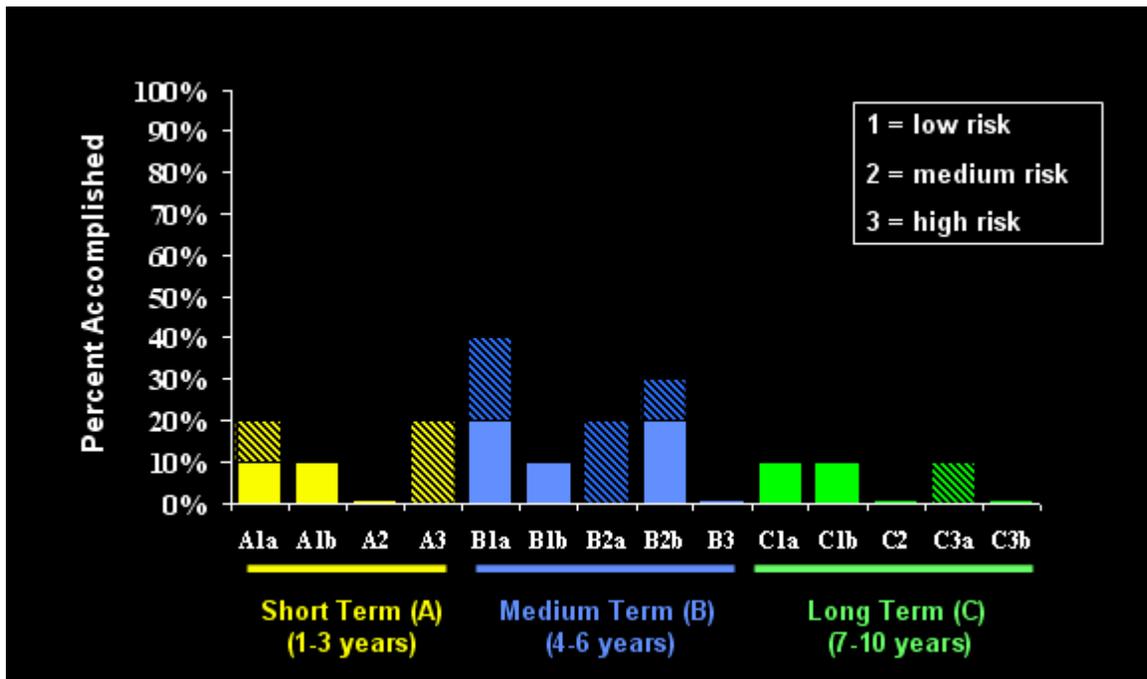
**C2. Based upon molecular pathogenesis, identify small molecule therapies that might alleviate neonatal cholestatic syndromes.** Targets for small molecule

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-07-001). (2006 0%; Total 0%)

**C3a. Define the etiology of biliary atresia.** This goal is the major focus of the BARC network, which is enrolling patients and collecting clinical data, serum DNA, and liver and biliary tissue for investigation of the etiology of this disease. In a mouse model of biliary atresia (neonatal rotavirus infection), activated T cells were responsible for bile duct injury, suggesting either autoimmune reactions or T cell responses to virus-infected biliary epithelial cells might underlie the pathogenesis of biliary atresia (Mack CL. *Hepatology* 2006;44:1231). (2006 10%; Total 10%)

**C3b. Develop gene, siRNA, cell transfer, or stem cell therapy for pediatric metabolic disease.** Both NIH- and industry-funded research investigators are active in this area. (2006 0%; Total 0%)

**Figure 12. Estimated Progress on Pediatric Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year’s progress.]**



## 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% of whites, but only 0.12% of African-Americans and less than 0.1% of Asian Americans. Among persons with C282Y homozygosity identified in screening studies who undergo liver biopsy, hepatic iron overload is common and significant fibrosis is found in 13% of women and 29% of men (Powell LW. *Arch Intern Med* 2006;13:294). Modifying factors for disease expression include viral hepatitis, alcoholism, and possibly nonalcoholic steatohepatitis (Walsh A. *Clin Gastroenterol Hepatol* 2006;4:1403). (2006 20%; Total 40%)
- A1b. Identify a cohort of patients with congenital hepatic fibrosis to study its natural history and optimal management.** Studies from cohorts seen at a major medical center between 1961 and 2004 identified 65 cases of autosomal recessive polycystic kidney disease (ARPKD), 10 of whom had isolated congenital hepatic fibrosis (CHF) with no or minimal renal involvement. Mutations in the *PKHD1* gene were found in 81% of cases and did not correlate with clinical phenotypes (Adeva M. *Medicine* 2006; 85:1). (2006 10%; Total 30%)
- A2a. Establish DNA evaluation centers of excellence for Wilson disease, the porphyrias, and hemochromatosis.** No such centers have yet been established. (2006 0%; Total: 0%)
- A2b. Develop a reliable animal model for the liver disease of cystic fibrosis.** Studies in a mouse model lacking the *CFTR* gene to assess therapies for CF suggest that chronic therapy with docosahexaenoic acid (an omega-3 fatty acid) is beneficial in decreasing inflammatory liver disease (Beharry SA. *Am J Physiol Gastrointest Liver Physiol* 2006;9: epub). These results have yet to be extended to humans. (2006 10%; Total 30%)
- A3. Fully elucidate the molecular mechanisms of intestinal absorption, hepatic metabolism, and biliary excretion of copper.** Copper absorption is largely accomplished by the human intestinal copper transport protein 1 (Ctr1), which is closely regulated by copper status (Kuo YM. *J Nutr* 2006;136:21); mice lacking the *Ctrl* gene develop severe copper deficiency (Nose Y. *Cell Metab* 2006;4:235). The crystallographic structure of human CTR1 has been defined, revealing a novel channel structure. Similarly, NMR spectroscopy of the Wilson ATPase (ATP7B) has defined the structure of the ATP-binding N-terminal domain, which is affected by at least 30 of the known Wilson disease mutations (Dmitriev O. *Proc Natl Acad Sci USA* 2006;103:5302). (2006 10%; Total 20%)
- B1a. Develop and apply practical and accurate screening methods for identifying hemochromatosis before significant tissue injury has occurred.** Potential screening tests for hemochromatosis include transferrin saturation, unsaturated

iron-binding capacity, and serum ferritin, all of which have shortcomings in terms of biological variability. The practicality of genetic testing for *HFE* gene mutations remains to be shown. (2006 0%; Total 0%).

**B1b. Define the role of heterozygosity for Wilson ATPase and *HFE* mutations in other liver diseases.** Persons heterozygous for Wilson ATPase and classical *HFE* mutations (C282Y and H62D) appear healthy and without tissue injury from copper or iron overload. Heterozygosity for *HFE* mutations is associated with mild increases in hepatic iron, but is not associated with worsening of hepatitis C (Bonkovsky HL. *Gastroenterology* 2006;131:1440) or alcoholic hepatitis (Gleeson D. *Am J Gastroenterol* 2006;101:304). (2006 10%; Total 20%)

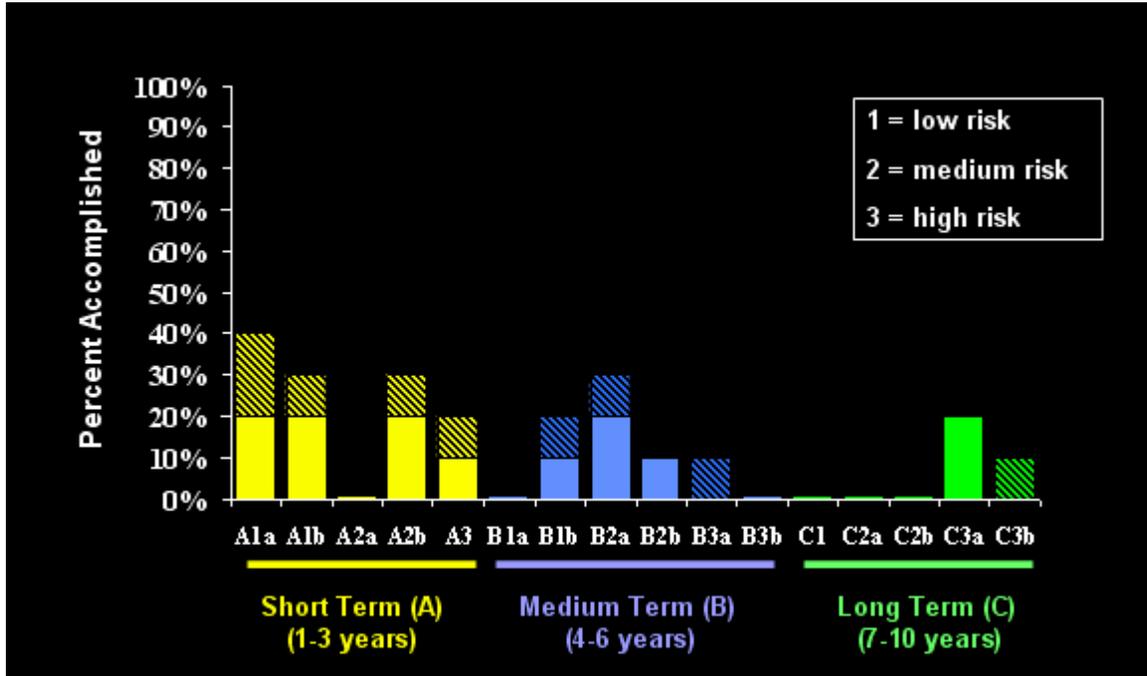
**B2a. Fully define the normal molecular pathways of iron metabolism in humans with specific definition of the roles of *HFE* and hepcidin.** Major advances continue to be made in the elucidation of the role of hepcidin and other molecules in iron metabolism (Nemeth E. *Blood* 2006;107:328). Targeted disruption of hepcidin results in severe hemochromatosis (Lesbordes-Brion JC. *Blood* 2006; 108:1402), and chronic overexpression of hepcidin causes iron deficiency and iron retention in macrophages (Viatte L. *Blood* 2006;107:2952). The mechanisms by which hemojuvelin mutations result in iron overload (juvenile hemochromatosis) have been elucidated. Hemojuvelin is a co-receptor for bone morphogenetic protein (BMP) and disruption of BMP signaling results in lowering of hepcidin expression and consequent iron overload (Babitt JL. *Nat Genet* 2006;38:531). Multiple other factors regulate hepcidin production by the liver including inflammatory signals through interleukin 6 (IL-6), which leads to STAT3 activation, hepcidin promoter engagement, and increased hepcidin expression (Wrighting DM. *Blood* 2006;108:3204). Inflammation and IL-6 also induces increases in the plasma membrane transporter Zip14, which mediates uptake of zinc and non-transferrin bound iron into hepatocytes (Liuzzi JP. *Proc Natl Acad Sci USA* 2006;103:13612). (2006 10%; Total 30%)

**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 cause of iron overload is likely due to deficient hepcidin production. Defining the possible role of iron depletion in ameliorating the course of chronic liver diseases awaits further study. (2006 0%; Total 10%)

**B3a. Identify the major genetic causes of inherited iron overload among African Americans, Asian Americans, and Hispanics.** Despite low rates of *HFE* mutations among African Americans, Asian Americans, and Hispanics, elevations in iron saturation and serum ferritin are not uncommon. In these groups, iron overload and serum aminotransferase elevations are frequently associated with high rates of hepatitis C (18-33%) and hepatitis B (2.5-5%) (Adams PC. *Clin Gastroenterol Hepatol* 2006; 4:918). The prevalence of actual iron overload has not been well defined in non-Caucasian populations, and may be low. Genetic causes for iron overload in the absence of co-existing liver disease in these cohorts have not been identified. (2006 10%; Total 10%)

- 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 yet been defined. (2006 0%; Total 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. More than 200 different mutations in ATP7B have been associated with Wilson disease, and testing for the most common mutations would identify less than half of cases. (2006 0%; Total 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. (2006 0%; Total 0%)
- C2b. Develop an improved therapy for amelioration of acute crises in porphyria.** A preparation of recombinant porphobilinogen deaminase is now in phase I/II human trials for acute intermittent porphyria. (2006 0%; Total 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.** Special MRI algorithms have been approved for clinical use in detecting marked iron overload. At present, however, accurate determination of mild and moderate iron or copper overload requires quantitative analysis of liver biopsy tissue. (2006 0%; Total 20%)
- C3b. Develop practical gene or stem cell therapy for AIP and EPP.** Sequential liver and bone marrow transplantation has been found to reverse EPP (Rand EB. Pediatrics 2006;118:1896). Gene therapy research is promoted by NIH-funded Molecular Therapy Core Centers. (2006 10%; Total 10%)

Figure 13. Estimated Progress on Genetic Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]



## 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 for accuracy and reliability. Extra points are given in these systems if hepatocellular carcinoma (HCC) is present. Recent analyses indicate that alpha-fetoprotein levels are ineffective in predicting the presence of HCC in patients with end-stage liver disease awaiting transplantation (Kemmer N. *Liver Transplant* 2006;12:1519), and radiological evidence is often unreliable, suggesting a need for more formal criteria for diagnosis of HCC in this setting (Freeman RB. *Liver Transpl* 2006;12:1504). (2006 10%; Total 30%)
- 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, which has initiated a program to find biomarkers for tolerance after liver transplantation. An NIH program announcement PA-07-052 (“Development of Disease Biomarkers”) specifically mentions the need for a non-invasive means of detecting acute rejection, immune suppression, and tolerance. (2006 0%; Total 0%)
- A3. Elucidate pathways of liver regeneration and identify targets for drug or cytokine/anticytokine therapy.** Several small molecules and growth factors have promise as a means of promoting regeneration in humans. Platelet-derived serotonin promotes liver regeneration in the mouse apparently through serotonin receptors on hepatocytes, which might be a target to stimulate regeneration (Lesurtel M. *Science* 2006;312:104). Also in mice, nuclear receptor (farnesoid-X-receptor [FXR])-dependent bile acid signaling is required for normal regeneration, suggesting that administration of bile acids or FXR ligands might be used to promote regeneration (Huang W. *Science* 2006;312:233). (2006 10%; Total 20%)
- B1a. Define efficacy of peginterferon and ribavirin in pre- and post-transplant HCV infection.** Small studies performed in the U.S. and Spain indicated that a proportion of patients treated with peginterferon and ribavirin before transplant do not suffer re-infection of the graft, which occurs in most patients without treatment. The Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) has initiated a prospective, randomized controlled trial of this approach in patients with hepatitis C awaiting transplantation. (2006 0%; Total 20%)
- B1b. Improve safety and define role of living donor liver transplantation.** Defining the role of living donor liver transplantation—as a means to overcome the shortage of organs, to decrease the waiting list mortality rate, and to improve post-transplant survival and quality of life—represents a major aim of the A2ALL cohort study. The long-term safety of liver donation requires prospective and long-term follow up. (2006 0%; Total 20%)

**B2a. Delineate molecular pathways of immune tolerance to allografts in humans.**

The NIH-sponsored Immune Tolerance Network is specifically focused on studies to delineate the mechanisms of immune tolerance after transplantation and to develop a means of achieving tolerance in patients. A small case series from Belgium has suggested that infusions of donor peripheral stem cells into living donor liver recipients can promote development of tolerance and allow early withdrawal of immunosuppression (Donckier V. *Liver Transpl* 2006; 12:1523). (2006 10%; Total 10%)

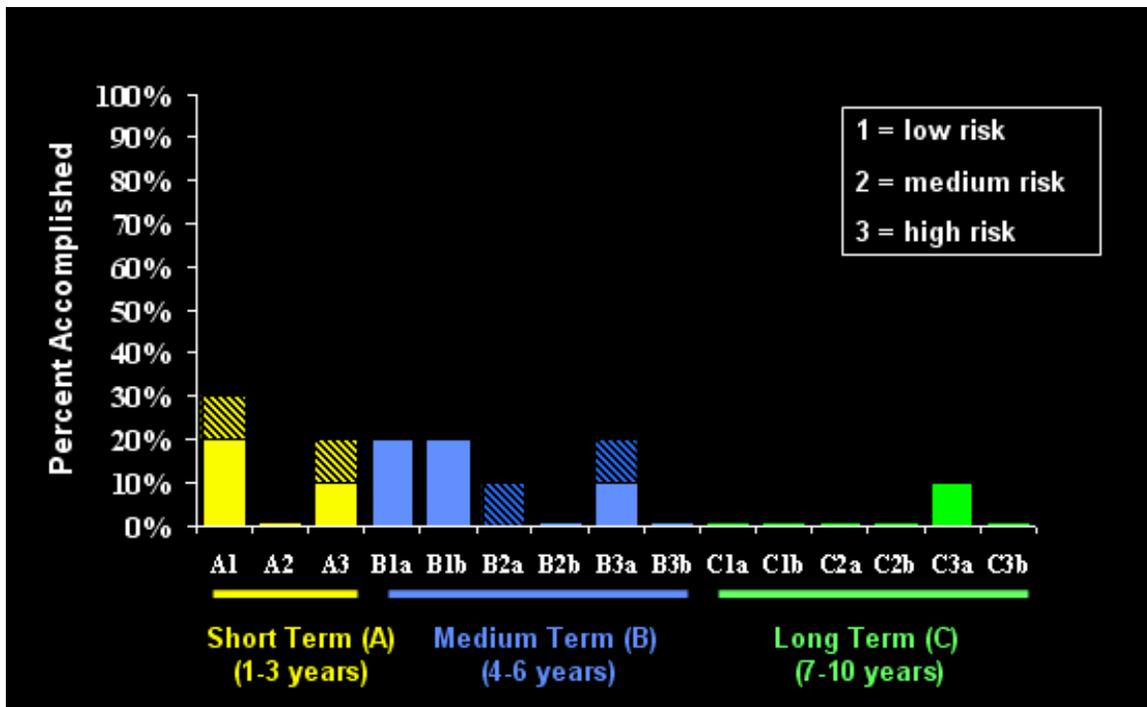
**B2b. Develop new therapies for hepatitis C that are effective in the transplant situation.** Several HCV-specific protease and polymerase inhibitors have been developed and are in phase I/II trials in patients with uncomplicated hepatitis C, but none have yet been evaluated in transplant patients. (2006 0%; Total 0%)**B3a. Elucidate the pathogenesis of post-transplant lymphoproliferative disease (PTLD) and means of prediction, prevention, and control.** Prospective monitoring of children undergoing liver transplantation indicates that PTLT occurs in the setting of primary Epstein-Barr virus (EBV) infection with high levels of viremia and immunosuppression (Loginov R. *J Clin Virol* 2006;37:104). Withdrawal of immunosuppression can reverse PTLT, and when this fails, chemotherapy including rituximab can be effective (Elstrom RL. *Am J Transplant* 2006;6:569). (2006 10%; Total 20%)**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. While potential targets for therapy have been identified in mouse models, none have yet been applied to humans. (2006 0%; Total 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 consortium 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. An NIH-sponsored meeting specifically dealing with this issue on “Improving Long-Term Outcomes for Pediatric Liver Transplantation” was held on February 12, 2007. (2006 0%; Total 0%).**C1b. Determine efficacy of chemotherapy and local ablative treatment of HCC done in the peri-transplant period.** The trans-NIH program announcement entitled, “Etiology, Prevention, and Treatment of Hepatocellular Carcinoma” (PA-07-258) encourages research in local ablative therapy. (2006 0%; Total 0%)**C2a. Based upon molecular mechanisms, develop and assess tolerance-inducing regimens, including studies in children.** The NIH held an *ad hoc* advisory meeting on new tolerance-inducing regimens for liver transplantation on December 19, 2006, and new approaches have been proposed to the A2ALL Consortium and SPLIT database. (2006 0%; Total 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 for a trans-NIH effort to clinically characterize immunological tolerance in transplantation. (2006 0%; Total 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, but development of new agents for hepatitis C is needed to fully address this need. (2006 0%; Total 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, progressive familial intrahepatic cholestasis (PFIC), and Crigler-Najjar syndrome. Gene therapies are being evaluated in animal models, but not in human subjects with these diseases. (2006 0%; Total 0%)

**Figure 14. Estimated Progress on Liver Transplantation Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year’s progress.]**



## Chapter 13

### Complications of Liver Disease

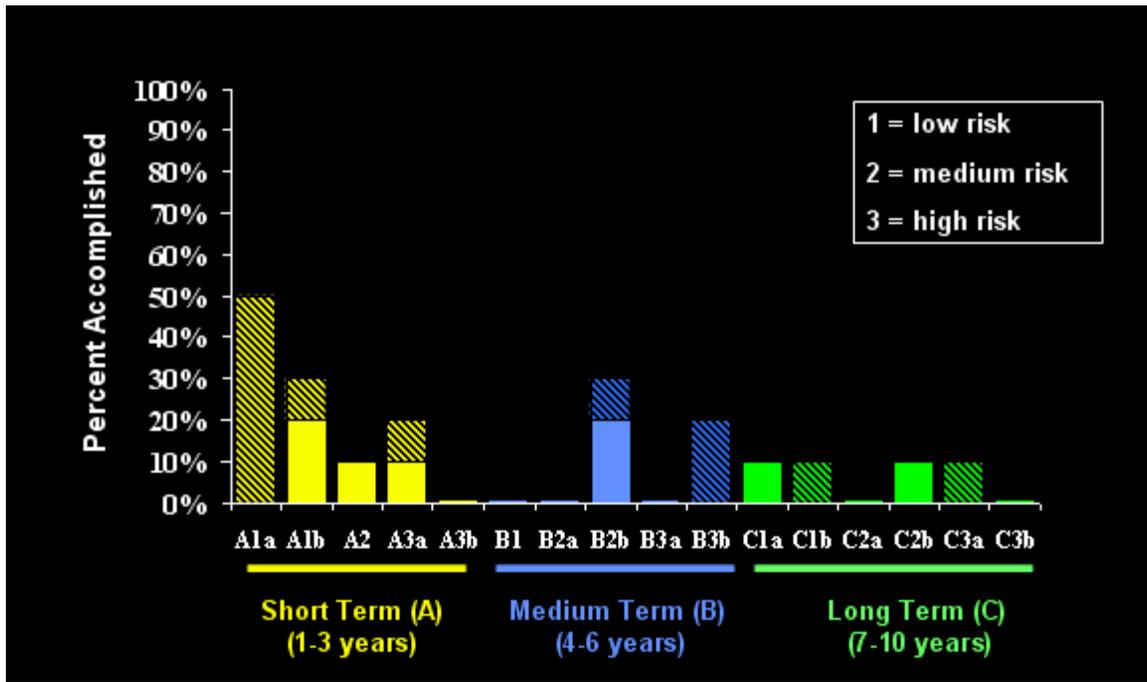
- A1a. Hold a research workshop on improvement and standardization of clinical measurements of cirrhosis and portal hypertension.** A workshop entitled “Measurement of Hepatic Vein Pressure Gradient: Role in Management of Portal Hypertension” was held on June 16-17, 2006. A summary publication is being drafted that will include recommendations for future research. (2006 50%; Total 50%)
- 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. The trial in adults has received been completed, and results are expected within the next year. The trial in children is still ongoing. (2006 10%; Total 30%)
- A2. Better define the natural history of hepatopulmonary syndrome and whether early detection is beneficial.** An NIH-funded National Network on Hepatopulmonary Syndrome continues to enroll patients in a prospective study of this syndrome. (2006 0%; Total 10%)
- A3a. More fully elucidate the pathophysiology of portal hypertension.** Patients with cirrhosis have a hyperdynamic state in the splanchnic bed that is caused at least in part by nitric oxide (NO). Vasodilation induced by NO may be mediated by vascular endothelial growth factor (VEGF) (Abralde JG. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G980). In the liver, the vasodilator response to NO is blunted, which in a rat model appears to be due to upregulation of endothelial phosphodiesterase-5, the enzyme which is responsible for degrading NO (Loureiro-Silva MR. *J Hepatol* 2006;44:886). Both phosphodiesterase-5 and VEGF may be targets for therapy of portal hypertension. (2006 10%; Total 20%)
- 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. (2006 0%; Total 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 has recently been completed, and results will be published in the next year. (2006 0%; Total 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. (2006 0%; Total 0%)

- B2b. Define natural history and identify predictors of development and growth of varices.)** Ultrasound elastography as a measure of liver stiffness may be a reliable and noninvasive means of following the development of varices (Kazemi F. *J Hepatol* 2006;45:230) (2006 10%; Total 30%)
- 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 yet been approved for use. (2006 0%; Total 0%)
- B3b. Develop a noninvasive means of measuring portal pressure.** Studies from Europe have shown that ultrasonic elastography is accurate in defining the degree of liver stiffness associated with a raised portal pressure (Carrion JA. *Liver Transpl* 2006;12:1791). Thus, elastography may be an excellent means of monitoring patients with advanced fibrosis and identifying when endoscopic documentation and intervention might be appropriate. This area of research is encouraged through a program announcement for small business (SBIR/STTR) grants: “New Technologies for Liver Disease” (PA-06-396/397). (2006 20%; Total 20%)
- C1a. Elucidate the optimal approach to manage patients with varices that have not bled (primary prevention).** Further studies are warranted comparing band ligation, beta blocker therapy, and more innovative approaches. (2006 0%; Total 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” held on June 16-17, 2006 dealt directly with this issue. In studies of patients with hepatitis C after liver transplantation, HVPG was shown to be more predictive of clinical decompensation than liver biopsy, suggesting that it may be a reliable surrogate marker for progression of liver disease (Blasco A. *Hepatology* 2006; 43:492). (2006 10%; Total 10%)
- 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 an NIH-funded initiative on “Development of Disease Biomarkers” (PA-07-052). (2006 0%; Total 0%)
- C2b. Develop and evaluate better drugs for portal hypertension.** Endothelin (ET)-1 is believed to contribute to portal hypertension in patients with cirrhosis. However, in investigational studies in 16 patients with cirrhosis, systemic and pulmonary but not portal pressures were decreased by inhibition of ET-A and ET-B receptor activity (Tripathi D. *Gut* 2006;55:1290). (2006 0%; Total 10%)
- C3a. Develop an artificial or bioartificial hepatic support and demonstrate that it prolongs survival in acute liver failure.** The status of hepatic assist devices as therapy of acute liver failure was considered at an NIH-sponsored meeting on “Acute Liver Failure” held December 4-5, 2006. At least five different bioartificial hepatic support devices are being evaluated in animal models and in phase 1 studies. In addition, several non-cell based hepatic assist devices are

being evaluated in acute liver failure and may become commercially available in the next few years. (2006 10%; Total 10%)

**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. Elastography may also be valuable as a tool to predict the presence of large varices. (2006 0%; Total 0%)

**Figure 15. Estimated Progress on Complications of Liver Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



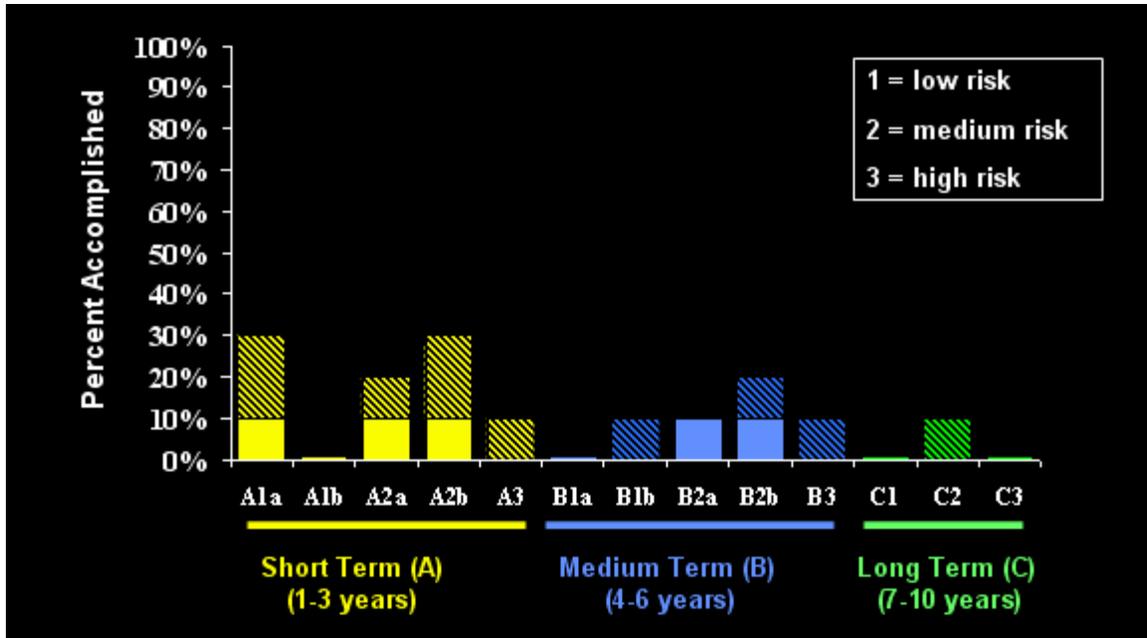
## Chapter 14 Liver Cancer

- A1a. Establish liver cancer serum and tissue bank.** Serum banks of samples from patients with early hepatocellular carcinoma (HCC) and liver disease controls are being established through several mechanisms, including the NIH-supported Early Detection Research Network (EDRN), HALT-C Trial, and HCC Genomic Consortium, all of which are being used to develop and validate early markers for liver cancer. The EDRN anticipates completion of enrollment during 2007 with targets of collecting 190 early-stage HCC and 400 cirrhosis control samples of tissue, serum, and plasma. (2006 20%; Total 30%)
- 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 NCI and NIDDK. Databases have been initiated by the American Society of Clinical Oncology and other academic hepatology groups. (2006 0%; Total 0%)
- A2a. Identify potential biomarkers for early HCC.** A one-day workshop on biomarkers for HCC was held in Princeton, New Jersey under the auspices of the Hepatitis B Foundation, a summary of which is being prepared for publication. Transcriptional profiles done on patient samples have identified three genes that reliably distinguish HCC from dysplasia (those coding for glypican-3, lymphatic vessel hyaluronan receptor 1, and survivin); these gene expression patterns may thus serve as biomarkers for early HCC (Llovet JM. *Gastroenterology* 2006;131:1758). (2006 10%; Total 20%)
- A2b. Define the molecular signatures and heterogeneity of HCC and determine how they correlate with clinical features.** Analysis of gene expression arrays from a heterogeneous collection of HCC cases demonstrated a subtype of HCC associated with expression patterns typical of fetal hepatoblasts, which had high levels of expression of downstream products of AP-1 transcription, as well as poor prognosis; defining these patterns may lead to tumor type-specific therapies (Lee JS. *Nat Med* 2006;12:410). Cluster analyses also show major differences in gene expression between HCC and normal, as well as adjacent non-tumor, tissue; major genes that were overexpressed in HCC included those coding for glypican-3, alpha-fetoprotein, and osteopontin. (Luo JH. *Hepatology* 2006; 44:1012). (2006 20%; Total 30%)
- A3. Develop functional imaging techniques that can distinguish HCC from benign lesions.** A trans-NIH program announcement (PA-07-258 “Etiology, Prevention, and Treatment of Hepatocellular Carcinoma”) encourages research on functional imaging of HCC. A refinement of PET scanning, using fluorocholine instead of fluorodeoxyglucose, has been found to improve detection of HCC (Talbot JN. *Eur J Nucl Med Mol Imaging* 2006; 33:1285); the specificity of this approach requires further study. (2006 10%; Total 10%)

- B1a. Demonstrate the relative efficacy, safety, and benefits of local ablative therapies for HCC.** A prospective study of percutaneous radiofrequency ablation in patients with HCC and cirrhosis has been initiated by the American College of Radiology Imaging Network (<http://www.acrin.org/RFAprotocol.html>). (2006 0%; Total 0%)
- B1b. Develop standardized terms and nomenclature for diagnosis, staging, and grading of HCC.** A conference organized by the American Association for the Study of Liver Diseases on “Endpoints in Clinical Trials for Hepatocellular Carcinoma” was held on Dec 8-9, 2006, which focused on standardization of design and endpoints in trials of therapy for HCC. A summary of the conference is being prepared for publication. (2006 10%; Total 10%)
- B2a. Validate reliability of biomarkers for early detection of HCC.** Analyses of several biomarkers for HCC (e.g., des-gamma-carboxy prothrombin [DCP] and alpha-fetoprotein [AFP]-L3) are underway as part of the HALT-C trial, and the EDRN is sponsoring a validation study of DCP. Results should be made available within the next year. (2006 0%; Total 10%)
- B2b. Identify risk factors for HCC associated with NASH.** Prospective studies of nonalcoholic fatty liver disease, such as the NIH-funded NASH Clinical Research Network, incorporate screening tests for HCC. In a study from Sweden of 129 patients with nonalcoholic fatty liver disease who were followed for an average of 13 years, seven developed decompensated cirrhosis (all had fibrosis on initial biopsy), and three developed HCC (Ekstedt M. *Hepatology* 2006;44:865). (2006 10%; Total 20%)
- 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, including downstream mediators of the transcription factor AP-1 and the MAPK/ERK pathway. A phase II study of Sorafenib, an oral multikinase inhibitor, has demonstrated modest efficacy and manageable toxicity (Abou-Alfa GK. *J Clin Oncol* 2006;24:4293). (2006 10%; Total 10%)
- C1. Demonstrate an effective strategy for prevention of HCC in high-risk populations.** The HALT-C trial and a similar study supported by industry (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 oltipraz and chlorophyllin. (2006 0%; Total 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-07-258. In a study of 80 cases of HCC in humans, activation of Ras and JAK/STAT signaling was enhanced in all samples, and cellular inhibitors of these pathways led to cancer cell apoptosis (Calvisi DF. *Gastroenterology* 2006; 130:1117). (2006 10%; Total 10%)

**C3. Based upon molecular analyses, develop effective, noncytotoxic therapy for HCC.** Development of non-cytotoxic therapies of HCC targeted at cellular and molecular pathways awaits demonstration of the importance of these pathways in hepatic carcinogenesis. (2006 0%; Total 0%)

**Figure 16. Estimated Progress on Liver Cancer Research Goals, 2006 (Year 2)**  
 [Cross-hatching indicates recent year's progress.]



## Chapter 15 Gallbladder and Biliary Disease

- A1. Fully characterize at least 10 murine *Lith* genes related to cholesterol gallstones.** A total of 23 murine *Lith* genes have been identified using quantitative trait locus analyses of inbred strains of mice susceptible to diet-induced cholesterol gallstones. Several candidate *Lith* genes involved in lipid uptake and secretion have been identified, but none definitely linked to these genetic sites. Recently, genes that regulate inflammation as well as innate and adaptive immune responses have been found in the same regions as several of the *Lith* genes (Lyons MA. *Biochim Biophys Acta* 2006;1761:1133). (2006 0%; Total 20%)
- A2. Develop small animal model for cholangiopathies that would allow analysis of effects of chronic necroinflammation on biliary epithelium.** Further use of the *Mdr2* knockout (-/-) mouse suggests that the side-chain modified bile acid, nor-ursodeoxycholate, is more effective than standard ursodeoxycholate in decreasing biliary inflammation and the chronic cholestasis of this animal model of sclerosing cholangitis. Thus, this animal model may provide means of screening for therapies for chronic cholestatic liver disease (Fickert P. *Gastroenterology* 2006;130:465). (2006 10%; Total 30%)
- 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.** Studies of positron emission tomography have provided further support for the reliability of this means of detection of cholangiocarcinoma (Prytz H. *Hepatology* 2006;44:1572). (2006 0%; Total 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.** In a cohort of normal adult volunteers, gallbladder dysmotility was found to correlate with insulin resistance (Nakeeb A. *J Gastrointest Surg* 2006;10:940), providing a potential link between obesity, diabetes, and gallstone disease, as well as affording new approaches to prevention. This cohort is also being evaluated for genetic factors linked to gallstones and is being used to assess plasma markers of lithogenicity of bile. (2006 10%; Total 20%)
- B2. Characterize the role of enterohepatic species of *Helicobacter* and other candidate bacteria in development of cholesterol gallstones in both mice and humans.** Several *Helicobacter* species have been linked to formation of gallstones in both murine models and humans. *Helicobacter* species associated with gallstone susceptibility produce urease, an enzyme that breaks down urea into ammonia and bicarbonate, thus creating an alkaline pH locally, which might promote calcium precipitation, a factor known to play a role in gallstone

formation (Belzer C. Gut 2006;55:1678). *Helicobacter pylori*, the well-known human pathogen that is linked to duodenal ulcer disease, does not promote gallstones in murine models (Maurer KJ. Am J Physiol Gastrointest Liver Physiol 2006; 290:175). (2006 10%; Total 20%)

**B3. Identify plasma or urine markers for lithogenicity of bile using proteomics or metabolomics.** Cohorts are being developed for assessment of plasma and urine markers that might correlate with lithogenicity of bile using proteomic approaches. Grant applications in this area are encouraged through program announcement (PA 07-016), “Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases.” (2006 0%; Total 0%)

**C1. Establish prospective database on cohort of patients with high risk of gallbladder cancer to allow development and assessment of means of early diagnosis and management.** No prospective studies have yet been initiated. (2006 0%; Total 0%)

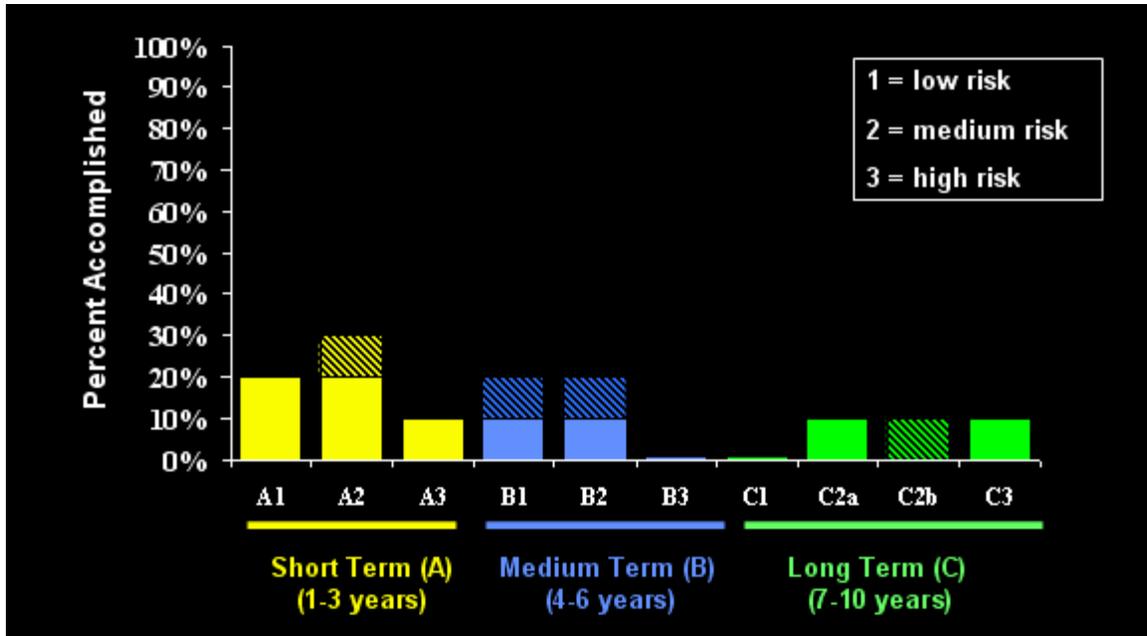
**C2a. Identify at least 5 human *LITH* genes associated with increased risk of gallstones, based upon homology with murine genes and family studies.** While several rare human gene variants have been linked to familial gallstone disease, no specific sites have been linked to typical cholesterol gallstone disease in humans. Investigation of human homologues to several candidate murine *Lith* genes (*ABCB11* and *LXRA*) found that they were not associated with typical gallstone disease (Schafmayer C. Hepatology 2006;44:650; Puppala S. Am J Hum Genet 2006; 78:377). (2006 0%; Total 10%)

**C2b. Develop noninvasive biomarkers for cholangiocarcinoma.** Proteomic profiling of serum and tissue from patients with cholangiocarcinoma have identified several patterns of protein peaks that correlate with the presence of cholangiocarcinoma, but no specific serum protein(s) has been identified that reliably predicts the presence of this cancer (Scarlett CJ. Hepatology 2006;44:658). Research grant applications on development of biomarkers are encouraged in program announcement (PA 07-052), “Development of Disease Biomarkers.” (2006 10%; Total 10%)

**C3. Develop practical and effective approach to or means of prevention of cholesterol gallstones in high-risk populations.** Human trials on prevention of gallstones have not yet been initiated, but several studies in animal models have suggested potential novel approaches. Fibroblast growth factor (FGF)-15 has been identified as playing an important role in gallbladder filling (Choi M. Nat Med 2006;12:1253). FGF-15 is induced in the terminal ileum as a result of bile acid signaling through FXR and then leads to gallbladder filling by acting on cAMP-linked receptors on smooth muscle cells in the biliary tract. Lack of gallbladder filling may predispose to gallstones, which perhaps explains the link between diseases of the terminal ileum and gallstone formation. Furthermore, agonists of FGF-19 (the homologue in humans) might play a role in prevention of

gallstones. In another study in mice, targeted deletion of *Gpbar1* (a gene involved in regulation of cholesterol secretion) led to resistance to gallstone formation in response to a high-fat diet (Vassileva G. *Biochem J* 2006;398:423). Thus, inhibitors of this cell-surface receptor for bile acids may be a means of decreasing the likelihood of gallstones. (2006 0%; Total 10%)

**Figure 17. Estimated Progress on Gallbladder and Biliary Disease Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



## Chapter 16

### Liver Imaging and Biotechnology

**A1a. Develop standardized definitions, diagnostic criteria, and methodology for liver imaging.**

A workshop entitled “Measurement of Hepatic Vein Pressure Gradient: Role in Management of Portal Hypertension” was held in June 2006. One of the recommendations from this workshop was to standardize the procedure and methods of measuring the pressure gradient. The Society of Interventional Radiology has published standards for terminology and reporting of image-guided tumor ablation (Goldberg SN. *Radiology* 2005;235:728). (2006 10%; Total 10%)

**A1b. Better define the role, efficacy, and safety of image-guided local therapies for HCC, such as radiofrequency and thermal ablation.**

A prospective study of percutaneous radiofrequency ablation in patients with hepatocellular carcinoma (HCC) and cirrhosis has been initiated by the American College of Radiology Imaging Network (<http://www.acrin.org/RFAprotocol.html>). (2006 0%; Total 10%)

**A2a. Create a liver tissue bank with correlative imaging data to facilitate clinical research.**

Serum and tissue banks of patients with early HCC and liver disease controls are being established through the NCI-supported Early Detection Research Network (EDRN), which will enroll 190 early-stage HCC and 400 cirrhosis control samples of tissue, serum, and plasma. Radiological images are also included for the HCC cases. Pathological imaging correlations of small nodules in patients with cirrhosis have demonstrated that focal nodular hyperplasia occurs not infrequently in patients with cirrhosis and can be misdiagnosed as HCC based on CT or MR imaging (Libbrecht L. *Am J Gastroenterol* 2006;101:2341). (2006 10%; Total 20%)

**A2b. Develop improved techniques for established imaging methods for liver disease, such as optical, MRI, or PET/CT scanning.**

PET scanning is more accurate than MR, CT, or ultrasound in detecting small and unsuspected cholangiocarcinoma in patients with sclerosing cholangitis awaiting liver transplantation (Prytz H. *Hepatology* 2006;44:1572). Fluorocholine was superior to fluorodeoxyglucose as a tracer in PET scanning for detection of HCC (Talbot J-N. *Eur J Nucl Med Mol Imaging* 2006;33:1285). Diffusion-weighted MR imaging and measurement of choline levels by MR spectroscopy have been used to detect HCC and to assess responses to therapeutic embolization of HCC in humans (Deng J. *J Vasc Interv Radiol* 2006; 17:1195; Chen CY. *Radiology* 2006;239:448; Nasu K. *Radiology* 2006;239:122). (2006 10%; Total 30%)

**A3. Evaluate molecular imaging techniques in animal models of liver disease.**

Use of micro-bubble contrast agents was found to be helpful in visualizing small bile ducts in a pig model (Roberts JP. *Clin Transplant* 2006;20:740). Radio-labeled antibody to vascular endothelial growth factor (VEGF) has anti-tumor effects against human HCC in immunodeficient mice (Chen J. *Cancer Lett*

2006;231:169). Finally, an animal model using implantation of rhabdomyosarcoma into liver provides a means of assessing the accuracy of MR techniques in detecting morphologic and functional characteristics of tumors (Chen F. *Radiology* 2006; 239:554). (2006 0%; Total 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. (2006 0%; Total 0%)

**B1b. Extend studies on validation to international populations.** This goal will follow development of definitions and diagnostic criteria. (2006 0%; Total 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 2006 including the Roadmap Bioinformatics and Computational Biology initiatives. Evaluation of liver disease can benefit from these non-disease specific initiatives. (2006 0%; Total 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 developed in animal models deserve evaluation in humans with liver disease. (2006 0%; Total 0%)

**C1a. Apply definitions, criteria, and methodology for liver imaging as surrogate endpoints to therapy of liver diseases.** A conference organized by the American Association for the Study of Liver Diseases (AASLD) on “Endpoints in Clinical Trials for Hepatocellular Carcinoma” was held on December 7-8, 2006, which focused on standardization of design and endpoints, including use of imaging in trials of therapy for HCC. (2006 0%; Total 0%)

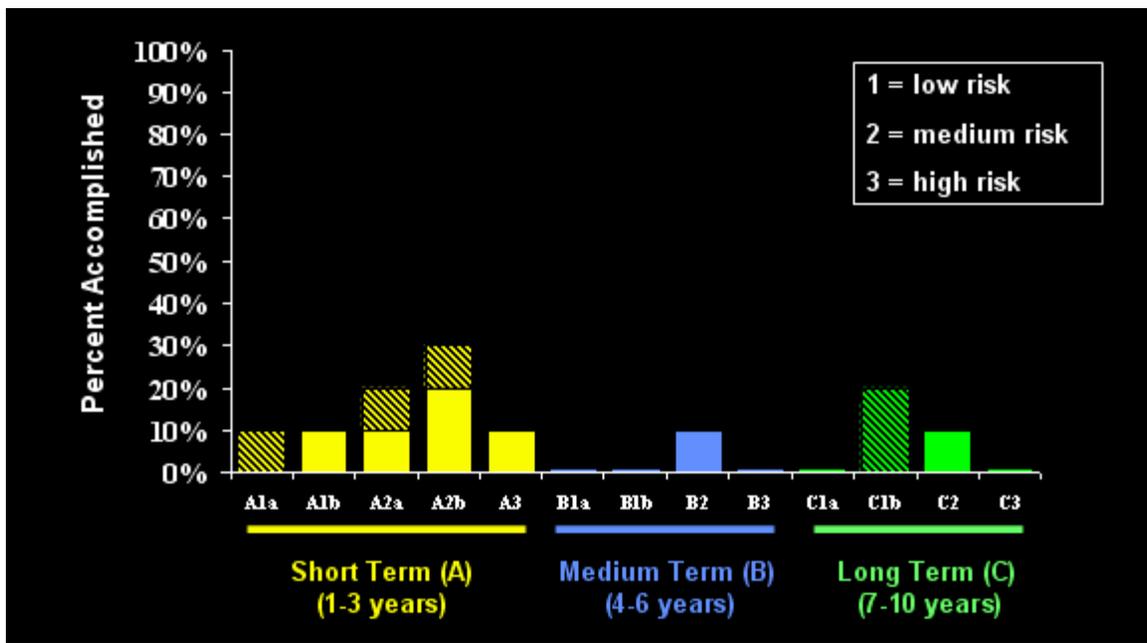
**C1b. Develop practical means of assessing liver (fat content, fibrosis, inflammation, functionality) for population-based studies.** Studies of double contrast MR imaging suggests that it is reasonably reliable in separating advanced from early or mild fibrosis (Aguirre DA. *Radiology* 2006; 239:425). More promising is ultrasound elastography as a means of assessing liver stiffness, which appears to be accurate in assessing degree of fibrosis, the presence of portal hypertension and esophageal varices (Carrion JA. *Liver Transpl* 2006;12: 1791; Kazemi F. *J Hepatol* 2006;45:230). Preliminary studies indicate that MR elastography, by reflecting whole-liver stiffness, may be more accurate than ultrasound (Rouvière O. *Radiology* 2006; 240:440). Elastography may be less accurate in assessing fibrosis in fatty liver disease. Multiple studies of MR assessment of hepatic fat are underway, and are attempting to correlate MR measurement of percent hepatic fat with clinical and histological features of nonalcoholic fatty liver disease. Doppler ultrasound evaluation of hepatic vein waveforms also shows promise as a noninvasive means of assessing degree of portal hypertension (Baik SK. *Radiology* 2006;240:574). (2006 20%; Total 20%)

**C2. Develop imaging techniques that are fully integrated into therapy of liver disease.** Real-time, three-dimensional ultrasound has been applied to robotic

laparoscopic abdominal surgery in animals in preparation for studies in humans (Pua EC. IEEE Trans Ultrason Ferroelectr Freq Control 2006;53:1999). (2006 0%; Total 10%)

**C3. Develop molecular imaging methods that provide individualized information for monitoring and therapy of liver disease, including pharmacokinetics and pharmacodynamics of targeted therapies.** Accurate molecular imaging methods are needed before they can be applied to individualized monitoring and therapy of liver disease. (2006 0%; Total 0%)

**Figure 18. Estimated Progress on Liver Imaging and Biotechnology Research Goals, 2006 (Year 2) [Cross-hatching indicates recent year's progress.]**



## Conclusions

Thus, important advances in liver disease research have continued in 2006, the second year after publication of the trans-NIH Action Plan for Liver Disease Research. Progress is reported in each of the 16 topic areas and in 106 of the 214 research goals. Since the start of the Action Plan, progress has been made on 155 goals. None of the research goals has been completely achieved, but important advances have been made and several goals 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 with liver disease research to plan initiatives for the year 2007 and beyond. Progress is reviewed yearly by the Liver Disease Subcommittee of the Digestive Diseases Interagency Coordinating Committee. At five and ten years, the Action Plan 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 of the Action Plan to assess progress and the need for further efforts.

## 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 measurable. Importantly, these benchmark goals could be used ultimately 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.

**Goal 1. Improve success rate of therapy of hepatitis C.** The current optimal regimen of therapy for hepatitis C 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, 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 are now in phase I/II studies, and response rates are likely to be reported in the next year. Furthermore, new insights have been made into the causes for a lack of response to peginterferon and ribavirin therapy. 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.

**Goal 2. Develop effective therapies for fatty liver disease, both alcoholic and non-alcoholic.** Prospective randomized controlled trials of pioglitazone, metformin, and vitamin E are underway under the auspices of the NIH-funded NASH Clinical Research

Network. During 2006, a pilot study of pioglitazone in patients with diabetes and NASH was published and demonstrated clear cut improvements in serum aminotransferase levels and liver histology. Smaller pilot studies have starting using silymarin and trials of S-adenosylmethionine and weight loss agents, including the cannabinoid receptor 1 antagonist, rimonabant, are being designed. Several of these approaches may also be applicable to alcoholic liver disease. Clearly, therapies for nonalcoholic steatohepatitis will be developed in the next few years; their degree of efficacy and general applicability remain to be defined.

**Goal 3. Develop regimens of antiviral therapy that are effective in long-term management of hepatitis B.** A total of six medications have been approved for use in chronic hepatitis B in the United States, one during the last year. Licensed therapies include standard interferon (IFN)- $\alpha$ , peginterferon, lamivudine, telbivudine, 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 needs careful prospective study. A NIH Consensus Development Conference on management of hepatitis B has been scheduled for 2008. Thus, there have been major advances in the therapy of hepatitis B and achievement of this goal is in sight.

**Goal 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; the most promising method being ultrasound-based elastography, which measures the degree of stiffness of the liver. Cross sectional and early results of prospective studies of elastography have shown that this technique is reproducible and provides relatively reliable estimation of advanced fibrosis and cirrhosis. The presence of portal hypertension and varices are also predicted by elastography. The role of this technique in monitoring of chronic liver disease and decision-making regarding interventions requires prospective study. Improvements in elastography (using MRI) are also under evaluation.

**Goal 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.

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

**Goal 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. The Consortium has also received funding for three ancillary studies directed at the etiology of biliary atresia, one focusing on genetics, one on proteomics, and one on gene expression arrays. The current understanding of etiology of biliary atresia was reviewed in an NIH research workshop “Screening and Outcomes of Biliary Atresia” held September 19-20, 2006, a summary of the meeting that includes recommendations for future research has been submitted for publication.

**Goal 8. Improve the safety and define optimal use of living donor liver transplantation.** Living donors are used in approximately 30 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. Preliminary, as yet unpublished results demonstrate that the availability of living donor liver transplantation improves survival in end-stage liver disease. Further investigations from this study group and in pediatric liver transplantation are likely to further define optimal use and safety of this life-saving procedure.

**Goal 9. Develop standardized and objective diagnostic criteria of major liver diseases and their grading and staging.** Diagnostic criteria and standardized means of grading and staging liver disease are being developed in several liver disease conditions, including pediatric cholestatic syndromes, nonalcoholic steatohepatitis, drug-induced liver disease, and liver cancer.

**Goal 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 the 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 from 2004. 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 2000-2004 are given in Table 3, which includes the final data from 2003, but only the preliminary data for 2004.

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

<b>Diagnosis</b>	<b>2000</b>	<b>2001</b>	<b>2002</b>	<b>2003</b>	<b>2004*</b>
Chronic Liver Disease and Cirrhosis	9.5	9.4	9.4	9.3	8.8
Viral Hepatitis	1.9	1.8	2.0	1.8	1.8
Malignant Neoplasms of the Liver and Intrahepatic Bile Ducts	4.7	4.7	5.0	4.9	5.0
Cholelithiasis and Disorders of the Gallbladder	1.0	1.0	1.0	1.0	1.0
Total: Liver and Biliary Disease	17.1	16.9	17.4	16.9	16.6
<b>Total Number of Deaths</b>	<b>47,635</b>	<b>48,068</b>	<b>50,076</b>	<b>50,588</b>	<b>50,119</b>

\* Preliminary results.

Thus, the total numbers of deaths from liver and biliary disease have been stable, but the age-adjusted death rate continues to fall. Death rates from chronic liver disease and cirrhosis are at an all-time low.

## 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
AAV	adeno-associated virus vector
ABC	ATP-binding cassette
AFP	alpha-fetoprotein
AIH	autoimmune hepatitis
AIP	acute intermittent porphyria
ALFSG	Adult Acute Liver Failure Study Group
AP-1	activator protein-1
ARC	arthrogryposis-renal dysfunction-cholestasis
ARPKD	autosomal recessive polycystic kidney disease
ATP	adenosine triphosphate
BARC	Biliary Atresia Research Consortium
BMP-4	bone morphogenetic protein-4
BSEP	bile salt export pump
cAMP	adenosine 3',5'-cyclic monophosphate
CAR	constitutive androstane receptor
CB1	cannabinoid receptor 1
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
DHBV	duck hepatitis B virus
DILIN	Drug-Induced Liver Injury Network
EBV	Epstein-Barr virus
EDRN	Early Disease Recognition Network
EPIC3	Evaluation of PegIntron in Control of Hepatitis C Cirrhosis study
EPP	erythropoietic protoporphyria
ER	endoplasmic reticulum
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FXR	farnesoid X receptor
FY	fiscal year
GLP-1	glucagon-like peptide 1
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
HDV	hepatitis D virus
HEIRS	Hemochromatosis and Iron Overload Screening study
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRSA	Health Resources and Services Administration
HURSO	High Dose Ursodiol for Primary Sclerosing Cholangitis study
HVPG	hepatic venous pressure gradient
IFN	interferon
IL	interleukin
IP <sub>3</sub>	inositol 1,4,5-trisphosphate
IRG	initial review group
JAK	janus kinase
JNK	c-Jun N-terminal kinase
LABS	Longitudinal Assessment of Bariatric Surgery
LADR	Low Dose Accelerating Regimen study (of A2ALL)
LCMV	lymphocytic choriomeningitis virus
LXR	liver X receptor
MAPK/ERK	mitogen-activated protein/extracellular signal-regulated kinase
MAVS	mitochondrial antiviral signaling protein
MDR	multi-drug resistance
MELD	Model for End-stage Liver Disease
MRI	magnetic resonance imaging
MRP	multidrug-resistance-associated protein
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
NCRR	National Center for Research Resources
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
NO	nitric oxide
NTCP	Na <sup>+</sup> /taurocholate co-transporting polypeptide
OATP	organic anion transporter
ODS	Office of Dietary Supplements
OLT HBV	Orthotopic Liver Transplantation for Hepatitis B Study
ORD	Office of Rare Diseases
OST	organic solute transporter
PA	program announcement
PACTG	Pediatric AIDS Clinical Trials Group
PAI-1	plasminogen activator inhibitor-1
PALFSG	Pediatric Acute Liver Failure Study Group
PBC	primary biliary cirrhosis
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-adenosylmethionine
SBIR	Small Business Innovation Research Program
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
SPLIT	Study of Pediatric Liver Transplantation
SREBP	sterol-regulatory element binding protein
START	STERoids in biliary Atresia Randomized Trial
STAT	signal transducer and activator of transcription
STTR	Small Business Technology Transfer Program
SyNCH	Silymarin in NASH and Chronic Hepatitis C
TONIC	Treatment of Nonalcoholic Fatty Liver Disease in Children trial
TGF	transforming growth factor
TLR	toll like receptor
TNF- $\alpha$	tumor necrosis factor- $\alpha$
TNFR1	tumor necrosis factor receptor 1
TPN	total parenteral nutrition
VEGF	vascular endothelial growth factor
Virahep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C