

Progress Review for 2007 (Year Three Analysis):
Action Plan for Liver Disease Research

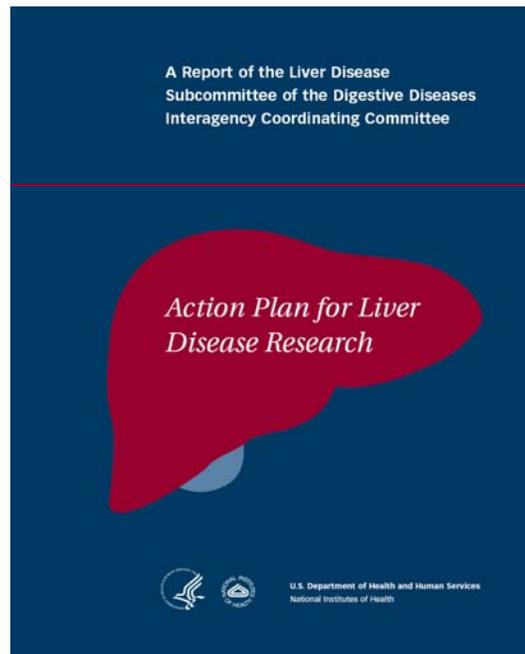


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Objective

This Progress Review of the trans-NIH Action Plan for Liver Disease Research describes progress made toward reaching the Action Plan's research goals during 2007, the third year following its release. It builds upon the previous two yearly Progress Reviews, which surveyed progress during 2005 and 2006, both of which are 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 private entities such as industry and foundations), and (4) support for specific initiatives by 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) at the NIH. 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 was recently 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 includes 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 have helped to coordinate these efforts.

The current document is a Progress Review of the Action Plan for the year 2007, approximately 3 years after its release. It follows the 2005 and 2006 Progress Reviews, which were released during the first and second years, respectively, 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 2007 that are apropos to each research goal. Finally, the degree of progress made toward each research goal during the 3 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 made during 2007, as well as cumulative progress made in the past 3 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 in this document, within sections organized around each of 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, M. 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, Kenneth Sherman and Katherine Davenny
- **Fatty Liver Disease:** Drs. Anna Mae Diehl, David Crabb, Joannes Hoek, Craig McClain, and Samir 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, I. Nicholas Crispe, M. Eric Gershwin, James Gorham, Keith Lindor, Barbara Rehermann, and Stephen James
- **Pediatric Liver Disease:** Drs. Jorge Bezerra, Saul Karpen, David Perlmutter, Ron Sokol, Frederick Suchy, Barbara Haber and Tonse Raju
- **Genetic Liver Disease:** Drs. Bruce Bacon, Nancy Andrews, Herbert Bonkovsky, Joseph Bloomer, Jonathan Gitlin, Caroline Philpott, Paul Adams, and Jean Jenkins
- **Liver Transplantation:** Drs. Jean Emond, Michael Lucey, John Roberts, Hugo Rosen, Robert Merion and James Everhart
- **Complications of Liver Disease:** Drs. Thomas Boyer, Michael Fallon, Roberto Groszmann, Kevin Mullen, William Lee, and Leonard Seeff
- **Liver Cancer:** Drs. Adrian Di Bisceglie, Gregory Gores, Snorri Thorgeirsson, Josep Llovet, Jordi Bruix, and Jaye Viner
- **Gallbladder and Biliary Disease:** Drs. Sum Lee, 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 scientific understanding and to 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 2007 that encourage research applications directed at specific research goals delineated in the trans-NIH Action Plan for Liver Disease Research.

Table 1. NIH-Sponsored Program Announcements and Requests for Applications Relevant to Action Plan in 2007

Initiative Number	Title	Sponsoring ICs
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, NIMH
RFA-RM-08-003	Pilot-Scale Libraries (PSL) for High-Throughput Screening (P41)	Roadmap
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-07-360 and -361	Molecular Mechanisms of Development and Reversal of Alcohol-Induced Liver Fibrosis	NIAAA
RFA-AA-08-001	The Role of Mitochondria in Alcohol-Induced Tissue Injury	NIAAA
PA-07-403, -404, and -405	Nutrition and Alcohol-Related Health Outcomes	NIAAA, NCI, ODS
PA-06-270	Mechanisms of Alcohol-Associated Cancers	NIAAA, NCI, ODS
PA-06-295 (now PA-07-258)	Etiology, Prevention and Treatment of Hepatocellular Carcinoma	NCI, NIDDK, NIBIB, NIAAA
RFA-DK-07-012	Continuation and Expansion of the Drug Induced Liver Injury Network (DILIN)	NIDDK
RFA-DK-07-011	Hepatitis B Clinical Research Network	NIDDK
PA-07-027	Health Disparities in NIDDK Diseases	NIDDK
PA-06-151	Secondary Analyses in Obesity, Diabetes, Digestive and Kidney Diseases	NIDDK
PA-06-396 and -397	New Technologies for Liver Disease SBIR/STTR	NIDDK, NLM, NIEHS, NCI, NCCAM, NIBIB, NIDA, NIAAA
PAR-07-024	Ancillary Studies to Major Ongoing NIDDK and NHLBI Clinical Research Studies	NIDDK, NHLBI
PA-06-301	Pilot and Feasibility Clinical Research Studies in Digestive Diseases and Nutrition	NIDDK

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. NIH-Sponsored Clinical and Epidemiologic Studies in 2007

Short Title/ Initiative #	Full Title	Sponsoring ICs/Agencies
A2ALL	Adult-to-Adult Living Donor Liver Transplantation Cohort Study (including "LADR" study below)	NIDDK, HRSA
<i>LADR</i>	Low Dose Accelerating Regimen study	NIDDK, HRSA
AACTG	Adult AIDS Clinical Trials Group, Hepatitis Subcommittee	NIAID
PACTG	Pediatric AIDS Clinical Trials Group	NIAID
ALFSG	Adult Acute Liver Failure Study Group	NIDDK
PALFSG	Pediatric Acute Liver Failure Study Group	NIDDK
ARPKD Network	Autosomal Recessive Polycystic Kidney Disease and Congenital Hepatic Fibrosis Study	NHGRI
BARC	Biliary Atresia Research Consortium	NIDDK, ORD
CLiC	Cholestatic Liver Disease Consortium	NIDDK, ORD
DILIN	Drug-Induced Liver Injury Network	NIDDK, FDA
EDRN	Early Detection Research Network	NCI
EPISOD	Evaluating Predictors and Interventions in Sphincter of Oddi Dysfunction trial	NIDDK
HALT-C	Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial	NIDDK, NCI, NIAID
HBV-OLTS	Hepatitis B Orthotopic Liver Transplantation Study	NIDDK
HHCATF	HIV/HCV Coinfection Antiretroviral Therapy and Fibrosis (USA)	NIDA
HURSO	High Dose Ursodiol for Primary Sclerosing Cholangitis	NIDDK
LABS	Longitudinal Assessment of Bariatric Surgery	NIDDK
LCNAHI	Longitudinal Cohort of Newly Acquired HCV Infection (Australia)	NIDA
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
NHHIHD	Natural History of HCV Infection in HIV Disease (USA)	NIDA
PEDS-C	Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C	NIDDK, FDA
PGRN	Pharmacogenetics Research Network	NIGMS, NHLBI, NCI, NIDA, NIEHS, NHGRI, NLM, NIMH, ORWH
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
WIHS	Women's Interagency HIV Study (including studies of HCV/HIV co-infection)	NIAID, NIDA, NICHD, NCI

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 2007 was approximately \$423 million. The liver-related research budget represents approximately 1.5% of the total NIH budget, which was \$29 billion in FY 2007. The amount of growth in liver disease-related research funding compared to the growth in the total NIH budget is shown graphically in Figure 1 below, expressed as the percent growth from a baseline in FY 1993. The proportion of liver disease-related funding by NIH Institutes and Centers for FY 2007 is shown in Figure 2. The majority of funding in liver disease research continues to derive from 8 Institutes or Centers: NIDDK, NIAID, NCI, NIAAA, NIDA, NIEHS, NCRR, and the NHLBI. Another 10 NIH Institutes or Centers provide 4 percent more of the liver disease research budget.

Figure 1. Growth in Liver Disease-Related Research Funding Relative to the Overall NIH Budget: FY 1993-2007

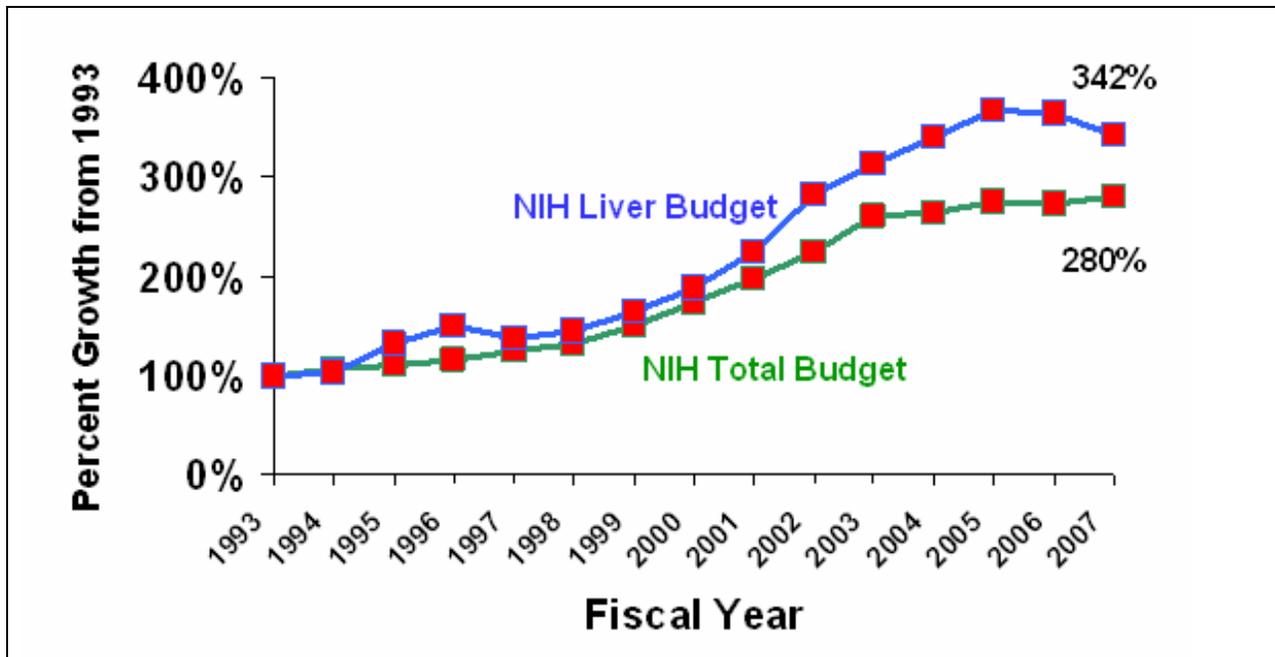
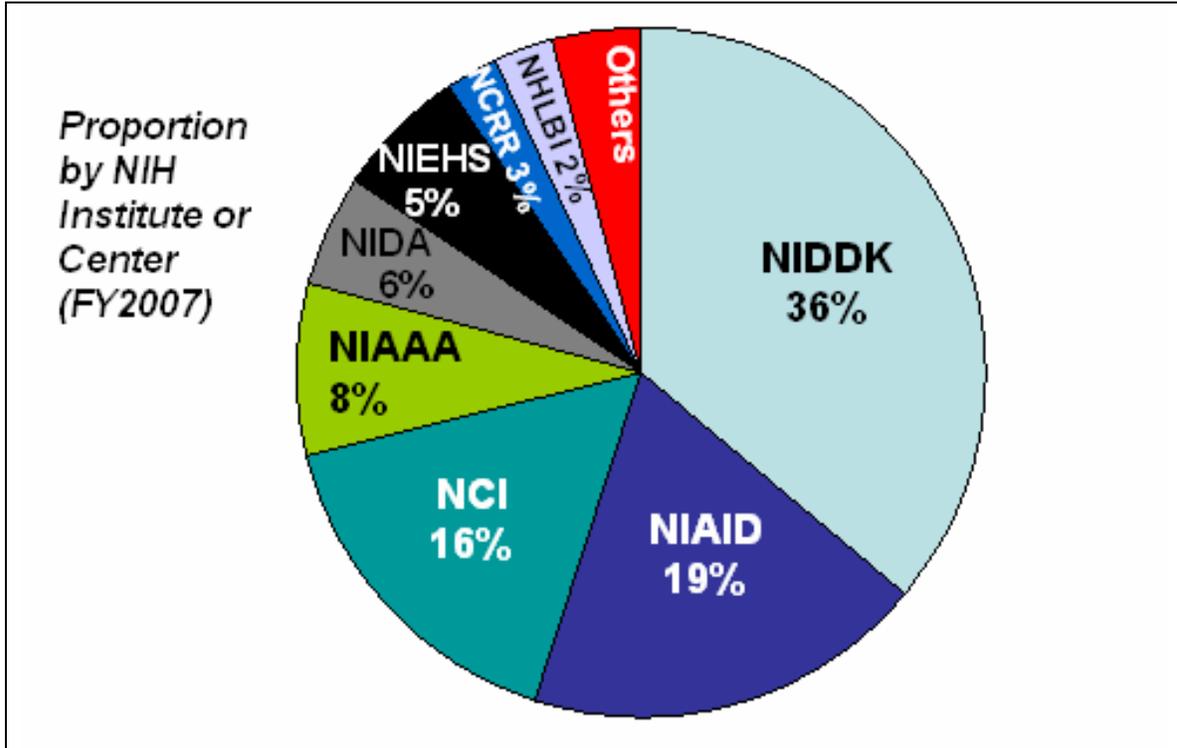


Figure 2. NIH Support of Liver Disease-Related Research



Progress Review: Year Three Analysis (2007)

The following 16 sections describe the third 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 private entities such as industry and foundations. 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.** Calcium signaling has been shown to play a central role in cell activation and gene transcription in the hepatocyte. During stellate cell activation, inositol trisphosphate (IP₃) receptors shift into the nucleus and cell extensions where they play important roles in calcium signaling and cell contractile activity (Kruglov EA. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: G975). Furthermore, calcium signaling affects the permeability of nuclear pores, allowing access to transcription factors and other proteins (O'Brien EM. *J Biol Chem* 2007; 282: 4210). (2007 10%; Total 30%)
- A2. Elucidate the mechanisms of lipid metabolism and transport in liver as it relates to whole body lipid homeostasis.** The role of the Niemann-Pick C1-Like 1 protein (NPC1L1) in cholesterol metabolism has been further defined by studies demonstrating its presence and role in hepatocytes where it is found on canalicular membranes and acts to reabsorb cholesterol from bile. Thus, the anti-cholesterol activity of ezetimibe may be due to its effects in blocking NPC1L1 in the liver as well as cholesterol absorption by the intestine (Temel RE. *J Clin Invest* 2007; 117: 1968). Coordinated control of lipid metabolism between intestine and liver has also been shown in studies of the farnesoid X receptor, which is activated by bile acids and represses transcription of the CYP7A1 gene, the rate-limiting enzyme in bile acid synthesis, and CYP8B1, which is required for production of cholic acid, partially through intestinal-specific effects (via the hormone FGF15) and partially by direct hepatic effects (Kim I. *J Lipid Res* 2007; 48: 2664). (2007 10%; Total 40%)
- A3. Determine how intra- and inter-cellular signals are integrated *in vivo* to regulate liver function.** The extracellular matrix of the liver is essential for normal function, survival, and regeneration. Integrin-linked kinase (ILK) has been shown to be essential in maintaining normal liver function and proliferation, and its loss results in rapid hepatocyte apoptosis (Gkretsi V. *Hepatology* 2007; 45: 1025). (2007 10%; Total 30%)
- B1. Elucidate physiological importance of liver plasma membrane transporters and mechanisms of action.** The cystic fibrosis transmembrane conductance regulator (CFTR), which is mutated in cystic fibrosis, is present on the plasma membrane of cholangiocytes and appears to be involved in ductular secretion of bicarbonate, probably not via chloride transport, but via ATP secretion into bile—a pathway that is activated by the choleric agent ursodiol (Minagawa N. *Gastroenterology* 2007; 133: 1592; Fiorotto R. *Gastroenterology* 2007; 133: 1603). (2007 10%; Total 20%)
- B2. Develop cell culture model that reflects different liver cell interactions (e.g., hepatocyte with Kupffer cell, cholangiocyte, stellate cell, or endothelial cell).** Activation of stellate cells and portal fibroblasts results in collagen secretion and

hepatic fibrosis. A new cell culture system using polyacrylamide supports has shown that increasing stiffness of matrix leads to myofibroblastic transformation of stellate cells and fibroblasts (Georges PC. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: G1147). (2007 10%; Total 30%)

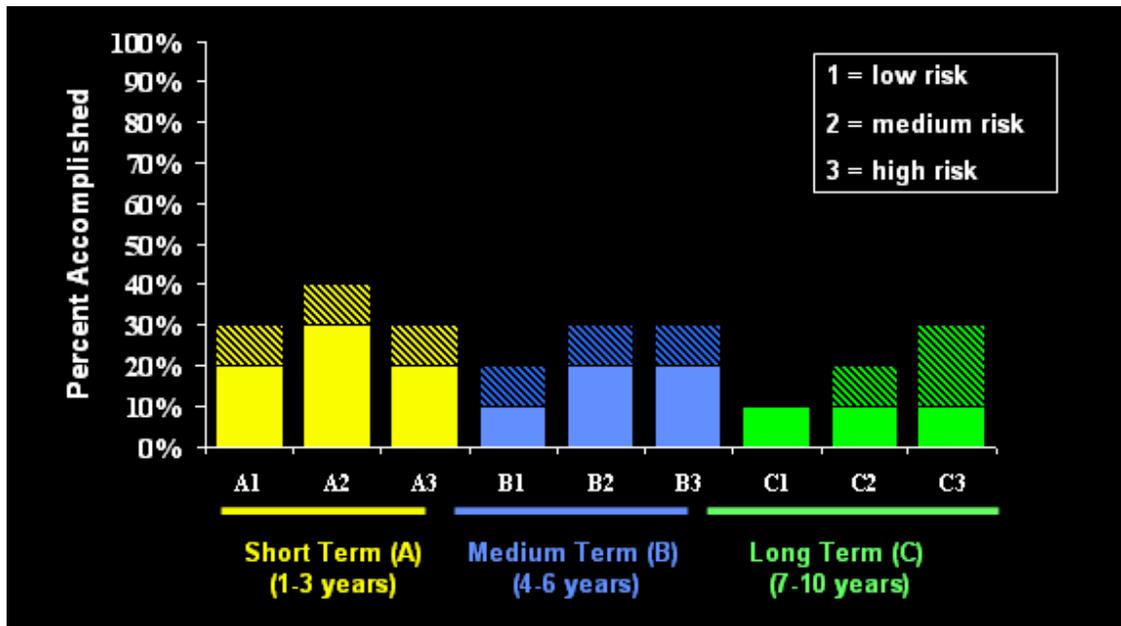
B3. Elucidate intra- and extra-cellular events that determine hepatocyte polarity. The functional polarity of hepatocytes relies upon separation of the functional machinery of the bile canalicular (apical) from that of the sinusoidal (basolateral) membrane. The presence of intact lipid rafts have been found to be important for establishment of polarity in hepatocytes by correctly orienting calcium signaling and IP3 receptor targeting (Nagata J. *Gastroenterology* 2007; 133: 256) Hepatocytes have a different pattern of polarity than other cells, such as renal tubular cells with their apical borders between lateral membranes (creating canaliculi). The hepatic-type polarity is dependent upon an intracellular kinase Par1b, which acts via E-cadherin signaling (Cohen D. *Mol Biol Cell* 2007; 18: 2203). (2007 10%; Total 30%)

C1. Elucidate major elements in process of transcellular vesicle trafficking in the hepatocyte. Uptake and intracellular transport in hepatocytes is mediated by receptor-activated clathrin- and caveolae-mediated endocytosis, but also by more non-specific micro- and macro-pinocytosis. Dynamin 2, a well known intracellular motor protein, is important for receptor-mediated endocytosis and now has been found to be important in micro- (but not macro-) pinocytosis (Cao H. *J Cell Sci* 2007; 120: 4167). The proteins responsible for movement of vesicles along microtubules in hepatocytes have been further characterized (Nath S. *Mol Biol Cell* 2007; 18: 1839; Stockert RJ. *Biochim Biophys Acta* 2007; 1768; 1741). (2007 0%; Total 10%)

C2. Elucidate how cells interact with each other (e.g., via gap junctions, ECM, paracrine, and endocrine signaling). Hepatic non-parenchymal cells respond to hepatocyte injury in a multitude of ways, including stellate cell activation and release of cytokines and collagen. The paracrine signals responsible for these interactions are not fully defined. Succinate is released by injured hepatocytes; recent investigations have shown that stellate cells express succinate receptors, and engagement by succinate accelerates stellate cell activation (Correa PR. *J Hepatol* 2007; 47: 262). (2007 10%; Total 20%)

C3. Develop knowledge base of normal liver proteome, including analysis of individual cell types, subcellular compartments, and changes along hepatic acinus. The Human Liver Proteome Project (HLPP) has initiated construction of the normal human liver proteome expression profile, with over 6,000 proteins identified to date (Beretta L. *Mol Cell Proteomics* 2007; 6: 2043; Chen M. *Proteomics* 2007; 7: 2479). Similar profiles are being prepared for the mouse (Shi R. *J Proteome Res* 2007; 6: 2963), rat (Gazzana G. *J Proteome Res* 2007; 6: 3143), and zebrafish (Wang N. *J Proteome Res* 2007; 6: 263). Proteomic characterization of subcellular organelles is being pursued, and a major priority is being placed on integration of data and accessibility of results. (2007 20%; Total 30%)

Figure 3. Estimated Progress on Cell and Molecular Biology of the Liver Research Goals, 2007 (Year 3) [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. Several studies have shown that hepatocytes can undergo epithelial to mesenchymal transition into collagen-producing, fibroblast-like cells. Elucidation of the pathways for such a transition may help in defining important therapeutic targets in chronic liver disease (Kaimori A. *J Biol Chem* 2007; 282: 22089; Zeisberg M. *J Biol Chem* 2007; 282: 23337). (2007 10%; Total 30%)

A1b. Elucidate whether and how nutritional factors affect liver cytotoxic and fibrotic pathways. Free fatty acids can trigger inflammation and hepatocyte injury. The pathways involved include transcriptional regulation of the intracellular death mediator Bim by activation of FoxO pathways (Barreyro FJ. *J Biol Chem* 2007; 282: 27141) and sensitization of hepatocytes to TRAIL cytotoxicity by upregulation of death receptor 5 (Malhi H. *Gut* 2007; 56: 1124). Such findings may eventually provide therapeutic targets for alcoholic as well as nonalcoholic fatty liver disease. (2007 10%; Total 30%)

A2a. Define the role of anti-apoptotic therapy in liver injury, fibrosis, and regeneration. Pan-caspase inhibitors have the potential of decreasing hepatic injury from a variety of insults by blocking apoptotic pathways. The therapeutic potential of anti-apoptotic caspase inhibitor (IDN-6556) has been assessed in pilot studies of liver preservation injury (Baskin-Bey ES. *Am J Transplant* 2007; 7: 218) and chronic hepatitis C (Pockros PJ. *Hepatology* 2007; 46: 324), demonstrating significant effects to reduce serum aminotransferase elevations with minimal evidence of toxicity. Larger human studies with more clinically important endpoints are being planned. (2007 10%; Total 30%)

A2b. Identify the impact of individual leukocyte sub-populations and their mediators on liver injury, fibrosis, and regeneration. Interleukin-22 (IL-22) is an anti-inflammatory cytokine that is produced by T helper 17 cells, while the receptor for IL-22 is found on hepatocytes and not on immunocytes. Studies in rodent models have now shown that IL-22 protects hepatocytes from T-cell mediated immune injury, suggesting an important role of this molecule in inflammatory liver diseases (Zenewicz LA. *Immunity* 2007; 27: 647). (2007 10%; Total 30%)

A3. Develop noninvasive biomarkers for fibrosis. Further studies on transient elastography show that it can accurately distinguish mild from more advanced cirrhosis, thus providing guidance for when interventions to treat varices and prevent bleeding are likely to be needed (Vizzutti F. *Hepatology* 2007; 45: 1290). Magnetic resonance elastography may be more accurate than ultrasound in assessing liver stiffness and degree of hepatic fibrosis (Yin M. *Clin Gastroenterol Hepatol* 2007; 5: 1207). (2007 10%; Total 40%)

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. Recent data suggest that death receptor-mediated apoptosis contributes to fatty liver disease (Zou C. *Nat Med* 2007; 13:1078). (2007 10%; Total 30%)

B2a. Identify the integrative mechanisms mediating oxidative, nitrosative, hypoxic, and ischemic-reperfusion injury and the role of sinusoidal cells. The interactions of pro-inflammatory and fibrogenic signals have been further defined in animal models demonstrating that intestinal bacterial microflora and the toll like receptor 4 (TLR4) function on Kupffer cells are both required for activation of hepatic stellate cells and hepatic fibrogenesis. (Seki E. *Nat Med* 2007; 13: 1324). (2007 10%; Total 20%)

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 the possibility of reliable detection of changes with liver injury. (2007 0%; Total 10%)

B3. Develop gene-, cell-, or pharmacology-based therapies for hepatic injury. The gas nitric oxide (NO) is a potent signaling molecule that induces smooth muscle relaxation. In high concentrations, NO inhibits ischemia/reperfusion injury in many tissues, including liver. In a small, randomized, controlled trial, liver transplant recipients were given NO by inhalation during anesthesia for transplantation and were found to have significantly lower elevations of serum aminotransferase levels and coagulation times after transplant, as well as shorter hospital stays. NO appeared to act by decreasing hepatocyte apoptosis (Lang JD. *J Clin Invest* 2007; 117: 2583). Larger controlled trials of this approach are warranted. Recombinant human annexin (diannexin) binds to phosphatidylserine and prevents attachment of leukocytes and platelets to sinusoidal endothelial cells after ischemia/reperfusion injury and has now been shown to ameliorate liver injury in two animal models of liver transplantation (Shen XD. *Am J Transplant* 2007; 7; 2463; Teoh NC. *Gastroenterology* 2007; 133: 632). In a large, randomized, double-blind controlled trial, therapy with recombinant interferon gamma (a potent inhibitor of fibrosis in animal models) had no effect on progression of fibrosis in chronic hepatitis C (Pockros PJ. *Hepatology* 2007; 45: 569). (2007 20%; Total 40%)

C1. Develop relevant and robust animal models of hepatic injury, inflammation, and fibrosis progression and resolution. The xenobiotic 3,5-diethoxycarbonyl-1,3-dihydrocollidine induces injury, inflammation, and fibrosis of the biliary tract in mice, providing a potentially valuable animal model of cholangiopathy (Fickert P. *Am J Pathol* 2007; 171: 525). (2007 10%; Total 40%)

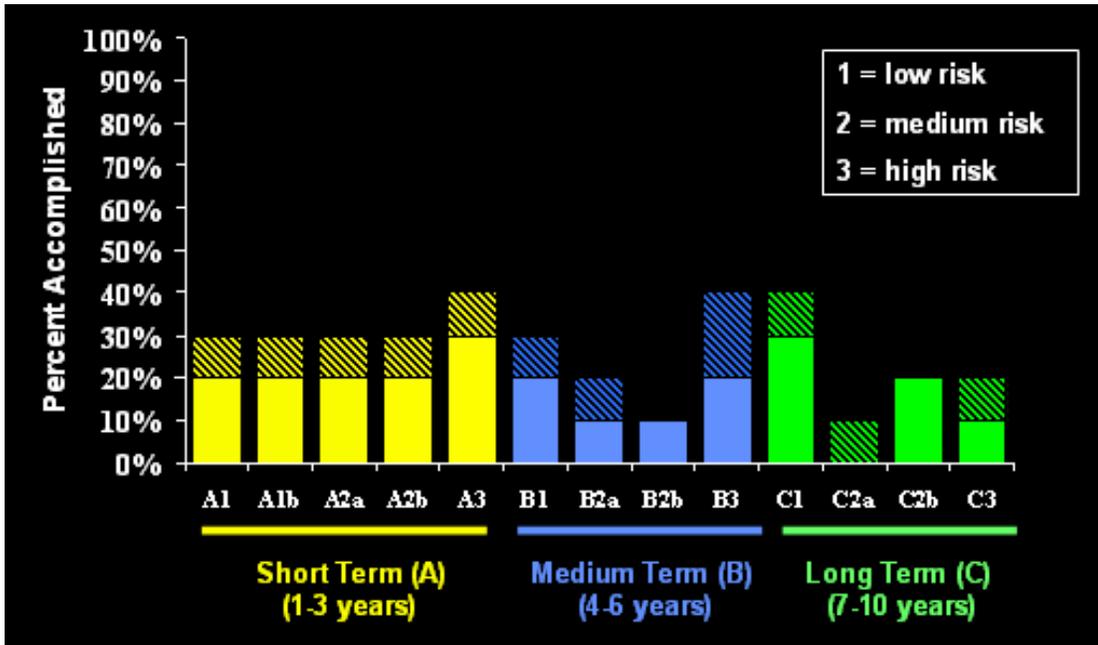
C2a. Using high-throughput screens, identify candidate small molecules that modify cytotoxic and fibrotic pathways in liver cells. Progress in this area will require development of methods for screening small molecules that augment or

impede cell signaling pathways related to apoptosis and fibrosis. Potential targets for such screens have been identified, including the RSK-C/EBP β pathway, which is important in fibrogenesis (Buck M and Chojkier M. PLoS ONE 2007; 2: e1372) and acid sphingomyelinase, which mediates cell injury from excess copper in Wilson disease (Lang PA. Nat Med 2007; 13: 164). This area is the focus of NIH Roadmap initiatives, including an RFA on “Pilot-Scale Libraries for High-Throughput Screening” (RFA-RM-08-003). (2007 10%; Total 10%)

C2b. Define genetic determinants of disease risk and progression in acute/chronic liver injury, fibrosis, and regeneration. This area is being actively studied in large cohorts of patients with chronic hepatitis C and NASH. (2007 0%; Total 20%)

C3. Develop mechanism-based drug therapy in fibrotic disease, targeting pro-fibrogenic and fibrosis resolution pathways. Several attractive approaches to prevention or reversal of hepatic fibrosis have been suggested in studies in animal models. Activation of stellate cells is a central step in hepatic fibrogenesis, and the pathway to activation has been shown to be mediated, at least in part, by activation of RSK and phosphorylation of C/EPB β —intracellular signals that might be targeted by small molecule inhibitors (Buck M and Chojkier M. PLoS ONE 2007; 2: e1372). Furthermore, angiogenic signals probably play a major role in the development and progression of fibrosis; in animal models of cirrhosis, combined inhibition of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) pathways reduced neovascularization of the splanchnic bed and decreased portal pressures, suggesting that anti-angiogenic medications might have a role in the therapy of portal hypertension (Fernandez M. Hepatology 2007; 46: 1208). (2007 10%; Total 20%)

Figure 4. Estimated Progress on Liver Injury, Inflammation, Repair, and Fibrosis Research Goals, 2007 (Year 3) [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. Oval cells are pluripotent liver cell precursors found in the adult liver that can proliferate in response to liver injury and may play a role in hepatocarcinogenesis. Recent data show that oval cells are less sensitive than differentiated, mature hepatocytes to the growth inhibitory actions of transforming growth factor- β , providing a mechanism for their proliferation in severe liver injury when hepatocytes fail to provide adequate regeneration (Nguyen LN. *Hepatology* 2007; 45: 31). The ability of oval cells to evolve into either hepatocytes or bile ductular cells has been demonstrated in human cirrhotic livers using triple staining with markers of the different lineages (Zhou H. *Hepatology* 2007; 45: 716). Hepatic stem cells that can differentiate into hepatoblasts have been isolated from both fetal and adult liver tissue, and markers have been identified for their immunoselection and expansion (Schmelzer E. *J Exp Med* 2007; 204: 1973). (2007 10%; Total 40%)

A1b. Profile transcriptional network during endodermal specification, liver growth, and regeneration. New cell-surface markers for identification of stem cells in liver have been developed using microarray analyses comparing adult to fetal hepatocytes (Nierhoff D. *Hepatology* 2007; 46: 535). Serial analysis of gene expression in murine embryos has revealed multiple genes that are important in endodermal development, some of which were not previously known (Hou J. *BMC Dev Biol* 2007; 7: 92). (2007 10%; Total 10%)

A2a. Identify noninvasive biomarker or imaging methods for assessing liver regeneration. Whole liver imaging for 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"). (2007 0%; Total 0%)

A2b. Define role of inflammation, fibrosis, and cell injury in regeneration. Recent research has further defined the role of the signal transducers and activators of transcription (STATs) in hepatic regeneration and their modulation by growth stimuli such as hepatocyte growth factor and inhibition by inflammatory cytokine signaling (Cui Y. *Hepatology* 2007; 46: 504; Paranjpe S. *Hepatology* 2007; 45: 1471). (2007 10%; Total 30%)

A3a. Define role of nonparenchymal cells in liver regeneration and liver development. Extracellular matrix is essential for normal function, survival, and regeneration of the liver. Integrin-linked kinase (ILK) has been shown to be one of the mediators of this effect and is essential in regeneration; loss of ILK activity results in rapid hepatocyte apoptosis (Gkretsi V. *Hepatology* 2007; 45: 1025). (2007 10%; Total 20%)

A3b. Develop new animal model systems to study liver development. *Drosophila* (fruit flies) has served as a key model system for study of genetics and protein function. While fruit flies do not have a liver, several hepatic functions appear to be served by oenocytes; for example, in a manner similar to hepatocytes, oenocytes accumulate lipid after fasting. Oenocytes also express lipid metabolizing genes and are required for larval growth and development. Thus, development of liver functions can be assessed in this model system (Gutierrez E. Nature 2007; 445: 275). (2007 10%; Total 30%)

B1a. Develop methods to select transplanted donor cells and induce homing and engraftment of transplanted cells to the liver. A rodent model with a humanized liver has been developed based upon the severely immunodeficient (Rag 2^{-/-} and Il2rg^{-/-}) fumarylacetoacetate hydrolase knockout (Fah^{-/-}) mouse that can be reconstituted with human hepatocytes from multiple sources including donor grafts and even liver biopsies (Azuma H. Nat Biotechnol 2007; 25: 903). This model can serve to expand human hepatocytes and may be helpful in gene therapy, as well as studies of regeneration, viral hepatitis, and drug-induced liver disease. (2007 20%; Total 30%)

B1b. Identify how deregulation of genes and pathways involved in normal regeneration contributes to carcinogenesis. The cytokine IL-6 contributes to normal regeneration and acts through activation of STAT-3, which has many actions that promote cell proliferation but also causes upregulation of myeloid cell leukemia 1 (Mcl-1) protein, which inhibits apoptosis. In cholangiocarcinoma there is sustained IL-6/STAT-3 signaling and increased expression of Mcl-1, which has now been shown to be caused by methylation and inactivation of the suppressor of cytokine signaling 3 (SOCS-3) promoter, which normally acts as a feedback control mechanism to terminate IL-6 signaling. These findings provide potential targets for non-cytolytic therapy of cholangiocarcinoma (Isomoto H. Gastroenterology 2007; 132: 384). (2007 10%; Total 30%)

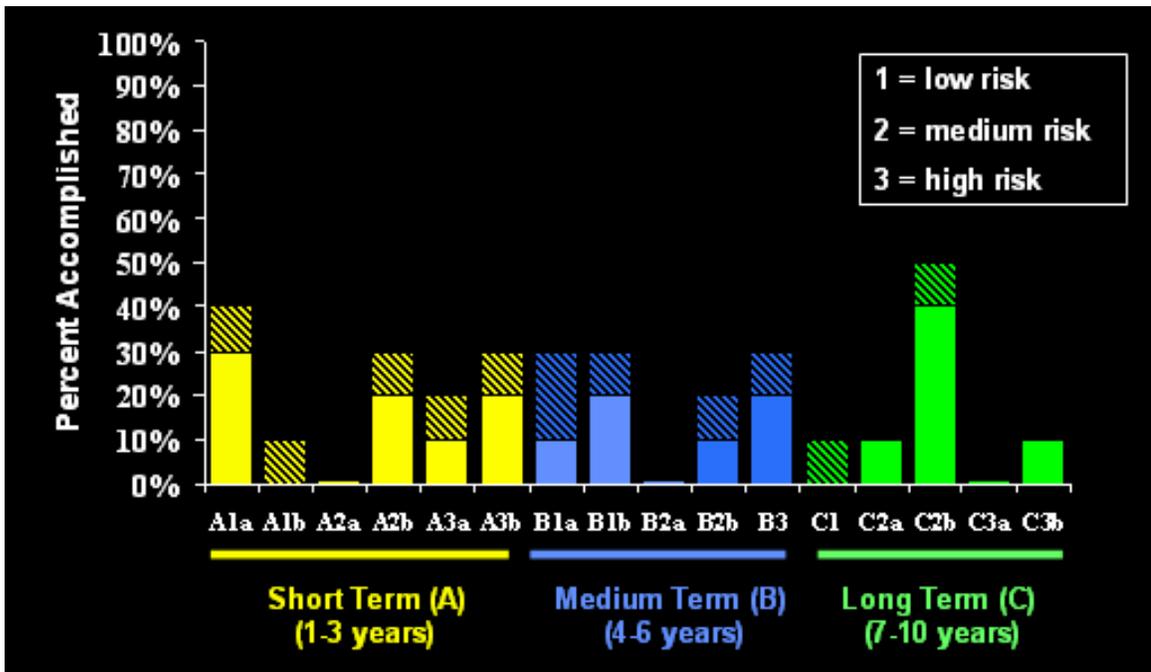
B2a. Validate biomarkers of regeneration in living donor liver donation and acute liver failure. This area of research is encouraged directly in the program announcement PA-07-052 (“Development of Disease Biomarkers”) and is the focus of ancillary studies in the A2ALL cohort study of living donor liver transplantation. (2007 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 recently begun to be characterized. Studies in *Drosophila* have identified a kinase cascade (“Hippo”) as a checkpoint involved in regulating organ size, leading to phosphorylation and inactivation of Yorkie, the mammalian homologue of which is YAP. Dysregulation of YAP leads to liver hypertrophy and cancer. These results suggest that this pathway is important in regulating organ size (Dong J. Cell 2007; 130: 1120). (2007 10%; Total 20%)

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 into mature hepatocytes are likely to be multiple,

- interrelated, and redundant. Fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) promote differentiation of hepatocyte-like cells from endodermal precursors and can be used to induce differentiation of human embryonic stem cells (Cai J. *Hepatology* 2007; 45: 1229). Similar requirements are essential for liver specification in zebrafish (Shin D. *Development* 2007; 134: 2041). (2007 10%; Total 30%)
- C1. Develop *ex vivo* and *in vivo* vectors for liver-directed gene therapy.** Hybrid vectors using adenoviruses and a bacteriophage-derived integrase have been developed that provide increased transgene expression (Ehrhardt A. *Mol Ther* 2007; 15: 146). (2007 10%; Total 10%)
- 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, but none have been tested in human patients. (2007 0%; 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 multiple transcription factors that must be properly expressed at the proper times. Studies in *Xenopus* embryos show that the Wnt/ β -catenin pathway must be repressed in the early somite stage for the foregut to maintain its identity and allow liver and pancreatic development; later in development, β -catenin has the opposite effect and enhances liver development (McLin VA. *Development* 2007; 134: 2207). Early stages of both liver and pancreas development are dependent upon GATA4, and other GATA factors may play supportive roles (Watt AJ. *BMC Dev Biol* 2007; 7: 37). (2007 10%, Total 50%)
- C3a. Develop practical gene or cell therapy for metabolic liver disease.** Pilot studies have been conducted of innovative gene and cell therapy for several metabolic liver diseases, but practical approaches have yet to be developed. (2007 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*. (2007 0%; Total 10%)

Figure 5. Estimated Progress on Developmental Biology and Regeneration Research Goals, 2007 (Year 3) [Cross-hatching indicates recent year's progress.]



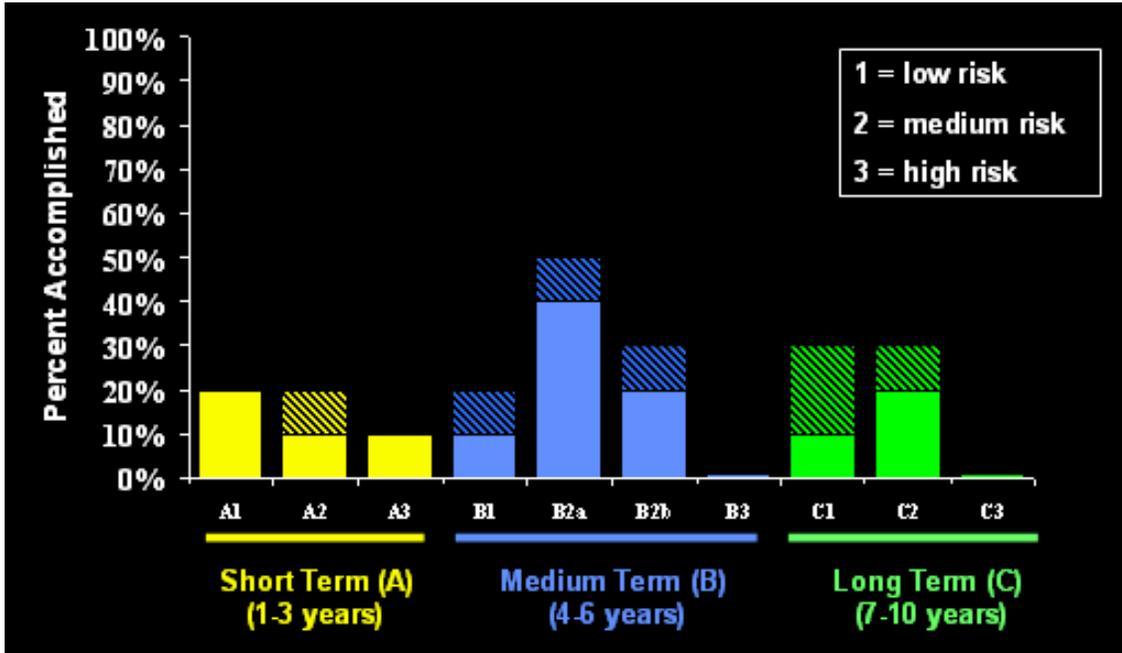
Chapter 4

Bile, Bilirubin, and Cholestasis

- A1. Further identify molecular causes of various forms of PFIC.** The molecular causes of most forms of progressive familial intrahepatic cholestasis (PFIC) have been identified: PFIC1 (classic Byler disease) is due to mutations in FIC1, an aminophospholipid translocase; PFIC2 is due to mutations in the bile salt export pump (BSEP); and PFIC3 is due to mutations in the multidrug resistance protein 3 (MDR3), a canalicular phospholipid translocase. Some forms of PFIC cannot be attributed to any of these three genes. Careful assessment of genetic mutations that are not associated with changes in amino acid sequence (silent or synonymous nucleotide substitutions) indicate that these variants can have altered function and substrate specificity (Kimchi-Sarfaty C. *Science* 2007; 315: 525). (2007 0%; Total 20%)
- A2. Define structure-function relationships of genes involved in cholestatic liver diseases and identify potential targets for therapy.** Different mutations in the bile salt export pump (BSEP) are associated with cholestatic liver diseases of variable severity (PFIC2, BRIC2 and cholestasis of pregnancy). These different mutations have unique effects on localization and function of the BSEP protein, providing potential approaches to alleviating the associated diseases (Lam P. *Am J Physiol Cell Physiol* 2007; 293: C1709). For instance, 4-phenylbutyrate increases the cell surface expression of BSEP in cells with wild-type as well as cholestasis-associated BSEP mutations (Hayashi H and Sugiyama Y. *Hepatology* 2007; 45: 1506). (2007 10%; Total 20%)
- 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. (2007 0%; Total 10%)
- B1. Define whether polymorphisms of major bile transporters are involved in drug-induced cholestatic liver disease.** Several mutations and single nucleotide polymorphisms in BSEP and MDR3 have been found to be associated with drug-induced cholestatic liver injury (Lang C. *Pharmacogenet Genomics* 2007; 17: 47), but no single mutation appeared to be responsible for more than a few instances. The biological bases of the associations require further investigation. (2007 10%; Total 20%)
- B2a. More fully elucidate the normal pathways of bile salt, lipid, and organic solute uptake, synthesis, transport, and secretion in hepatocytes.** The feedback regulation of bile acid synthesis by the intestine has been further characterized using genetic approaches. Activation of the farnesoid X receptor (FXR) in liver and intestine inhibits bile acid synthesis, and FXR in these two sites have complementary actions at different steps in the bile acid synthesis pathway (Kim I. *J Lipid Res* 2007; 48: 2664; Houten SM. *Mol Endocrinol* 2007; 21: 1312). Bile acid uptake by the liver is increased during fasting and decreased by feeding, mediated through changes in the bile salt transporters (NTCP,

- OATPs). The level of expression of these transporters is regulated by the transcription factors known as hepatic nuclear factor 4 α (HNF4 α) and PPAR γ coactivator (PGC) 1 α —factors that coordinate metabolic changes at different states of nutrition (Dietrich CG. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: G585). (2007 10%; Total 50%)
- B2b. Define the pathways and regulation of hepatic cholesterol synthesis and secretion.** The Niemann-Pick C1-like 1 protein (NPC1L1) is required for intestinal cholesterol absorption and is blocked by ezetimibe, a potent cholesterol lowering agent. Recent studies show that this protein is also present in liver and localizes to canalicular membranes where it acts to allow retention of biliary cholesterol by hepatocytes. Thus, ezetimibe may act not just on cholesterol absorption, but also by increasing biliary secretion of cholesterol (Temel RE. *J Clin Invest* 2007; 117: 1968). (2007 10%; Total 30%)
- B3. Develop drug therapy that stimulates bilirubin metabolic pathways or interferes with bilirubin production in the newborn.** Additional information is needed on these metabolic pathways and their status at birth before interventions can be designed. (2007 0%; Total 0%)
- C1. Define molecular basis of pruritus and identify targets for potential therapies.** Gastrin-releasing peptide (GRP) is detectable in selected neurons in the spinal cord, the distribution of which matches that of pain and itch sensation pathways. Mice with deletion of GRP receptors have reduced itch responses to pruritogenic stimuli, but no apparent decrease in pain sensation, and intraspinal administration of GRP receptor agonists induce itching behavior, suggesting that GRP pathways mediate itching and providing a target for possible therapeutic agents (Sun YG and Chen ZF. *Nature* 2007; 448: 700). (2007 20%; Total 30%)
- 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.** Several genetic variants of bile acid metabolic or transport genes have been found to be associated with the occurrence or severity of cholestatic liver diseases, such as primary biliary cirrhosis (Zollner G. *Liver Int* 2007; 27: 920) and cholestasis of pregnancy (Van Mil SW. *Gastroenterology* 2007; 133: 507; Wasmuth HE. *Gut* 2007; 56: 265. (2007 10%; Total 20%)
- C3. Develop effective gene therapy for at least one form of severe, neonatal cholestasis or hyperbilirubinemia.** Diseases of particular focus for future research in this area should include Crigler-Najjar syndrome and Byler disease (PFIC-1), both of which are usually fatal to children without liver transplantation. (2007 0%; Total 0%)

Figure 6. Estimated Progress on Bile, Bilirubin, and Cholestasis Research Goals, 2007 (Year 3) [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. The biologic basis for non-response to interferon and ribavirin treatment in chronic hepatitis C was the focus of a research workshop held in March 2007, sponsored by the American Association for the Study of Liver Diseases (AASLD). Analysis of gene expression arrays in liver biopsies from patients given either interferon alone or interferon and ribavirin before undergoing biopsy one day later showed that interferon-stimulated genes are typically elevated before treatment in non-responders to therapy and that levels increase minimally with treatment. Ribavirin appears to augment the interferon response largely through downregulation of genes involved in interferon inhibition and hepatic stellate cell activation (Feld JJ. *Hepatology* 2007; 46: 1548). (2007 10%; Total 30%)
- A1b. Define efficacy of interferon and ribavirin in subgroups of HCV patients.** Studies of peginterferon and ribavirin therapy in patients with recurrent hepatitis C after liver transplantation indicate that it is poorly tolerated, but that sustained responses can be achieved in one-third of patients, particularly those with earlier stages of fibrosis. Therapy was associated with improved histological outcomes and better portal hemodynamics (Carrión JA. *Gastroenterology* 2007; 132: 1746). (2007 10%; Total 40%)
- 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. Recently, the type I interferons have been shown to induce non-coding microRNAs (miRNAs) that act in the early innate immune response against viral infections. Some of these miRNAs have sequence-predicted targets against the HCV genome, and blocking their effects reduces the antiviral actions of interferon against HCV (Pedersen IM. *Nature* 2007; 449: 919). Thus, miRNAs may play an important role in the control of HCV infection, and their modulation may improve means of both prevention and treatment of hepatitis C. (2007 10%; Total 50%)
- 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. Recent studies using modifications of the infectious inocula with reporter genes have allowed for rapid assessment of replication and synthesis of chimeric viruses to define the roles of different viral genes in viral cell entry, processing, replication, assembly, and release and to elucidate the molecular basis for differences among genotypes. (2007 0%, Total 60%)
- B1a. Fully define early events during HCV and HBV infection.** Mathematical analyses of viral kinetics based upon testing of serial HBV DNA levels during acute hepatitis B indicate that after almost universal infection of hepatocytes during early phases of infection, cells cleared of virus remain uninfected and are protected against re-infection, probably as a result of local cytokine production by

infiltrating T cells (Ciupe SM. Proc Natl Acad Sci USA 2007; 104: 5050). (2007 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 CoPilot). Publication of results of both trials is expected in 2008. (2007 10%; Total 10%)

B2a. Identify new targets in viral replication and the host for development of small molecule therapeutics (HCV, HBV, HDV). Use of the replicon and cell culture systems for HCV replication has identified a number of host cell proteins that are essential in the HCV life cycle, many of which may be appropriate targets for therapy (Randall G. Proc Natl Acad Sci USA 2007; 104: 12884). Proteins newly shown to be important in HCV infection include: claudin-1, a component of hepatocytes' tight junctions that is required for cell entry (Evans MJ. Nature 2007; 446: 801); very low density lipoproteins (VLDL) and apolipoprotein B (apoB), which are required for viral replication and assembly (Huang H. Proc Natl Acad Sci USA 2007; 104: 5848); and host cell miRNAs, many of which interact with HCV or are associated with increase or decrease in HCV replication (Pedersen IM. Nature 2007; 449: 919). The HCV p7 protein has been further characterized as being crucial for viral assembly and release and having properties of a viroporin, viral proteins that form membrane pores that can promote viral entry or release (Steinmann E. PLoS Pathog 2007; 3: e103). (2007 10%; Total 20%)

B2b. Define the molecular basis for antiviral resistance of HBV. Telbivudine, a potent L-nucleoside, has been shown to be effective in treatment of chronic hepatitis B and more potent than either lamivudine or adefovir (Chan HLY. Ann Intern Med 2007; 147: 745; Lai CL. N Engl J Med 2007; 357: 2576). Resistance patterns to adefovir and lamivudine have been further defined during long-term therapy. The role of entecavir, a highly potent agent against hepatitis B, has been further studied. Long-term treatment with entecavir is associated with very low rates of resistance (Gish RG. Gastroenterology 2007; 133: 1437); however, entecavir resistance develops not uncommonly in patients with pre-existing lamivudine resistance. Furthermore, because entecavir has effects against HIV that can promote resistance, it should not be used as monotherapy against HBV in HIV-positive patients (McMahon MA. N Engl J Med 2007; 356: 2614). Entecavir-resistant mutations of HBV are sensitive to adefovir and tenofovir both *in vitro* and *in vivo* (Yatsuji H. J Med Virol 2007; 79: 1811). (2007 10%, Total 20%)

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. A rodent model with a humanized liver has been developed based upon the severely immunodeficient (Rag 2^{-/-} and Il2rg^{-/-}) fumarylacetoacetate hydrolase knockout (Fah^{-/-}) mouse, which can be reconstituted with human hepatocytes from multiple sources including donor grafts and even liver biopsies (Azuma H. Nat Biotechnol 2007; 25: 903; Bissig KD. Proc Natl Acad Sci USA 2007; 104: 20507). This model can serve to

expand human hepatocytes and can be used to study replication of HCV in a small animal model. (2007 10%; Total 20%)

B3b. Better characterize the HBV life cycle, virus-host interactions, basis for generation, and stability of cccDNA and viral state of HBV in humans.

A Request for Applications for establishment of a long-term Hepatitis B Clinical Research Network was published in 2007 (RFA-DK-07-011) and will be awarded in 2008 to establish a cohort of well-studied patients to be followed for virological, immunological, and clinical features over time. (2007 0%; 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 that target the RNA polymerase and serine protease. At present, two orally available, potent HCV protease inhibitors (VX-950/telaprevir and SCH 503034/boceprevir) have entered phase III trials of efficacy and safety, and preliminary results should be available within the next one to two years (Forestier N. *Hepatology* 2007; 46: 640; Prongay AJ. *J Med Chem* 2007; 50: 2310). Hepatitis A and E account for a large proportion of acute hepatitis that occurs worldwide; no specific therapies have been developed for either. However, two recent demonstrations of vaccine efficacy and safety—one of a recombinant hepatitis E vaccine (Shrestha MP. *N Engl J Med* 2007; 356: 895) and another of hepatitis A vaccination as an approach to post-exposure prophylaxis (Victor JC. *N Engl J Med* 2007; 357: 1685)—both promise to provide means of decreasing the morbidity and mortality of these important causes of acute hepatitis. (2007 10%; Total 30%)

C1b. Evaluate long-term benefits and risks of combination therapy of HBV.

Rates of resistance during 3 to 5 years of mono-therapy with lamivudine, telbivudine, and adefovir have been shown to be high. In contrast, studies of entecavir given for up to 4 years and tenofovir for up to 3 years show low rates of resistance despite monotherapy. Prospective studies are needed to define the rates of resistance with various agents and their virological, immunological and clinical correlates. (2007 0%; 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. The possibility of prevention of re-infection using monoclonal antibodies to HCV has not been assessed; several human monoclonal antibodies have been developed that broadly neutralize HCV *in vitro*, which promise to have applicability in humans (Johansson DX. *Proc Natl Acad Sci USA* 2007; 104: 16269). (2007 10%; Total 20%)

C2b. Achieve sustained response rate of over 90 percent in chronic hepatitis C.

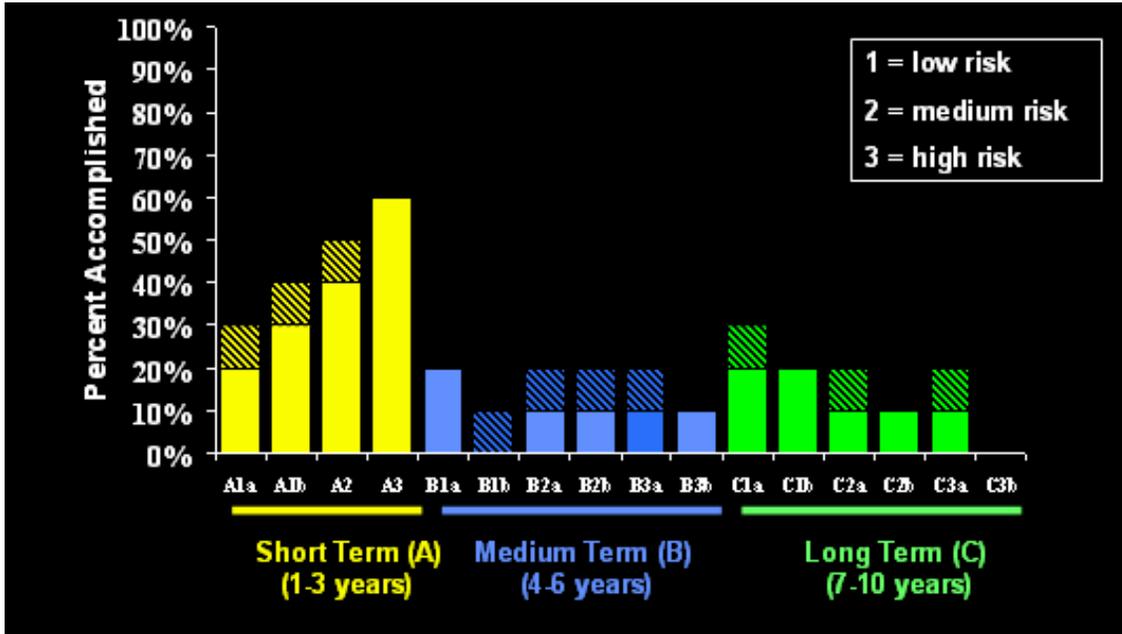
The sustained virological response rate to current therapy is 50-60%, and is higher in patients with genotypes 2 and 3 (approximately 80%) than genotype 1 (approximately 45%). Recent studies have shown that 24 weeks is the optimal duration of therapy in patients with genotypes 2 and 3 (Shiffman ML. *N Engl J*

Med 2007; 357: 124). Several promising new HCV protease and polymerase inhibitors with potent activity against HCV have been described. Antiviral resistance develops rapidly when these agents are used, particularly as monotherapy (Sarrazin C. Gastroenterology 2007; 132: 1767); for this reason, in future clinical trials these new agents will be given in combination with peginterferon and ribavirin. Two large-scale, industry-supported phase III trials have been initiated of protease inhibitors (telaprevir; boceprevir) in combination with peginterferon and ribavirin, but results have not yet been reported. (2007 0%; Total 10%)

C3a. Develop HCV vaccine. An experimental vaccine consisting of recombinant HCV-like particles produced in insect cells has been evaluated in chimpanzees and shown to induce broadly reactive T cell activity against the envelope and core regions of HCV and to ameliorate, but not prevent, infection with HCV. Thus, the three experimental HCV vaccines that have been evaluated in the chimpanzee model have demonstrated at least some degree of amelioration of infection and decrease in chronicity but not prevention of infection, suggesting that the focus of vaccination should be prevention of chronicity rather than complete immunity to infection (Elmowalid GA. Proc Natl Acad Sci USA 2007; 104: 8427). (2007 10%; Total 20%)

C3b. Develop therapeutic HBV vaccine. The first line of therapy for chronic hepatitis B will clearly be use of the oral nucleoside analogues with potent activity against HBV. Therapy with these agents, however, rarely leads to clearance of HBsAg and development of anti-HBs, which serve as markers of full recovery from chronic hepatitis B. Adjuvant use of a therapeutic HBV vaccine during long-term nucleoside therapy might help with clearance of HBsAg in patients with adequate viral suppression without loss of viral antigen; this area warrants prospective study. (2007 0%; Total 0%)

Figure 7. Estimated Progress on Viral Hepatitis Research Goals, 2007 (Year 3)
[Cross-hatching indicates recent year's progress.]



Chapter 6 HIV and Liver Disease

- A1a. Develop improved regimens of HAV and HBV vaccination.** Rates of response to HAV vaccine are reduced in HIV-infected adults compared to non-infected adults (approximately 65% vs 95% response rates, respectively) (Shire NJ. Vaccine 2006; 24: 272). Higher rates of response have been found in pilot studies using a virosome-formulated HAV vaccine (approximately 92%; Loutan L. Vaccine 2007; 25: 6310). The Adult AIDS Clinical Trials Group (AACTG) has fully enrolled a study on improving responses to HBV vaccine using GM-CSF, and results should be available in 2008. (2007 10%; Total 20%)
- A1b. Define short- and long-term safety and efficacy of peginterferon and ribavirin in different subpopulations of patients with HIV-HCV co-infection.** Rates of response to peginterferon and ribavirin are lower among HIV-HCV co-infected than HCV mono-infected individuals (Soriano V, AIDS 2007; 21: 1073). More extended courses of therapy and use of full doses of ribavirin (with or without erythropoetin) yield higher rates of response that approach the rates in mono-infected individuals (Nunez M. AIDS Res Hum Retroviruses 2007; 23: 972; Kevans D. Haemophilia 2007; 13: 593). (2007 10%; Total 10%)
- A2. Define safety and efficacy of peginterferon therapy for acute hepatitis C in HIV co-infection.** Observational studies have shown that peginterferon and ribavirin can induce clearance of HCV during acute infection in HIV-infected individuals, but relapse is frequent. Recent small outbreaks of acute hepatitis C among HIV-positive men who have sex with men have been shown to be linked to high-risk sexual behaviors (Danta M. AIDS 2007; 21: 983). (2007 0%; Total 30%)
- A3. Define effects of HIV infection on the liver, including on different populations of liver cells.** The susceptibility of different populations of liver cells to HIV infection is under investigation. (2007 0%; Total 20%)
- B1a. Define whether long-term peginterferon slows progression of disease in chronic hepatitis C with HIV co-infection.** The AACTG is sponsoring a clinical trial entitled “Suppressive Long-term Antiviral Management of Hepatitis C Virus (HCV) in HIV-1 Co-infected Subjects” to evaluate the safety and efficacy of long-term antiviral treatment. (2007 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. Further data have demonstrated that hepatitis C tends to progress more rapidly in HIV-coinfected than in HCV-monoinfected individuals (Posthouwer D. Blood 2007; 109: 3667; Sulkowski MS. AIDS 2007; 21: 2209). Other causes of liver disease in HIV-positive persons include hepatitis B, nonalcoholic and alcoholic liver disease, and drug-induced liver injury. (2007 10%; Total 30%)

- B2a. Elucidate mechanisms by which HIV infection accelerates fibrosis and disease progression in HBV and HCV infection.** The mechanisms by which HIV infection accelerates liver fibrosis largely remain unknown, but hepatic stellate cells that produce collagen after activation are sensitive to circulating cytokines and activated immune cells. Active infection with the GBV-C virus (a flavivirus similar to HCV) is associated with an apparent decrease in the rate of fibrosis progression and liver decompensation in HCV-HIV co-infected individuals, although the mechanism of this protective effect remains elusive (Berzsenyi MD. *Gastroenterology* 2007; 133: 1821). Several NIH-funded prospective studies are actively assessing factors associated with progression of liver disease in patients with HIV/HCV co-infection (e.g., Natural History of HCV infection in HIV Disease [NHHHD]; HIV/HCV Co-infection, Antiretroviral Therapy and Fibrosis [HHCATF]; and Women's Interagency HIV Study [WIHS], a major aim of which is to study HCV/HIV co-infection). (2007 0%; Total 20%)
- B2b. Define factors that lead to reactivation of HBV in HIV co-infection and develop means of prevention.** Recent data suggest that improved regimens of therapy for hepatitis B have decreased the mortality rate of patients with HBV/HIV co-infection. (2007 0%; Total 30%)
- B3. Develop noninvasive means of detecting early hepatic mitochondrial dysfunction.** New methods of detecting early mitochondrial dysfunction have not been reported. Mice with genetically induced partial deficiency in mitochondrial superoxide dismutase 2 (Sod2^{+/-}) have increased susceptibility to mitochondrial toxins and may provide means of screening antiviral agents for this potential form of toxicity (Ong MM. *Toxicol Sci* 2007; 97: 205). (2007 10%; Total 10%).
- C1a. Develop optimal therapeutic regimens for chronic hepatitis B in different stages and patterns of disease in HIV-co-infected patients.** The combination of tenofovir and emtricitabine is now recommended as the standard of therapy for HBV-HIV co-infection and has excellent long-term efficacy. The potent anti-HBV drug entecavir has been found to have direct effects on HIV-1 replication and can lead to development of antiviral resistance; therefore, this agent should be avoided as monotherapy in HBV-HIV co-infected persons (McMahon MA. *N Engl J Med* 2007; 356: 2614). (2007 10%; Total 50%)
- 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 phase II clinical trials; HIV-HCV co-infected cohorts have not been included in early testing (Sherman KE. *Hepatology* 2007; 46: 2014). (2007 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 (Vergara S. *Clin Infect Dis* 2007; 45: 969). Determination of the reliability and reproducibility of these approaches will require further study. (2007 0%; Total 30%)

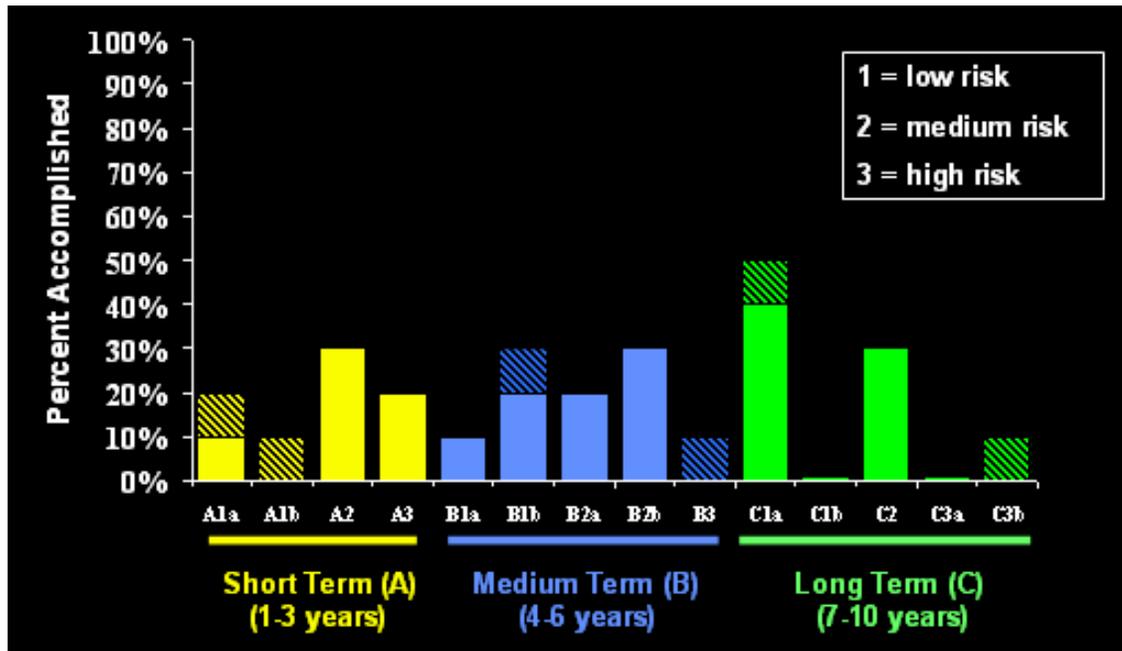
C3a. Develop *in vitro* or *in vivo* models of HIV-HCV and HIV-HBV co-infection.

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. (2007 0%; Total 0%)

C3b. Develop means to reliably attribute causality of drug-induced liver disease in HIV-infected persons.

In an analysis of nevirapine hypersensitivity, chronic HCV infection was found to be the only associated factor (Phillips E. AIDS 2007; 21:1561). An international, NIH-sponsored workshop on “Drug-Induced Liver Injury: Standardization of Nomenclature and Mechanisms of Causality Assessment,” scheduled for December 1-2, 2008, will include sessions addressing issues with anti-retroviral agents in HIV-infected individuals. (2007 10%; Total 10%)

Figure 8. Estimated Progress on HIV and Liver Disease Research Goals, 2007 (Year 3) [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.** The NIH-funded NASH Clinical Research Network has enrolled a cohort of more than 1200 individuals. These study participants have been fully characterized clinically and will be followed for 5 years, providing resources for studies of natural history, genetics, and biomarker development. (2007 10%; Total 60%)
- 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 completed and phase II trials initiated in both NASH and hepatitis C by the Silymarin in NASH and Chronic Hepatitis C (SynCH) Network. Small pilot studies of pentoxifylline and orlistat have reported promising results on biochemical and histological features of NASH (Satapathy SK. *J Gastroenterol Hepatol* 2007; 22: 634; Hussein O. *Dig Dis Sci* 2007; 52: 2512). (2007 10%; Total 20%)
- A3. Develop more accurate animal models of nonalcoholic fatty liver disease (including secondary forms) and define molecular characteristics.** While accumulation of fat is considered a central component of nonalcoholic fatty liver disease, inhibition of triglyceride synthesis in an animal model leads to a worsening of hepatic damage and fibrosis, indicating that accumulation of fat may be a protective mechanism rather than a necessary component of NASH (Yamaguchi K. *Hepatology* 2007; 45: 1366). Further studies in rodent models of nonalcoholic fatty liver have shown the potential importance of several factors, including: serotonin metabolism (Nocito A. *Gastroenterology* 2007; 133: 608), episodic hypoxemia (Savransky V. *Am J Physiol Gastrointest Liver Physiol* 2007; 293: G871), CEBP signaling (Rahman SM. *Hepatology* 2007; 45: 1108), protein kinase C (Samuel VT. *J Clin Invest* 2007; 117: 739), regulatory T cells of the innate immune system (Ma X. *Hepatology* 2007; 46: 1519), and toll like receptor signaling in Kupffer cells (Rivera CA. *J Hepatol* 2007; 47: 571). High-fat diet rabbit models of nonalcoholic hepatitis have been developed and used in helping to elucidate the pathogenesis of NASH (Kawada N. *J Gastroenterol Hepatol* 2007; 22: Suppl 1: S85; Otagawa K. *Am J Pathol* 2007; 170: 967). (2007 20%; Total 40%)
- 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. Gene profiling in small numbers of patients with either simple steatosis or NASH has revealed upregulation of platelet-derived growth factor (PDGF) receptor, STAT 5 and hepatocyte nuclear factor-3 and SMAD pathways (among others) and downregulation of pathways of glutathione and amino acid metabolism and in the peroxisome proliferator activated receptor (PPAR) pathways, providing insights into possible

means of treatment or prevention of NASH (Yoneda M. J Pharmacol Sci 2007; 105; 151; Baranova A. Obes Surg 2007; 17: 1111). Analysis of protein microarrays from omental fat in patients undergoing bariatric surgery for obesity demonstrated marked disruption of insulin signaling pathways in those with NASH compared to those with simple steatosis (Calvert VS. Hepatology 2007; 46: 166). (2007 10%; Total 20%)

B1b. Evaluate role and effects of bariatric surgery on NASH. Single-center studies continue to demonstrate improved biochemical and histological features of NASH after gastric bypass surgery for obesity. While fibrosis improves minimally, if at all, with the weight loss following bariatric surgery, it does not progress (Liu X. Obes Surg 2007; 17: 486; Furuya CK Jr. J Gastroenterol Hepatol 2007; 22: 510); long-term follow-up is needed to resolve whether fibrosis is improved and cirrhosis prevented by bariatric surgery. The specific role of bariatric surgery in management of NASH warrants elucidation through a prospective, randomized controlled trial. (2007 10%; Total 30%)

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 recent findings indicate that this may be mediated by induction of proinflammatory cytokines, such as IL-8. Lipidomic analyses of liver tissue demonstrate increases in diacyl- and triacylglycerols, but not free fatty acids, in nonalcoholic fatty liver compared to controls and decreases in omega-6 fatty acids in NASH (Puri P. Hepatology 2007; 46: 1081). (2007 10%; Total 30%)

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. (2007 0%, 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 Screening" (RFA-RM-08-003), and a PA for similar grants was published as "Development of Assays for High-Throughput Drug Screening" (PA-07-320). (2007 0%; Total 0%)

B3b. Develop therapy of acute alcoholic hepatitis that promotes recovery and decreases permanent injury. An NIH-sponsored trial of antibody to TNF soluble receptor in alcoholic hepatitis has recently been completed, and results are anticipated in early 2008. (2007 0%; Total 10%)

C1a. Establish the efficacy and safety of therapy with insulin-sensitizing agents and vitamin E in NASH. The NASH Clinical Research Network has completed enrollment in a placebo-controlled trial of two years of pioglitazone vs vitamin E vs

placebo in 247 adults and a similar trial of metformin vs vitamin E vs placebo in 173 children with NASH. Preliminary results are anticipated in 2008. (2007 0%; Total 40%)

C1b. Establish the efficacy and safety of therapy with SAMe in alcoholic liver disease. The rationale behind the use of S-adenosylmethionine (SAMe) and antioxidants in alcoholic liver disease was the topic of an NIH workshop recently summarized in the literature (Purohit V. *Am J Clin Nutr* 2007; 86: 14). Alcohol inhibits SAMe biosynthesis and thereby sensitizes the liver to injury and proinflammatory cytokines such as TNF α (Song Z. *Biochem Pharmacol* 2007; 74: 521). In an animal model of alcoholic fatty liver disease (the alcohol-fed, folate-deficient Yucatan micropig), SAMe therapy attenuated the increase in lipid synthesis caused by alcohol. (Esfandiari F. *Alcohol Clin Exp Res* 2007; 31: 1231) (2007 10%; Total 10%)

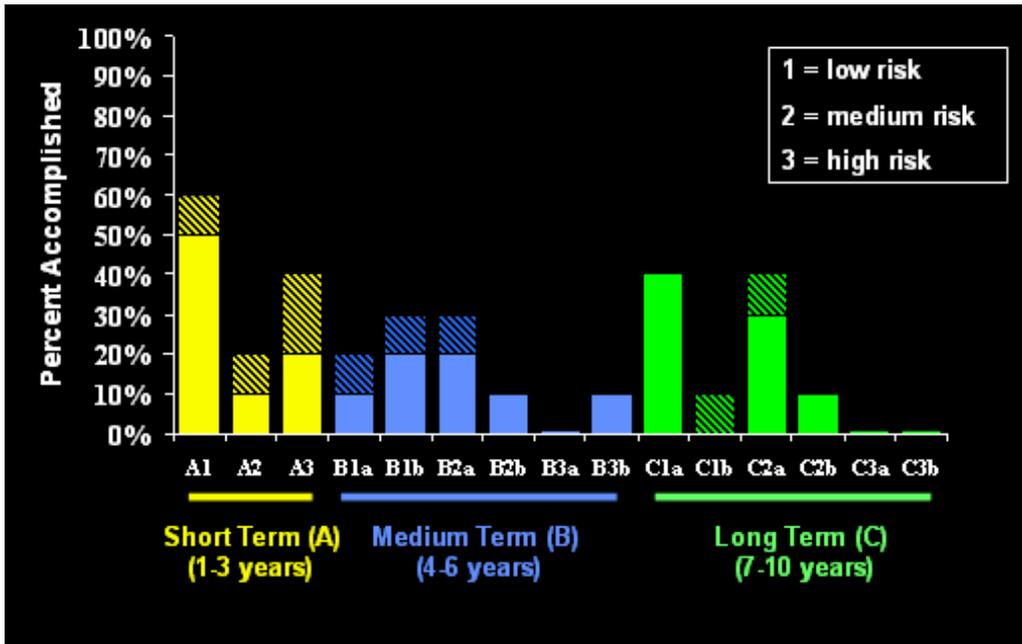
C2a. Establish the prevalence and incidence of NASH in the general population as well as special populations in the United States, such as children, minority groups, and patients with diabetes and other dysmetabolic syndromes. Studies using ultrasound indicate that 7% of persons with type 2 diabetes have fatty liver disease, and these individuals are at an increased risk for cardiovascular events (heart attack, coronary revascularization, stroke, or death) independent of other high risk factors (Targher G. *Diabetes Care* 2007; 30: 2119). The NIH-funded National Children's Study has been initiated, which will follow and assess 100,000 American children from birth to adulthood for childhood diseases and health conditions focusing on the role of genetics, environmental exposures, diet, and activity. This Study will likely yield information of relevance to assessing the impact of fatty liver disease and NASH on American children. (2007 10%; Total 40%)

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 the context of liver disease. (2007 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 will be needed on a greater number of patients to investigate an expanded set of polymorphisms. (2007 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. (2007 0%; Total 0%)

Figure 9. Estimated Progress on Fatty Liver Disease Research Goals, 2007 (Year 3) [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.** An international NIH-sponsored workshop entitled “Drug-Induced Liver Injury: Standardization of Nomenclature and Causality Assessment” has been scheduled for December 1-2, 2008. The Drug-Induced Liver Injury Network (DILIN) has continued to refine its causality assessment process and added severity and data completeness scales, which will be discussed at the workshop. Draft FDA guidance on standardizing procedures for detection and reporting of hepatotoxicity in clinical trials conducted by industry has been published in the Federal Registry, and a final version is expected in 2008. (2007 10%; Total 30%)
- A2. Develop positive diagnostic assay for acetaminophen toxicity.** Acetaminophen adducts are readily detectable in the serum of most patients with acetaminophen-induced acute liver failure. Adducts are also present in lower concentrations in serum of patients taking pharmacological doses. Definition of the reliability and accuracy of various levels of adducts in relationship to acetaminophen dose and liver injury requires further research. (2007 0%, Total 40%)
- A3a. Develop *in vitro* or *in vivo* systems for study of allergic and non-allergic idiosyncratic hepatotoxicity.** Animal models for idiosyncratic drug injury in humans would be extremely helpful in elucidating causes of this type of liver injury. Mice with genetically induced deficiency in mitochondrial superoxide dismutase 2 (Sod2^{-/-}) have increased susceptibility to mitochondrial toxins and may serve as a means of assessing agents with a potential for mitochondrial toxicity (Ong MM. Toxicol Sci 2007; 97: 205). Continuing work on the lipopolysaccharide-treated rodent has promise in defining the cause of idiosyncratic liver injury to some agents (Shaw PJ. Toxicol Sci 2007; 100: 259). Differences between humans and mice in bile salt uptake pump sensitivity to inhibition may account for why preclinical rodent studies sometimes fail to predict human toxicity (Leslie EM. J Pharmacol Exp Ther 2007; 321: 1170). (2007 10%; Total 20%)
- A3b. Identify chemical substructures that are protoxicant and could be avoided in design of new drugs.** New chemical substructures continue to be identified that may underlie drug-induced liver injury (Coe KJ. Chem Res Toxicol 2007; 20: 1277). (2007 10%, Total 10%)
- 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.** DILIN has enrolled more than 400 patients with idiosyncratic drug-induced liver injury into a database with careful collection of clinical information, serum, DNA, and tissue. Collaborations to further utilize these research resources are being pursued with other clinical and laboratory investigators in the field of drug-induced liver disease. (2007 10%, Total 30%)

B2a. Elucidate molecular mechanisms of common forms of hepatotoxicity.

Molecular mechanisms of acetaminophen toxicity have been further defined with identification of the protective roles of IL-10, IL-4 and IL-13 in downregulating the innate immune system and opposing proinflammatory cytokines such as IL-6 and TNF- α . (Yee SB. *Chem Res Toxicol* 2007; 20: 734; Bourdi M. *Chem Res Toxicol* 2007; 20: 208). Acetaminophen toxicity was shown to be mediated at least in part through neutrophils and the coagulation system (Ganey PE. *Hepatology* 2007; 46: 1177). (2007 10%; Total 30%)

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. Prospective studies of drug-induced liver injury with provisions for long-term follow-up and assessment of chronicity are underway in Europe, Asia, and the United States. (2007 0%; Total 10%)

B3a. Define the role of the innate immune system in both allergic and non-allergic forms of hepatotoxicity. Several animal models indicate that innate immune responses modulate drug-induced liver injury. Neutrophils are particularly important in injury caused by medications, and pathways of the neutrophil response may engage the innate immune response and IL-1 (Deng X. *J Pharmacol Exp Ther* 2007; 322: 852; Chen CJ. *Nat Med* 2007; 13: 851). (2007 10%; Total 30%)

B3b. Develop an animal model of adaptation to hepatotoxicity to help define the genes necessary for the adaptive response. Animal models are limited largely to studies of direct hepatotoxins and their effects on the liver. Recently, a rodent model has been developed with a fully humanized liver based upon the severely immunodeficient (Rag 2^{-/-} and Il2rg^{-/-}) fumarylacetoacetate hydrolase knockout (Fah^{-/-}) mouse, which can be reconstituted with human hepatocytes from donor grafts or even liver biopsies (Azuma H. *Nat Biotechnol* 2007; 25: 903). This model may allow for better analyses of the effects of drugs on human liver. (2007 10%; Total 10%)

C1. Identify genetic factors that contribute to hepatotoxicity of several major forms of drug-induced liver disease. Genes that are associated with susceptibility to idiosyncratic forms of drug-induced liver disease have been identified for several agents, including diclofenac (*UGT2B7*, *CYP2C8* and *ABCC2*: Daly AK. *Gastroenterology* 2007; 132: 272), ximelagatran (MHC *DRB1*07* and *DQA1*02*: Kindmark A. *Pharmacogenomics J* May 15, 2007; epub), anti-tuberculosis drugs (*MnSOD* and *GSTM1*: Huang YS. *J Hepatol* 2007; 47: 128) and drugs that cause cholestasis (*ABCB11* and *ABCB4*: Lang C. *Pharmacogenet Genomics* 2007; 17: 47-60). These findings require independent confirmation and analysis for determination of biologic and functional significance. (2007 10%; Total 10%)

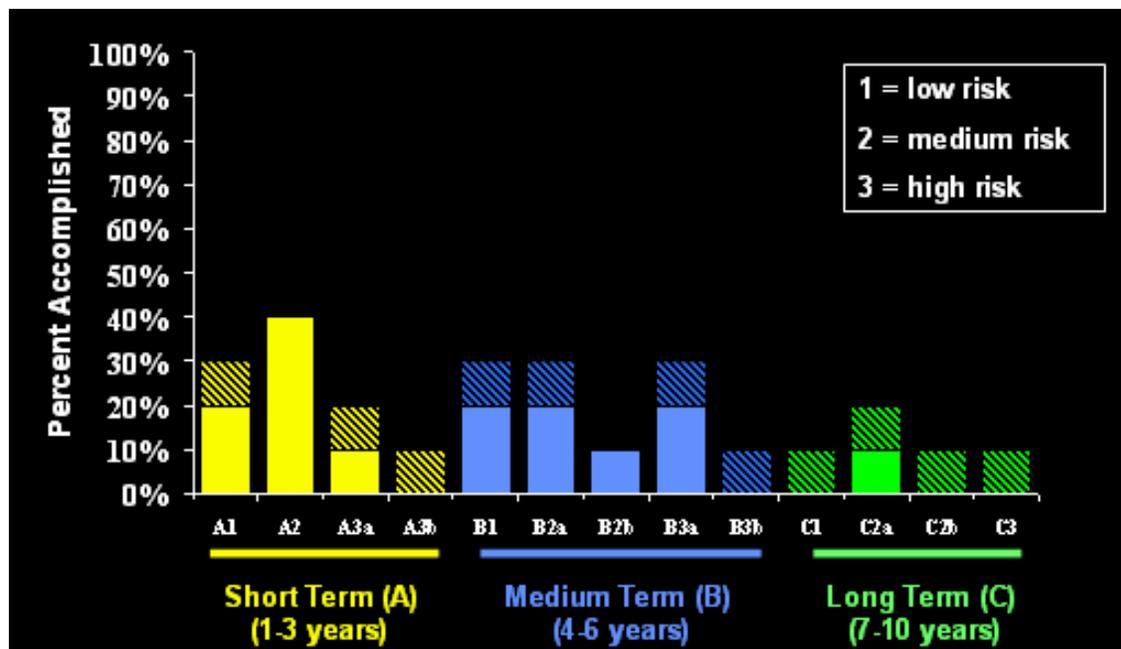
C2a. Determine the efficacy of nonspecific therapy of hepatotoxicity with antioxidants or hepatoprotective medications. Both the adult and the pediatric Acute Liver Failure Study Groups are evaluating N-acetylcysteine (NAC) as a therapy for drug-induced and other forms of acute liver failure. Preliminary presentations indicate that NAC improves spontaneous survival in patients who present with early stages of liver failure (hepatic encephalopathy [HE] grades 1 and

2), but not in those with more advanced stages (HE stages 3 and 4). The final results of this trial and subgroup analyses should be published in the next year. Further trials of antioxidant therapies are being planned. (2007 10%; Total 20%)

C2b. Develop and assess biomarkers or metabolites to predict the development of hepatotoxicity, and to distinguish between established hepatotoxicity and transient, adaptive enzyme elevations. Novel reactive metabolites that may underlie drug-induced liver injury and may be useful as biomarkers continue to be identified (Li F. *Chem Res Toxicol* 2007; 20: 1854; Kostrubsky SE. *Chem Res Toxicol* 2007; 20: 1503). In a study of 2500 patients receiving the anti-asthma agent zileuton, 4.4% developed serum ALT elevations, but most abnormalities resolved without major liver injury, either upon discontinuation or even with continuation of the drug (Watkins PB. *Drug Saf* 2007; 30: 805). Data collections such as these may help to identify early markers of clinically significant liver injury. (2007 10%; Total 10%)

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 to address this area. The blood transcriptome during acetaminophen hepatotoxicity in rats provides a diagnostic signature for this injury in humans as well (Bushel PR. *Proc Natl Acad Sci USA* 2007; 104: 18211). (2007 10%; Total 10%)

Figure 10. Estimated Progress on Drug- and Toxicant-Induced Liver Disease Research Goals, 2007 (Year 3) [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 clinical and basic research symposium entitled “Hepatic Inflammation and Immunity,” sponsored by the NIH and the US-Japan Immunology and Hepatitis Panels, and held in Galveston, Texas on January 25-27, 2008, featured talks on the role of animal models in elucidating the pathogenesis of autoimmune hepatitis. (2007 10%; Total 40%)
- A2. Develop multicenter networks of investigators to study natural history, pathogenesis, etiology, and therapy of autoimmune liver diseases.** A database has been developed with information on children with sclerosing and autoimmune cholangitis, under the auspices of the Morgan Foundation. This database will also incorporate serum and DNA samples and collaborations are being sought with genotyping groups. A U.S. primary biliary cirrhosis (PBC) epidemiology group has been developed and enrolled over 1000 patients with a similar number of controls; preliminary studies demonstrate that patients with PBC suffer from symptoms of fatigue and arthritis, but have well-preserved quality of life (Selmi C. *Hepatology* 2007; 46: 1836). (2007 10%; Total 20%)
- 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.** Patients with PBC often have activated and hyper-responsive B cells in the peripheral circulation. The B cells produce mitochondrial antibodies and exhibit enhanced signaling in response to stimulation that can be partially blocked by potassium channel blockers (Moritoki Y. *Hepatology* 2007; 45: 314). In autoimmune hepatitis type 2, CD8 T cells specific for CYP 2D6 correlate with the degree of hepatocyte damage (Longhi MS. *Hepatology* 2007; 46: 472). Patients with primary sclerosing cholangitis (PSC) have IgG antibodies against cholangiocytes, which can induce ERK1/2 signaling and increases in TLR-4 and -9 (Karrar A. *Gastroenterology* 2007; 132: 1504). (2007 10%; Total 20%)
- 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, randomized double-blind controlled trial of long-term, high-dose ursodiol therapy in PSC is fully enrolled and final results are expected in 2010. (2007 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. Ultrasound and magnetic resonance transient elastography are promising non-invasive approaches to assess liver stiffness and hence degree of fibrosis in chronic liver disease including PBC and PSC (Yin M. *Clin Gastroenterol Hepatol* 2007; 5: 1207; Talwalkar JA. *Clin Gastroenterol Hepatol* 2007; 5: 1214). (2007 10%; Total 10%)

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 a proposed autoimmune clinical research network. (2007 0%; Total 10%)

B3a. Identify genetic linkages in PBC and refine the HLA associations in autoimmune hepatitis and PSC.

Studies on 166 women with PBC and 226 controls demonstrate that there is excess X-chromosome loss in women with PBC (39%) compared to controls (17-24%). This loss is preferential for the chromosome of one parent, suggesting that the X chromosome may play an important role in the female predisposition of PBC (Miozzo M. *Hepatology* 2007; 46: 456). (2007 0%; Total 10%)

B3b. Develop animal models for each of the autoimmune liver diseases.

Several animal models have been developed for PBC and PSC, and promising approaches have been applied to development of a model of autoimmune hepatitis (Christen U. *Autoimmun Rev* 2007; 6: 306; Leung PS. *J Immunol* 2007; 179: 2651). Shortcomings of current models are that they typically are associated with acute inflammation and necrosis and do not lead to progressive loss of bile ducts or advanced cirrhosis and end-stage liver disease. (2007 10%; Total 50%)

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 the "Immune Tolerance Network," which encourages research on alternative maintenance therapy for autoimmune diseases. Further pilot and uncontrolled studies suggest that mycophenolate mofetil may be beneficial in patients with autoimmune hepatitis who have failed to respond to or are intolerant of prednisone and azathioprine (Inductivo-Yu I. *Clin Gastroenterol Hepatol* 2007; 5: 799). (2007 10%; Total 10%)

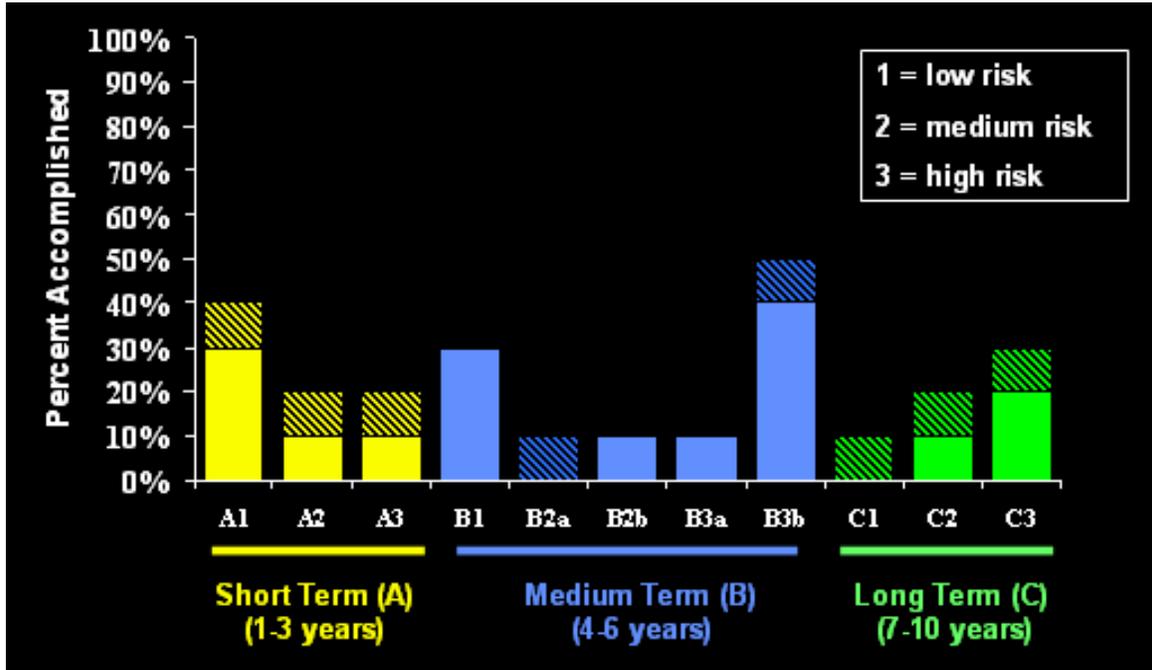
C2. Develop sensitive serum markers for early detection of cholangiocarcinoma in PSC.

A single center study from Italy reported elevated levels of insulin-like growth factor-I (IGF-I) in bile from patients with cholangiocarcinoma, but not from those with pancreatic cancer or benign biliary tract diseases (Alvaro D. *Ann Intern Med* 2007; 147: 451). Serum levels of IGF-1 were similar in all groups. These results need further confirmation, but promise to provide a means of early detection of this tumor in high-risk patients. (2007 10%; Total 20%)

C3. Identify modifiable environmental (with or without genetic) triggers for induction of autoimmune liver disease (from human studies or murine models).

Genetic factors are important in the pathogenesis of autoimmune liver diseases, but environmental factors also play a role, particularly in triggering disease onset. In guinea pigs, immunization with several chemical xenobiotics that share epitopes with the autoantigen of PBC (an immunodominant lipoylated peptide sequence) induces high titers of antimitochondrial antibodies and leads to a cholangitic injury similar to human PBC (Leung PS. *J Immunol* 2007; 179: 2651). These findings suggest that PBC may represent a genetic predisposition with a triggering exposure to an environmental immunogen. (2007 10%; Total 30%)

Figure 11. Estimated Progress on Autoimmune Liver Disease Research Goals, 2007 (Year 3) [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 adolescents in the United States. The NASH Clinical Research Network has enrolled over 300 children with nonalcoholic fatty liver into a prospective database and a clinical trial comparing metformin to vitamin E to placebo therapy. The database will allow for both clinical and basic laboratory studies on the etiology, natural history, and therapy of the disease. (2007 10%; Total 30%)
- 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. (2007 0%; Total 10%)
- A2. Develop systems to better characterize the frequency, medical burden, and epidemiology of pediatric liver disease.** Analysis of medical databases from the Calgary Health Region in Canada has provided population-based estimates of the frequency of primary sclerosing cholangitis (PSC) in children and adults, yielding an incidence of approximately 0.2 cases per 100,000 children and 1 case per 100,000 adults per year. In all, 73% of patients had concurrent inflammatory bowel disease and 16% had small duct PSC, and both associations were more common in children (Kaplan GG. *Am J Gastroenterol* 2007; 102: 1042). (2007 10%; Total 10%)
- 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 were assessed in a population-based cohort living in the Metropolitan Atlanta area between 2000 and 2004: 38% of cases were of unknown etiology (Bower WA. *Am J Gastroenterol* 2007; 102: 2459), a proportion similar to that reported from the NIH funded Pediatric Acute Liver Failure Study Group. A rare cause of idiopathic acute liver failure may be mitochondrial depletion syndromes. Two cases have been described that were attributable to mutations in the mitochondrial inner membrane protein MPV17; both individuals developed rapidly progressive liver failure and lactic acidosis in the newborn period (Wong LJ. *Hepatology* 2007; 46: 1218). (2007 10%; Total 30%)
- B1a. Define structural and functional development of the liver and biliary system.** Factors that lead to differentiation of the liver are multiple, interrelated, and redundant. The zebrafish model system continues to provide insights into liver specification during embryogenesis. Fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling are two essential pathways active during development of the hepatic bud from the endodermal foregut; thereafter, homeobox gene *Hhex* and *Prox 1* are essential for hepatoblast differentiation and bile duct morphogenesis (Shin D. *Development* 2007; 134: 2041; Hunter MP. *Dev Biol* 2007; 308: 355; Berg T. *Hepatology* 2007; 46: 1187; Meivar-Levy I. *Hepatology* 2007; 46: 898). Differentiation of embryonic stem cells into mature hepatocytes requires many

of these same factors (Soto-Gutierrez A. Nat Protoc 2007; 2: 347). The coordination of these signaling pathways is key to normal hepatogenesis. (2007 10%; Total 50%)

B1b. Evaluate long-term outcomes, complications, and tolerance-inducing regimens in children undergoing liver transplantation. New approaches to the study of long-term outcomes and immunosuppression-minimizing regimens in children are being pursued in the NIH-funded SPLIT registry. Late graft loss (occurring in 11% by 5 years) is usually due to rejection or technical and surgical complications; late mortality (6% at 5 years) is most frequently due to malignancy, infection, multi-organ failure, or post-transplant lymphoproliferative disorder (Soltys KA. Am J Transplant 2007; 7: 2165). Long-term follow-up and assessment of quality of life in children with liver transplants showed that they have excellent psychological and physical functioning, but decreased general health (Sundram SS. Am J Transplant 2007; 7: 982). These findings will help to direct efforts at improving outcomes and quality of life in children undergoing liver transplantation. (2007 10%; Total 20%)

B2a. Delineate the molecular pathogenesis of at least 3 of the neonatal cholestatic syndromes. Alagille syndrome is an autosomal dominant condition marked by a progressive loss of intrahepatic bile ducts along with malformations of the heart, eyes, and skeleton. Alagille syndrome is caused by mutations in the *JAGGED1* gene, which encodes a transmembrane protein that signals through the NOTCH pathway. Analysis of the biologic effects of several common *JAGGED1* mutations indicates that the mutated protein is abnormally shed from its extracellular domain then acts to antagonize normal NOTCH signaling, suggesting a “dominant-negative” effect of the mutation (Boyer-Di Ponio J. Hum Mol Genet 2007; 16: 2683). In a similar fashion, several mutations in the bile salt export protein (BSEP), encoded by the *ABCB11* gene that is affected in progressive familial intrahepatic cholestasis-2, benign recurrent intrahepatic cholestasis-2, and the cholestasis of pregnancy, have been analyzed in cell culture. Mutations associated with the greatest degree of cholestasis were associated with lower expression of BSEP on cell surface membranes, apparently due to protein instability and proteasome degradation—processes that could be approached therapeutically (Lam P. Am J Physiol Cell Physiol 2007; 293: C1709). Finally, use of a transgenic mouse model of alpha-1-antitrypsin (A1AT) deficiency has demonstrated that the amount of retained A1AT ZZ protein correlates with cell injury and that the liver responds via a distinct form of ER stress, which may constitute an attractive marker for the hepatic phenotype in this disorder (Lindblad D. Hepatology 2007; 46: 1228; Hidvegi T. J Biol Chem 2007; 282: 27769). (2007 20%; Total 40%)

B2b. Develop better animal models for neonatal cholestatic syndromes. Human citrin deficiency is due to loss-of-function mutations in the *citrin* gene; some of these mutations being associated with neonatal cholestasis. Mice with disruption of genes for citrin and a mitochondrial dehydrogenase develop citrullinemia, hyperammonemia, and fatty liver typical of human citrin deficiency, which is not simulated in a mouse model in which citrin alone is knocked out (Saheki T. J Biol Chem 2007; 282: 25041). (2007 10%; Total 40%)

B3. Identify biomarkers for diagnosis, staging, and grading of neonatal cholestatic syndromes. Two molecular tests for diagnosis of neonatal cholestatic syndromes

have been developed: first, a resequencing gene chip that is customized to detect major mutations in genes causing the 5 most common forms of neonatal intrahepatic cholestasis: Alagille syndrome, alpha-1-antitrypsin deficiency, and three forms of progressive familial intrahepatic cholestasis (PFIC-1, 2, and 3) (Liu C. *Gastroenterology* 2007; 132: 119); and second, a mutation scanning method for analysis of the entire coding region of *JAGGED1* and *NOTCH2*—genes responsible for the highly heterogeneous Alagille Syndrome (Samejima H. *Genet Test* 2007; 11: 216). (2007 20%; Total 20%)

C1a. Conduct clinical trials to optimize medical and surgical management of biliary atresia. Enrollment continues in the NIH-funded randomized, placebo-controlled trial of corticosteroids after hepatoportoenterostomy (Kasai procedure) in infants with biliary atresia called the STeroid for Biliary Atresia Randomized Trial (START), as part of the Biliary Atresia Research Consortium. A similar, but smaller prospective trial from the United Kingdom showed no apparent effect of perioperative corticosteroid therapy overall, but a subset of infants with biliary atresia undergoing the Kasai procedure before 70 days of age appeared to show benefit (Davenport M. *Hepatology* 2007; 46: 1821). A summary was published of an NIH-sponsored research workshop on biliary atresia with a research agenda for the future (Sokol RJ. *Hepatology* 2007; 46: 566). (2007 10%; Total 20%)

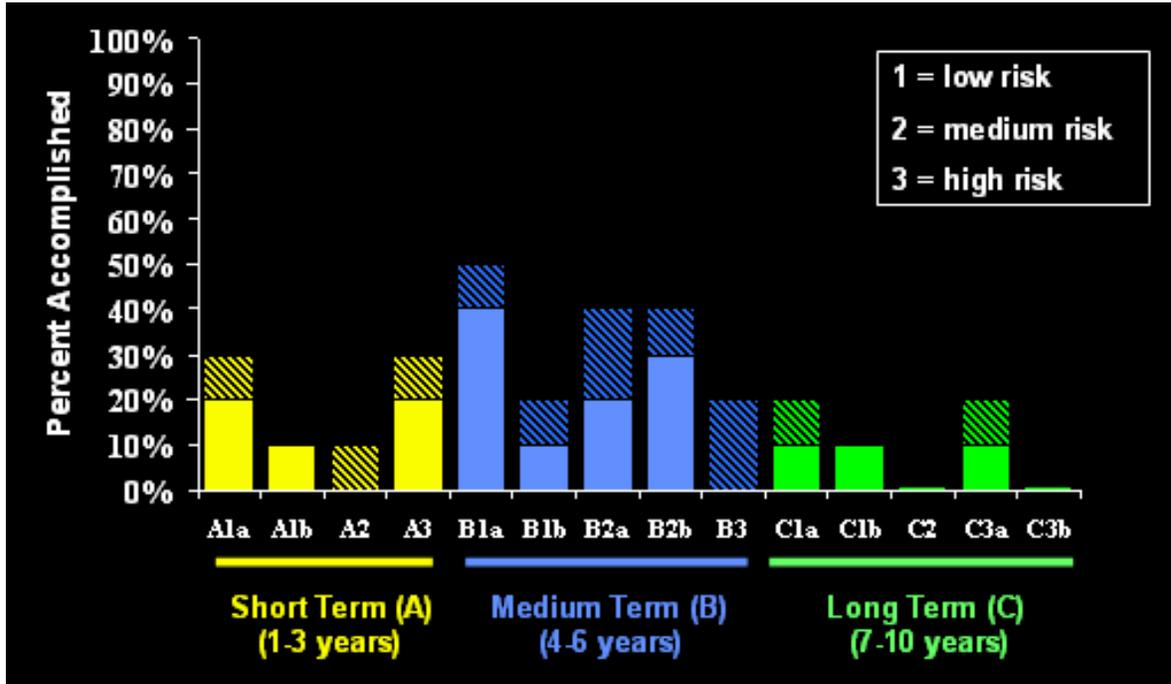
C1b. Evaluate therapies for acute liver failure in children. An NIH-funded trial of N-acetylcysteine in children with acute liver failure not due to acetaminophen has reached 50% of its planned enrollment and should be completed in two years. (2007 0%; Total 10%)

C2. Based upon molecular pathogenesis, identify small molecule therapies that might alleviate neonatal cholestatic syndromes. Targets for small molecule therapies are being identified in molecular studies of gene mutations associated with the various forms of neonatal cholestasis, but no such new approach has been applied to children with these diseases. (2007 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. This goal is an active area of investigator-initiated research, and several promising insights into the pathogenesis of biliary atresia have been published in the last year, including reports of higher rates of maternal microchimerism (Hayashida M. *J Pediatr Surg* 2007; 42: 2097), higher rates of alpha-1-antitrypsin polymorphic heterogeneity (Campbell KM. *J Pediatr Gastroenterol Nutr* 2007; 44: 99), and further evidence of heightened immune reactivity as a cause of bile duct injury in biliary atresia (Mack CL. *Gastroenterology* 2007; 133: 278; Shivakumar P. *Gastroenterology* 2007; 133: 268). (2007 10%; Total 20%)

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. (2007 0%; Total 0%)

Figure 12. Estimated Progress on Pediatric Liver Disease Research Goals, 2007 (Year 3) [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.** Both genetic and environmental factors appear to play a role in variation in iron overload among persons with *HFE* mutations associated with genetic hemochromatosis. Genome-wide scans for quantitative trait loci from the United States (Acton RT. *Clin Genet* 2007; 71: 518) and analysis of specific single nucleotide polymorphisms (SNPs) in iron regulatory genes from France (Milet J. *Am J Hum Genet* 2007; 81: 799) have identified several candidate chromosomal regions (16p, 17q, 5q) and genes (*BMP2*, *BMP4*, *HJV*) that may modulate iron overload. (2007 10%; Total 50%)
- A1b. Identify a cohort of patients with congenital hepatic fibrosis to study its natural history and optimal management.** The National Human Genome Research Institute (NHGRI) at the NIH has an ongoing intramural research protocol on autosomal recessive polycystic kidney disease (ARPKD)/congenital hepatic fibrosis (CHF) focusing on producing a comprehensive longitudinal dataset on natural history and pathogenesis, thus providing the basis for investigations of optimal management and treatment. (2007 0%; Total 30%)
- A2a. Establish DNA evaluation centers of excellence for Wilson disease, the porphyrias, and hemochromatosis.** No such centers have yet been established, but an online database has been developed for reporting mutations in the Wilson ATPase gene—*ATP7B*—which currently includes 379 probable disease-causing variants from populations worldwide (Kenney SM and Cox DW. *Hum Mutat* 2007; 28: 1171). A laboratory dedicated to analyses of porphyria-associated genes has been established at the Department of Genetics, Mount Sinai School of Medicine. (2007 10%; Total: 10%)
- A2b. Develop a reliable animal model for the liver disease of cystic fibrosis.** Studies in a mouse model lacking the *CFTR* gene suggest that chronic therapy with docosahexaenoic acid (an omega-3 fatty acid) is beneficial in decreasing inflammatory liver disease (Beharry S. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: G839). These results have yet to be extended to humans. (2007 0%; Total 30%)
- A3. Fully elucidate the molecular mechanisms of intestinal absorption, hepatic metabolism, and biliary excretion of copper.** Progress continues to be made in understanding copper metabolism. Copper transporter 1 (Ctr1) mediates high-affinity copper transport, but can also transport cisplatin by a distinct mechanism (Sinani D. *J Biol Chem* 2007; 282: 26775). Liver cell death and anemia in Wilson disease may be mediated by sphingomyelinase and ceramide (Lang PA. *Nat Med* 2007;13;164). (2007 10%; Total 30%)
- 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. Rather than general population screening, most current approaches advocate targeting high-risk groups, such as first-degree relatives of affected individuals and those with blood tests suggesting iron overload (Adams PC and Barton JC. *Lancet* 2007; 370: 1855). (2007 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 other liver diseases. (2007 0%; Total 20%)

B2a. Fully define the normal molecular pathways of iron metabolism in humans with specific definition of the roles of *HFE* and hepcidin. Progress continues to be made in the elucidation of pathways of iron regulation. The hepatic peptide hepcidin is the major iron-regulatory hormone, and new information is available on how hepcidin levels are controlled. The bone morphogenetic proteins (BMPs) play an important role in control of hepcidin expression *in vivo* (Babitt JL. *J Clin Invest* 2007; 117: 1933). Diferric transferrin regulates hepcidin in hepatocytes through BMP2/4 and hemojuvelin, for which the gene is altered in juvenile hemochromatosis (Lin L. *Blood* 2007; 110:2182). Transgenic mice with overexpression of hepcidin develop features of the anemia of inflammation and chronic disease (Roy CN. *Blood* 2007; 109: 4038). Growth differentiation factor 15 (GDF15) originating from an expanded erythroid compartment may act as the erythropoietic regulator of iron absorption by inhibiting hepcidin expression (Tanno T. *Nat Med* 2007; 13: 1096). (2007 10%; Total 40%)

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. In animal models, alcohol decreases hepatic hepcidin expression, which may facilitate iron accumulation (Harrison-Findik DD. *Hepatology* 2007; 46: 1979). In humans with chronic hepatitis C, hepatic oxidative DNA damage correlates with iron accumulation and is decreased by phlebotomy (Fujita N. *Free Radic Biol Med* 2007; 42: 353). The role of phlebotomy in managing chronic liver diseases awaits further study. (2007 10%, Total 20%)

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, although the genetic causes for iron overload in the absence of co-existing liver disease in these groups have not been identified. (2007 0%; Total 10%)

B3b. Define the molecular basis of the increase in HCC risk among persons with the porphyrias. Links have not yet been defined between the molecular abnormalities of

porphyrin metabolism in the inherited porphyrias and pathways of carcinogenesis. (2007 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. (2007 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. (2007 0%; Total 0%)

C2b. Develop an improved therapy for amelioration of acute crises in porphyria.

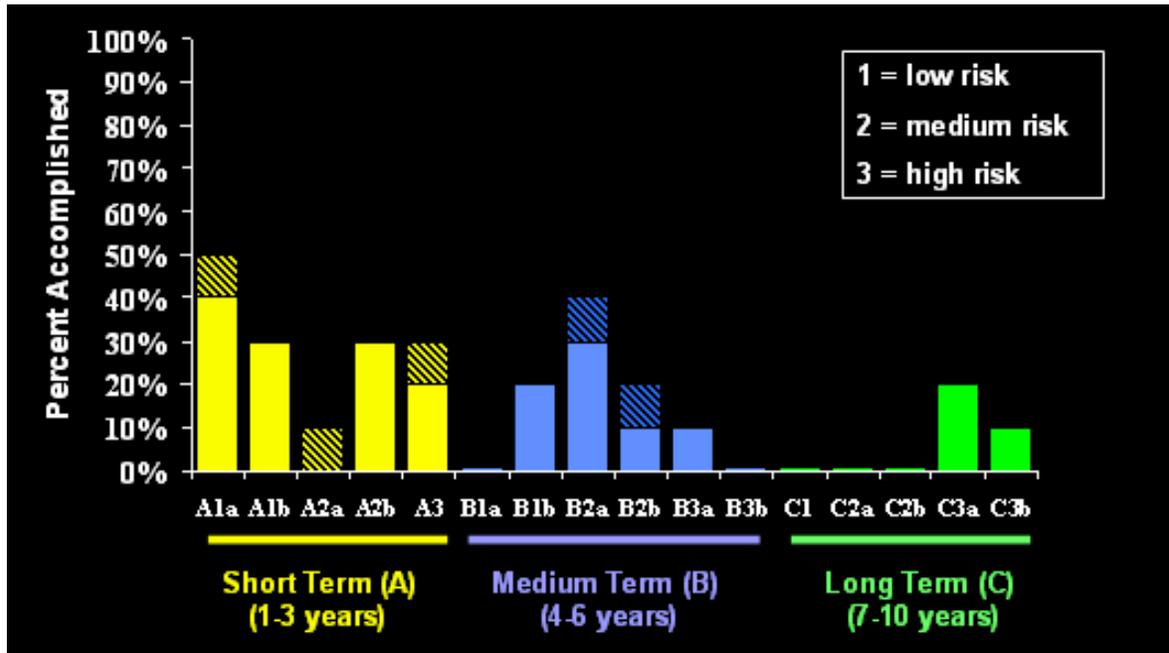
Intravenous hemin therapy remains the most effective treatment for acute porphyria. Porphyria cutanea tarda may be caused by a porphomethene inhibitor of uroporphyrinogen decarboxylase, providing a potential target for future therapy (Phillips JD. Proc Natl Acad Sci USA 2007; 104: 5079). (2007 0%; Total 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 continue to be refined to measure hepatic and myocardial iron levels (Positano V. Conf Proc IEEE Eng Med Biol Soc 2007: 2895; Song R. J Magn Reson Imaging 2007; 26: 208). At present, however, accurate determination of mild and moderate iron or copper overload requires quantitative analysis of liver biopsy tissue. (2007 0%; Total 20%)

C3b. Develop practical gene or stem cell therapy for AIP and EPP. Successful gene therapy for porphyria has yet to be accomplished. Gene therapy research is promoted by NIH-funded Molecular Therapy Core Centers. (2007 0%; Total 10%)

Figure 13. Estimated Progress on Genetic Liver Disease Research Goals, 2007 (Year 3) [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. Both systems use the international normalized ratio (INR) as a measure of coagulopathy and severity of liver disease. The INR, however, is standardized as a means of monitoring anticoagulant therapy and can be unreliable in assessing the coagulopathy of liver disease. Two studies have shown that calibration of the INR for end-stage liver disease may improve the accuracy of MELD and PELD in predicting survival (Tripodi A. *Hepatology* 2007; 46: 520; Bellest L. *Hepatology* 2007; 46: 528). In ongoing monitoring, the risk of waitlist mortality in relationship to estimates of added years of life is being assessed as a criterion for liver allocation. (2007 10%; Total 40%)
- A2. Identify biomarkers for acute cellular rejection and adequacy of immune suppression.** Identification and diagnosis of early rejection remains an unmet clinical challenge in liver transplantation, often requiring 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 encourages applications for development of noninvasive means of detecting acute rejection, immune suppression, and tolerance. (2007 0%; Total 0%)
- A3. Elucidate pathways of liver regeneration and identify targets for drug or cytokine/anticytokine therapy.** Several factors that are important in liver regeneration such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) and interleukin-6 (IL-6) interact with different cell surface receptors, but then signal through a common STAT3 pathway, which is counterregulated through feedback inhibition by suppressor of cytokine signaling 3 (SOCS-3) (Paranjpe S. *Hepatology* 2007; 45: 1471). These factors are appropriate targets for modulation of the regenerative process. (2007 0%; Total 20%)
- B1a. Define efficacy of peginterferon and ribavirin in pre- and post-transplant HCV infection.** Small studies performed to date indicate that combination therapy can be safely applied after liver transplantation with sustained response rates of approximately 40%, but with a slightly increased risk of rejection (Carrión JA. *Gastroenterology* 2007; 132: 1746). Careful attention to virological and biochemical responses during therapy can aid in adjusting treatment if it is ineffective and in promoting adherence if viral levels are responsive to treatment (Sharma P. *Liver Transpl* 2007; 13: 1100). (2007 10%; Total 30%)
- B1b. Improve safety and define role of living donor liver transplantation.** Initial analysis of retrospective data from the Adult-to-Adult Living Donor Liver Transplantation (A2ALL) cohort study has shown that availability of living donor liver transplantation improves survival in patients with end-stage liver disease, particularly at experienced centers (Berg CL. *Gastroenterology* 2007; 133: 1806).

Determining the long-term safety of liver donation requires prospective and long-term follow-up, which is the focus of the prospective component of A2ALL. (2007 20%; Total 40%)

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. (2007 0%; Total 10%)

B2b. Develop new therapies for hepatitis C that are effective in the transplant

situation. Several HCV-specific protease inhibitors (e.g., telaprevir, boceprevir) have been developed and are in phase III trials in patients with uncomplicated hepatitis C. Studies are now being planned to test these agents in patients awaiting transplantation or who have recurrence of hepatitis C after transplantation. (2007 0%; Total 0%)

B3a. Elucidate the pathogenesis of post-transplant lymphoproliferative disease (PTLD) and means of prediction, prevention, and control.

PTLD after liver transplantation typically represents primary Epstein-Barr virus (EBV) infection with high levels of viremia occurring in the face of immunosuppression. Withdrawal of immunosuppression reverses PTLD in some patients, and when that fails, rituximab (monoclonal antibody to CD20) is often effective, although several courses of treatment may be necessary to maintain remission. PTLD is still a cause of early and late morbidity and mortality after liver transplantation, and better means of prevention and treatment are needed (Avolio AW. *Transplant Proc* 2007; 39: 1956; Trappe R. *Transplantation* 2007; 83: 912). (2007 10%; Total 30%)

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. (2007 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.

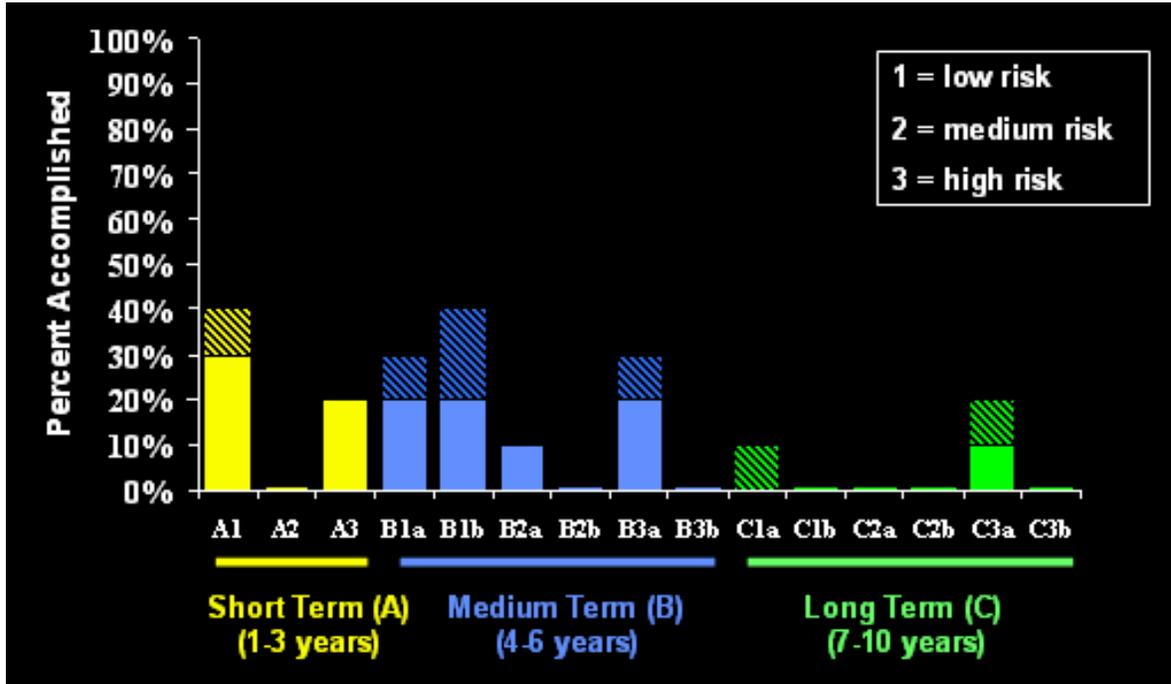
An NIH-sponsored meeting on February 12, 2007 specifically addressed this issue, entitled "Improving Long-Term Outcomes for Pediatric Liver Transplantation." Long-term follow-up and assessment of quality of liver in children enrolled in the SPLIT consortium showed that children who receive liver transplants have excellent psychological and physical functioning, but decreased general health (Sundram SS. *Am J Transplant* 2007; 7: 982). These findings will help to direct efforts at improving outcomes and quality of life in children undergoing liver transplantation. (2007 10%; Total 10%)

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. (2007 0%; Total 0%)

- C2a. Based upon molecular mechanisms, develop and assess tolerance-inducing regimens, including studies in children.** New approaches for induction of tolerance have been made a component of the A2ALL Consortium, and applications are now being accepted for prospective analysis of tolerance in children after transplantation. (2007 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. Future studies plan to include careful prospective collection of serum, liver, and DNA specimens from children undergoing liver transplantation with the objective of developing a repository to analyze biomarkers for tolerance. (2007 0%; Total 0%)
- C3a. Develop means to prevent recurrence of hepatitis C after liver transplantation.** Treatment of recurrent hepatitis C after transplantation has been assessed in a randomized controlled trial from Spain, which achieved reasonable response rates, though largely in patients with early stages of fibrosis (Carrión JA. *Gastroenterology* 2007; 132: 1746). Trials are underway in the A2ALL cohort study of peginterferon and ribavirin therapy in patients before transplantation aimed at prevention of recurrence, but development of new agents for hepatitis C is needed to fully address this need. (2007 10%; Total 20%)
- 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. (2007 0%; Total 0%)

Figure 14. Estimated Progress on Liver Transplantation Research Goals, 2007 (Year 3) [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, but recommendations have not been established for standardization of measurements and the role of hepatic vein pressure gradient determination. (2007 0%; Total 50%)
- A1b. Define whether N-acetylcysteine is beneficial in acute liver failure.** Two prospective, randomized, controlled trials have received NIH funding to study N-acetylcysteine (NAC) for non-acetaminophen induced acute liver failure. The trial in adults has been completed, and results will be published within the next year. The trial in children has enrolled more than half of the participants planned, and results will be available in two years. (2007 10%; Total 40%)
- A2. Better define the natural history of hepatopulmonary syndrome and whether early detection is beneficial.** Pulse oximetry can be easily and reliably used to screen for hepatopulmonary syndrome among patients with cirrhosis (with values less than 97% triggering a full diagnostic evaluation) (Arguedas MR. *Clin Gastroenterol Hepatol* 2007; 5: 749). Such screening is cost-effective (Roberts DN. *Liver Transpl* 2007; 13: 206). Portopulmonary hypertension is a rare complication of cirrhosis, but has a markedly adverse effect on outcome of liver transplantation. Vasodilation therapy with intravenous prostaglandin or inhaled prostacyclin analogues, with or without oral bosentan (an endothelin receptor antagonist), lowers pulmonary pressure in a proportion of patients, and those with a favorable response have excellent post-transplant outcomes (Hoeper MM. *Eur Respir J* 2007; 30: 1096; Ashfaq M. *Am J Transplant* 2007; 7: 1258). (2007 10%; Total 20%)
- A3a. More fully elucidate the pathophysiology of portal hypertension.** Portal hypertension results from an increase in hepatic resistance, partially due to sinusoidal vasoconstriction and partially due to increased hepatic fibrosis. Hepatic vasoconstriction in cirrhosis is characterized by both diminished production and reduced effects (resistance) of nitric oxide (NO), a major vasodilatory stimulus, as well as increases in vasoconstrictive pathways mediated by molecules such as endothelin. (2007 0%; Total 20%)
- A3b. Better characterize the cause of increased susceptibility to bacterial infections in cirrhosis.** Patients with cirrhosis have increased rates of bacterial translocation from the intestine and markedly increased susceptibility to systemic bacterial infections and spontaneous peritonitis. Translocation can be detected by testing for bacterial DNA in ascitic fluid and identifying patients with high risk for bacterial peritonitis (Francés R. *Clin Exp Immunol* 2007; 150: 230). During acute alcoholic hepatitis, neutrophils are defective in phagocytic and oxidative burst activity, which correlate with circulating endotoxin levels and are reversed with clinical improvement or neutralization of endotoxin (Mookerjee RP. *Hepatology* 2007; 46: 831). Means of

decreasing bacterial endotoxin exposure or inhibiting its effects in liver disease might improve clinical outcomes. (2007 10%; Total 10%)

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 randomized controlled trial from Spain has shown that prophylaxis of patients with cirrhosis and ascites (and low ascitic protein levels) using fluoroquinolones decreases the rate of bacterial peritonitis and improves survival (Fernández J. *Gastroenterology* 2007; 133: 818), indicating that antibiotic prophylaxis should be routinely used in this situation. In a small, prospective, randomized trial among patients with cirrhosis and minimal hepatic encephalopathy, lactulose improved health-related quality of life and cognitive function compared to no therapy, suggesting that patients with cirrhosis should be screened for hepatic encephalopathy and treated routinely (Prasad S. *Hepatology* 2007; 45: 549). For patients with severe hepatic encephalopathy, temporary improvement can be achieved with extracorporeal albumin dialysis, but it remains unclear whether this more expensive and invasive approach is cost-effective or improves survival (Hassanein TI. *Hepatology* 2007; 46: 1853). Improvements in thrombocytopenia in patients with cirrhosis can be achieved using eltrombopag, an oral thrombopoietin receptor agonist; this approach may be particularly helpful in improving platelet counts in preparation for surgery or during episodes of bleeding (McHutchison JG. *N Engl J Med* 2007; 357: 2227). These latter three approaches require more rigorous, placebo-controlled prospective study using meaningful endpoints to assess benefit. (2007 20%; Total 20%)

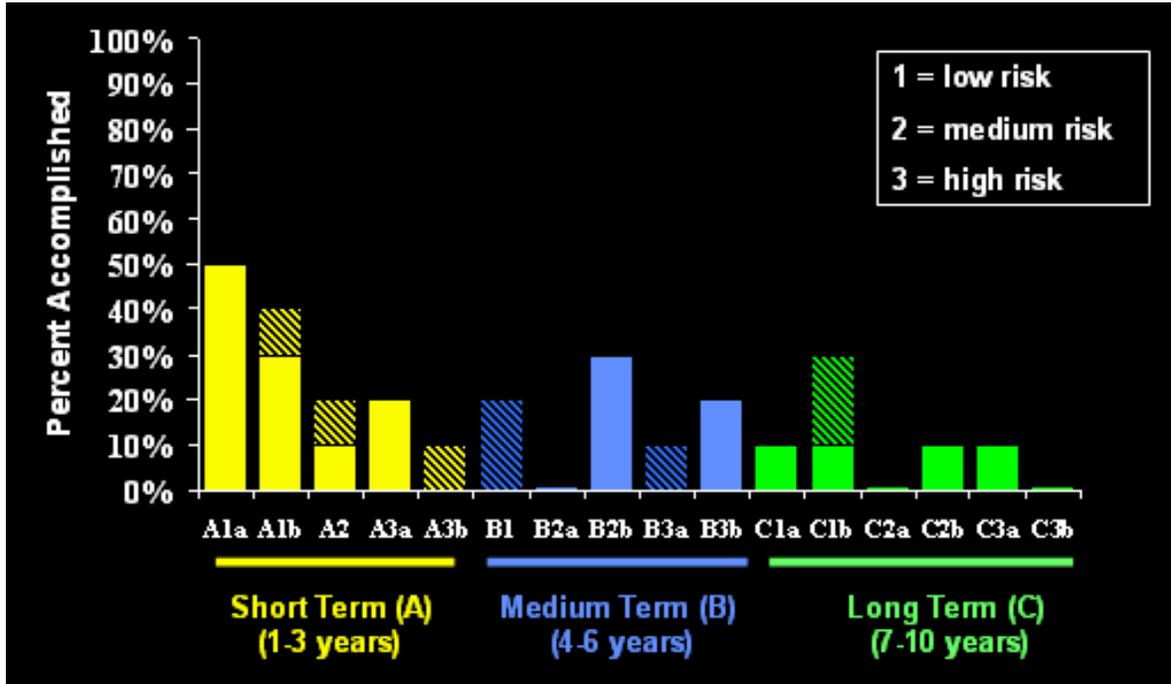
B2a. Define whether hypothermia is beneficial in acute liver failure for management of cerebral edema. A multi-center U.S. trial of hypothermia for acute liver failure has been designed by the adult Acute Liver Failure Study Group (ALFSG) and submitted for funding. The ALFSG has published guidelines for management of patients with acute liver failure; the effects of hypothermia were discussed, but no recommendations could be made for its routine use (Stravitz RT. *Crit Care Med* 2007; 35: 2498). (2007 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, but requires prospective critical evaluation for its reliability and accuracy in detecting progression of disease, particularly in comparison to simple clinical findings and laboratory tests (Lim JK and Groszmann RJ. *Hepatology* 2007; 45: 1087). (2007 0%; Total 30%)

B3a. Identify small molecule targets that would lead to better control of portal hypertension at different stages of disease. In a rat model of cirrhosis, atorvastatin lowered portal pressure and intrahepatic resistance, probably through activation of endothelial nitric oxide synthase via inhibition of a pathway known as Rho1/Rho-kinase, the activity of which is decreased by inhibition of cholesterol synthesis caused by statins. Prospective evaluation of effects of statins on complications of cirrhosis are warranted. (2007 10%; Total 10%)

- B3b. Develop a noninvasive means of measuring portal pressure.** Development of noninvasive devices for indirect or direct measurement of portal pressure is specifically encouraged in program announcements for small business grants (SBIR/STTR) entitled “New Technologies for Liver Disease” (PA-06-396/397). (2007 0%; Total 20%)
- C1a. Elucidate the optimal approach to manage patients with varices that have not bled (primary prevention).** Octreotide enhances the efficacy of beta blockade in reducing portal hypertension in patients with cirrhosis and deserves prospective study for efficacy in preventing complications such as bleeding (Vorobioff JD. *Am J Gastroenterol* 2007; 102: 2206). (2007 0%; Total 10%)
- C1b. Define whether monitoring portal pressure (HVPG) improves management of patients with chronic liver disease.** The reliability of HVPG measurement in assessing severity of fibrosis and predicting disease progression and hepatic decompensation has been documented in studies of acute alcoholic hepatitis (Rincon D. *Aliment Pharmacol Ther* 2007; 25: 841), compensated cirrhosis due to multiple causes (Ripoll C. *Gastroenterology* 2007; 133: 481), and recurrent hepatitis C after liver transplantation (Samonakis DN. *Liver Transpl* 2007; 13: 1305; Carrión JA. *Gastroenterology* 2007; 132: 1746). Such measurements should be incorporated into studies of therapy of advanced liver disease, and their clinical usefulness in patient management should be better defined. (2007 20%; Total 30%)
- 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). (2007 0%; Total 0%)
- C2b. Develop and evaluate better drugs for portal hypertension.** Better understanding is needed of the pathophysiology of portal hypertension and different stages of hepatic fibrosis and cirrhosis before new therapies can be attempted in this condition. (2007 0%; Total 10%)
- C3a. Develop an artificial or bioartificial hepatic support and demonstrate that it prolongs survival in acute liver failure.** Trials of artificial hepatic support using membrane albumin dialysis in acute liver failure are underway in Europe. (2007 0%; 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. Transient elastography can reliably predict clinically significant and severe portal hypertension, but is not accurate in predicting the presence of large varices (Vizzutti F. *Hepatology* 2007; 45: 1290). (2007 0%; Total 0%)

Figure 15. Estimated Progress on Complications of Liver Disease Research Goals, 2007 (Year 3) [Cross-hatching indicates recent year's progress.]



Chapter 14 Liver Cancer

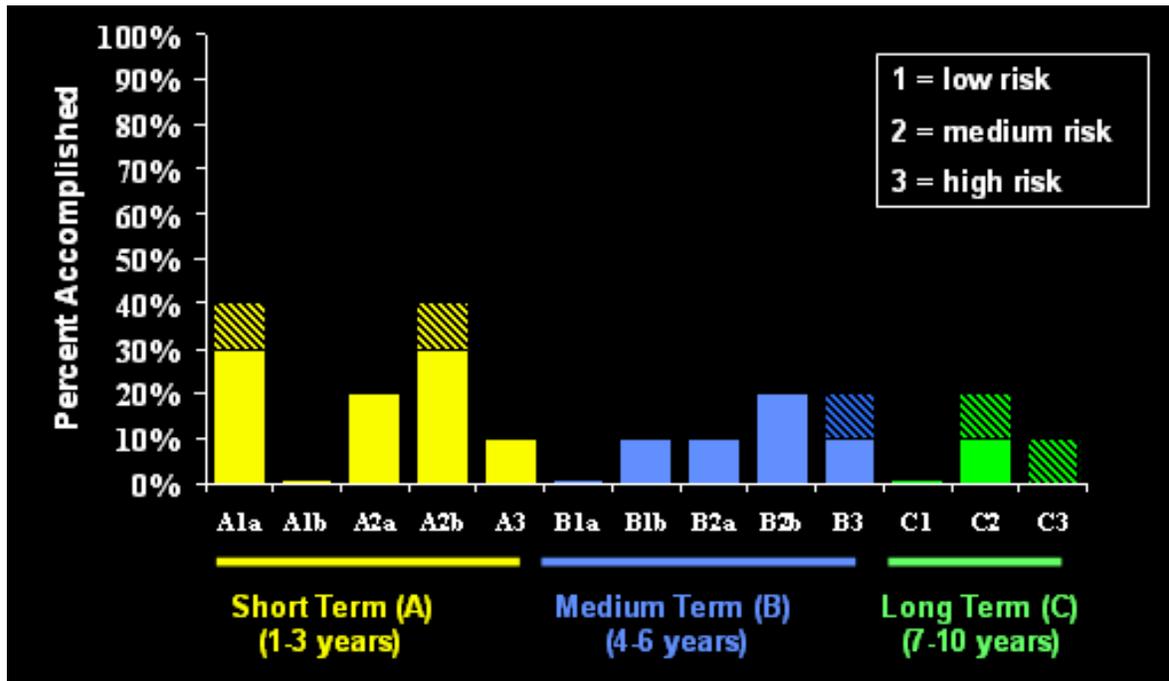
- A1a. Establish liver cancer serum and tissue bank.** Serum banks of samples from patients with early hepatocellular carcinoma (HCC) and liver disease controls are being established through several mechanisms, including the NIH-supported Early Detection Research Network (EDRN), the Hepatitis Antiviral Long-Term Treatment Trial against Cirrhosis (HALT-C) Trial, and HCC Genomic Consortium, all of which are being used to develop and validate early markers for liver cancer. The EDRN completed enrollment during 2007 with targets of collecting 190 early-stage HCC and 400 cirrhosis control samples of tissue, serum, and plasma. (2007 10%; Total 40%)
- A1b. Establish means of active surveillance of HCC in the United States.** Databases have been initiated by the American Society of Clinical Oncology and other academic hepatology groups. (2007 0%; Total 0%)
- A2a. Identify potential biomarkers for early HCC.** Gene expression arrays have provided many examples of genes that might serve as convenient biomarkers for HCC. Glypican-3 is highly expressed in HCC and usually undetectable in normal liver tissue (Kandil D. *Cancer* 2007; 111: 316). Serum assays for glypican-3 have been developed and are being evaluated for their sensitivity and specificity in identifying HCC. (2007 0%; Total 20%)
- A2b. Define the molecular signatures and heterogeneity of HCC and determine how they correlate with clinical features.** Analyses of gene expression arrays from liver tissue of patients with chronic hepatitis C ranging from cirrhosis to dysplasia to HCC have shown unique molecular markers of the individual stages of development of HCC, many of which might be appropriate targets for therapy or markers for early cancer (Wurmbach E. *Hepatology* 2007; 45: 938). Microarray analysis of gene expression in large numbers of patients with HCC has identified different patterns that correlate with clinical and etiological features of this cancer (Boyault S. *Hepatology* 2007; 45: 42). (2007 10%; Total 40%)
- 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. Markers such as glypican-3 might be used to help discriminate between tumorous and non-tumorous tissue in patients with cirrhosis or advanced fibrosis. (2007 0%; Total 10%)
- B1a. Demonstrate the relative efficacy, safety, and benefits of local ablative therapies for HCC.** A prospective, randomized, controlled trial from China compared radiofrequency ablation (RFA) alone to the use of both RFA and percutaneous ethanol injection simultaneously in patients with HCC mostly due to hepatitis B and found that the combination ablative approach yielded higher rates of tumor ablation and better long-term survival (Zhang YJ. *Radiology* 2007; 244: 599). (2007 0%; Total 0%)

- B1b. Develop standardized terms and nomenclature for diagnosis, staging, and grading of HCC.** The summary of a conference organized by the American Association for the Study of Liver Diseases (AASLD) on “Endpoints in Clinical Trials for Hepatocellular Carcinoma” has been prepared for publication and will appear in 2008. Prospective validation of these criteria are underway. (2007 0%; 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 EDNR is sponsoring a validation study of DCP. Results should be made available within the next year. (2007 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. Factors in patients with NASH that correlate with HCC development may help define the mechanism of carcinogenesis in fatty liver disease. (2007 0%; Total 20%)
- B3. Identify targets 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. Attractive targets are the cell signaling pathways of Wnt, β -catenin, Ras, which are all frequently altered in HCC (Boyault S. *Hepatology* 2007; 45: 42). The tumor suppressor gene *p53* is altered in many cancers including HCC; in mice, transient reactivation of *p53* can induce complete tumor regression (Xue W. *Nature* 2007; 445: 656). HCC is also characterized by disordered methylation of DNA, and, therefore, small molecules that alter methylation might affect tumor growth and progression (Calvisi DF. *J Clin Invest* 2007; 117: 2713). (2007 10%; Total 20%)
- C1. Demonstrate an effective strategy for prevention of HCC in high-risk populations.** The HALT-C trial and a similar study supported by industry (CoPilot) 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. In retrospective analyses, complete eradication of hepatitis C virus (HCV) by successful treatment using peginterferon and ribavirin appeared to improve survival and decrease the frequency of death from end-stage liver disease but did not decrease the rate of development of HCC, at least in the short term (Veldt BJ. *Ann Intern Med* 2007; 147: 677). (2007 0%; Total 0%)
- C2. Define the cellular and molecular pathways that lead to hepatocarcinogenesis.** Dysregulation of small non-coding RNAs (miRNAs) may play a role in human HCC. Microarray analyses indicate that miR-122a, the most common micro-RNA found in liver, is downregulated in the majority of cases of HCC. The target of miR-122a appears to be cyclin G1, higher levels of which are found in HCC and may lead to genomic instability (Gramantieri L. *Cancer Res* 2007; 67: 6092). In contrast, miR-21 levels are increased in HCC, and its target appears to be a tumor suppressor gene, *PTEN*, which is typically decreased in HCC (Meng F. *Gastroenterology* 2007; 133: 647). Large non-coding RNAs (ncRNA) are also found in the liver, but their function

and significance are not well defined. Recent results indicate that large ncRNAs are markedly elevated in rodent and human HCC and, therefore, may serve as molecular markers for neoplastic cells (Lin R. *Oncogene* 2007; 26: 851). Recent studies have identified disturbances of several other important pathways in HCC including the tumor suppressor gene *KLF6* (Kremer-Tal S. *J Hepatol* 2007; 46: 645), APE1/Ref-1 (Di Maso V. *Mol Med* 2007; 13: 89), and the p53 response to shortened telomeres (Feldser DM and Greider CW. *Cancer Cell* 2007; 11: 461). (2007 10%; Total 20%)

C3. Based upon molecular analyses, develop effective, noncytotoxic therapy for HCC. Small clinical trials have evaluated several non-cytolytic medications directed at intracellular targets involved in hepatocarcinogenesis. Agents that have been assessed in studies of advanced HCC with little evidence of effect on survival include octreotide and tamoxifen (Verset G. *Br J Cancer* 2007; 97: 582), erlotinib (a tyrosine kinase inhibitor; Thomas MB. *Cancer* 2007; 110: 1059), and cetuximab (a monoclonal antibody against epidermal growth factor receptor; Zhu AX. *Cancer* 2007; 110: 581), as well as more conventional chemotherapeutic regimens such as capecitabine (a prodrug of 5-FU) and oxaliplatin (Boige V. *Br J Cancer* 2007; 97: 862), as well as nolatrexed (a thymidine synthase inhibitor) (Gish RG. *J Clin Oncol* 2007; 25: 3069). The growth factor pathways of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and Raf-1 are the targets of the oral multi-kinase inhibitor sorafenib, which has been evaluated in a randomized controlled trial with promising results, which will be published within the next year. (2007 10%; Total 10%)

Figure 16. Estimated Progress on Liver Cancer Research Goals, 2007 (Year 3)
 [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.** Quantitative trait locus mapping has identified 23 *Lith* loci in mice, many of which have been linked to candidate genes related to bile canalicular transport and cholesterol metabolism (*Abcb11*, *Abcg5/8*, *Apobec1*, *Nr1h4*) (Lyons MA and Wittenburg H. *Gastroenterology* 2006; 131:1943). (2007 0%; Total 20%)
- A2. Develop small animal model for cholangiopathies that would allow analysis of effects of chronic necroinflammation on biliary epithelium.** The biliary tree can be disrupted by inflammation and fibrosis, and the role of biliary epithelial cells and myofibroblasts in this process can be studied using isolated cells in culture allowing for assessment of possible therapeutic agents (Choi HS. *J Surg Res* 2007; 141: 183). (2007 0%; 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.** Magnetic resonance imaging of the gallbladder has played an increasing role in diagnosis and management of gallbladder and biliary tract disease (Elsayes KM. *Acta Radiol* 2007; 48: 476). (2007 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.** An NIH-funded multicenter, prospective study entitled Evaluating Predictors and Interventions in Sphincter of Oddi Dysfunction (EPISOD) has been initiated, which is designed to enroll 214 number of patients at six U.S. centers in a prospective study of the clinical features, risk factors, natural history, and therapy of sphincter of Oddi dysfunction. (2007 10%; Total 30%)
- B2. Characterize the role of enterohepatic species of *Helicobacter* and other candidate bacteria in development of cholesterol gallstones in both mice and humans.** Infection with several *Helicobacter* species is associated with gallstones in mice. Recent studies demonstrate the gallstone formation is dependent upon intact T cell functioning, suggesting that inflammatory response to cholesterol crystals causes the development of gallstones and gallbladder disease (Maurer KJ. *Gastroenterology* 2007; 133: 1304). (2007 10%; Total 30%)
- B3. Identify plasma or urine markers for lithogenicity of bile using proteomics or metabolomics.** Cohort studies 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 a program announcement on “Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases” (PA 07-016). (2007 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, but several cross-sectional and case-control studies from Europe and Asia have identified the major risk factors for gallbladder and biliary tract carcinoma to be gallstone disease, obesity, and age (Ahrens W. *Eur J Gastroenterol Hepatol* 2007; 19: 623; Hsing AW. *Br J Cancer* 2007; 97: 1577). (2007 10%; Total 10%)

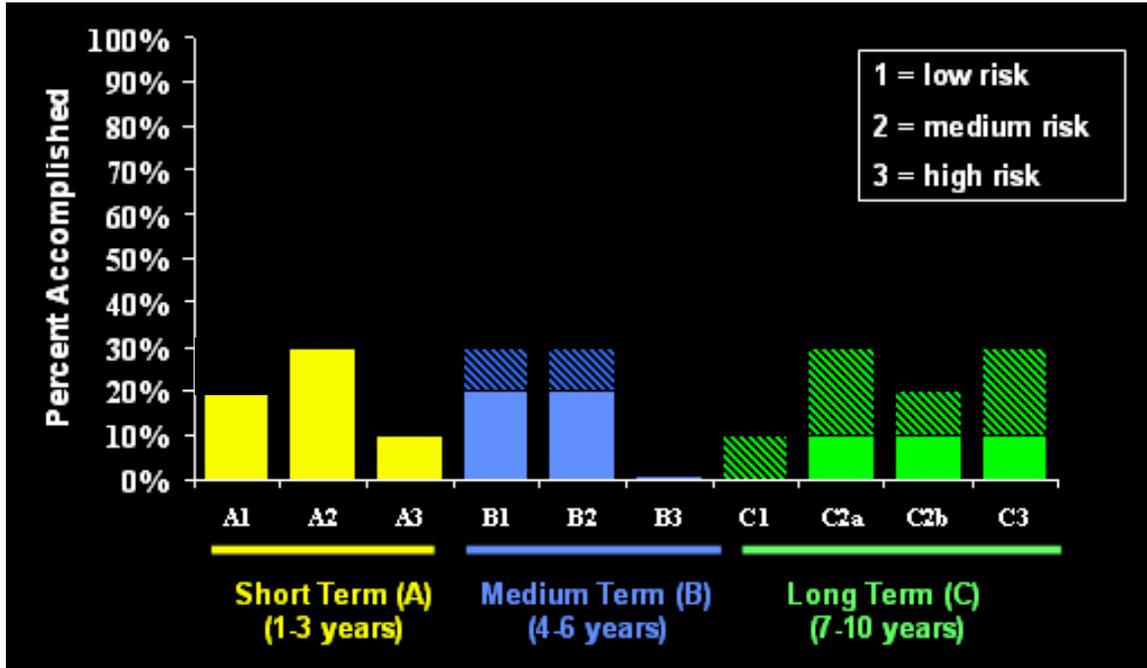
C2a. Identify at least 5 human *LITH* genes associated with increased risk of gallstones, based upon homology with murine genes and family studies.

Genome-wide scans and candidate gene approaches focusing on human homologues of murine Lith genes have linked specific polymorphisms in the human hepatic cholesterol transporter (ABCG5/G8) with increased susceptibility to cholesterol gallstones (Buch S. *Nat Genet* 2007; 39: 995; Grunhage F. *Hepatology* 2007; 46: 793). (2007 20%; Total 30%)

C2b. Develop noninvasive biomarkers for cholangiocarcinoma. A single center study from Italy reported elevated levels of insulin-like growth factor-I in bile from patients with cholangiocarcinoma, but not from those with pancreatic cancer or benign biliary tract diseases (Alvaro D. *Ann Intern Med* 2007; 147: 451). In contrast, serum levels of IGF-I and serum and biliary levels of other growth factors were similar in all groups. These results need further confirmation, but may provide a means of early detection of this tumor in high-risk patients. (2007 10%; Total 20%)

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 mice and humans have suggested potential novel approaches. Inhibition of cholesterol reuptake by hepatocytes and biliary epithelial cells by ezetimibe, which blocks the Niemann-Pick C1-like 1 protein (NPC1L1) transporter, decreases cholecystosteatosis in mice and may affect gallstone formation in humans (Mathur A. *Surgery* 2007; 142:228; Al-Azzawi HH. *J Gastrointest Surg* 2007; 11: 835). The finding that NPC1L1 is present in canalicular membranes, where it acts to allow retention of biliary cholesterol, reinforces the possibility that ezetimibe may prevent cholesterol gallstones (Temel RE. *J Clin Invest* 2007; 117: 1968). The role of decreased function of the canalicular cholesterol transporter (ABCG5/8), which has been linked to gallstone disease in genetic studies (Buch S. *Nat Genet* 2007; 39: 995) provides another potential target for prevention studies. Finally, epidemiologic research has suggested that increased iron consumption (Tsai CJ. *Am J Clin Nutr* 2007; 85: 518) and decreased physical activity (Kriska AM. *Med Sci Sports Exerc* 2007; 39: 1927) are associated with an increased risk of gallstone disease, suggesting potential nutritional and behavioral approaches to decreasing gallstone risk. (2007 20%; Total 30%)

Figure 17. Estimated Progress on Gallbladder and Biliary Disease Research Goals, 2007 (Year 3) [Cross-hatching indicates recent year's progress.]



Chapter 16

Liver Imaging and Biotechnology

- A1a. Develop standardized definitions, diagnostic criteria, and methodology for liver imaging.** This research need continues, as originally identified in a workshop entitled “Measurement of Hepatic Vein Pressure Gradient: Role in Management of Portal Hypertension” was held in June 2006. A major recommendation from this workshop was to standardize the procedure and methods for measuring the pressure gradient. (2007 0%; Total 10%)
- A1b. Better define the role, efficacy, and safety of image-guided local therapies for HCC, such as radiofrequency and thermal ablation.** Better imaging of cancer to allow for percutaneous radiofrequency ablation has been achieved using an integrated system with both real-time CT and ultrasound, yielding 90% response rates after a single session (Minami Y. *Oncology* 2007; 72 Suppl 1: 111). (2007 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) and the HALT-C trial, which will include early-stage HCC and cirrhosis control samples of tissue, serum, and plasma. Radiological images will also be included for the HCC cases. (2007 0%; Total 20%)
- A2b. Develop improved techniques for established imaging methods for liver disease, such as optical, MRI, or PET/CT scanning.** A system for optical imaging of the liver with fluorescent molecular probes has been developed in mice (Upadhyay R. *Radiology* 2007; 245: 523). (2007 0%; Total 30%)
- A3. Evaluate molecular imaging techniques in animal models of liver disease.** Intravital optical imaging was used to demonstrate initial entrapment and growth of colon cancer cells of varying propensity to form liver metastases in mice, allowing study of initial stages of metastasis formation (Kruskal JB. *Radiology* 2007; 243: 703). (2007 10%; Total 20%)
- 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. (2007 0%; Total 0%)
- B1b. Extend studies on validation to international populations.** This goal will follow development of definitions and diagnostic criteria. (2007 0%; Total 0%)
- B2. Develop bioinformatics such that computer-aided diagnostics are useful in evaluation of liver disease.** Integration of results from contrast-enhanced CT with results of gene expression arrays and response to doxorubicin in HCC was carried out using advanced bioinformatics, demonstrating that both gene array and tumor imaging correlate with response to chemotherapy (Kuo MD. *J Vasc Interv Radiol* 2007; 18: 821; Segal E. *Nat Biotechnol* 2007; 25: 675). (2007 10%; Total 20%)
- 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 warrant evaluation in humans with liver disease. (2007 0%; Total 0%)

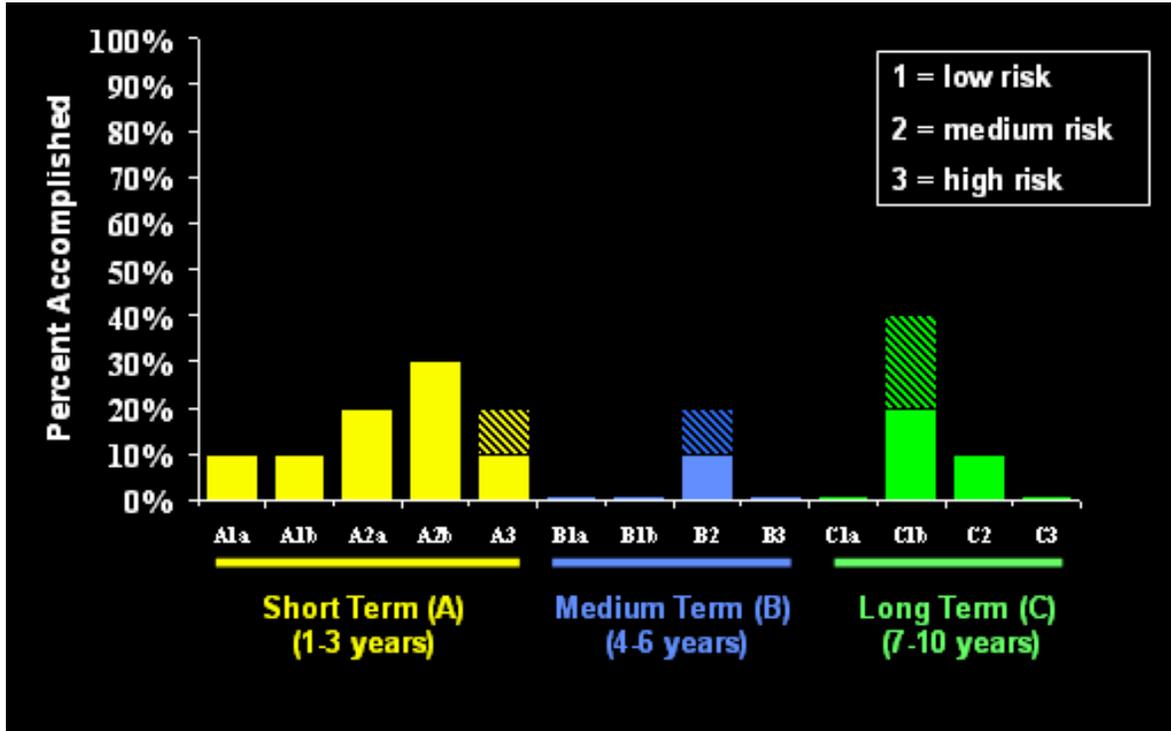
C1a. Apply definitions, criteria, and methodology for liver imaging as surrogate endpoints to therapy of liver diseases. Use of ultrasound elastography to assess hepatic fibrosis is gaining increasing acceptance as a means of screening and classifying patients based on the presence of advanced fibrosis (Barreiro P. *J Infect Dis* 2007; 195: 973), but it has not yet been used as a surrogate endpoint for therapy of chronic liver disease. (2007 0%; Total 0%)

C1b. Develop practical means of assessing liver (fat content, fibrosis, inflammation, functionality) for population-based studies. Ultrasound elastography has proven to have excellent sensitivity and specificity for identifying cirrhosis, but lesser reliability in identifying intermediate stages of fibrosis (Talwalkar JA. *Clin Gastroenterol Hepatol* 2007; 5: 1214). In preliminary studies, MR-based elastography has shown a higher degree of sensitivity and specificity than ultrasound in detecting earlier stages of fibrosis both in animal models (Yin M. *Magn Reson Med* 2007; 58: 346; Salameh N. *J Magn Reson Imaging* 2007; 26: 956) and in humans with chronic liver disease (Yin M. *Clin Gastroenterol Hepatol* 2007; 5: 1207; Huwart L. *Radiology* 2007; 245: 458). Other methods that may be useful in identifying cirrhosis and staging of fibrosis in the liver are ultrasound-based Doppler impedance of the spleen (Liu CH. *Clin Gastroenterol Hepatol* 2007; 5: 1199) and diffusion-weighted magnetic resonance imaging (Lewin M. *Hepatology* 2007; 46: 658). Improvements in accuracy of estimation of fat in the liver may be achieved using magnetic resonance gradient echo imaging (Liu CY. *Magn Reson Med* 2007; 58: 354). (2007 20%; Total 40%)

C2. Develop imaging techniques that are fully integrated into therapy of liver disease. Methods of assessing fat and fibrosis in the liver will need to demonstrate value as clinical endpoints for them to be fully integrated into patient care and treatment of liver disease. (2007 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. (2007 0%; Total 0%)

Figure 18. Estimated Progress on Liver Imaging and Biotechnology Research Goals, 2007 (Year 3) [Cross-hatching indicates recent year's progress.]



Conclusions

Thus, important advances in liver disease research have continued in 2007, the third year after publication of the trans-NIH Action Plan for Liver Disease Research. Progress is reported this year in each of the 16 topic areas and in 121 (56%) of the 214 research 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.

Some of the most exciting research advances identified in this area for 2007 include: (1) development of a transgenic mouse with a “humanized” liver, which has major implications for the study of many liver diseases, including viral hepatitis, fatty liver disease, drug-induced liver injury, liver transplantation, gene therapy, and genetic liver diseases; (2) further work on the human liver proteome project, which is providing a complete picture of the proteins of the liver, their structures, different forms, modifications, and interactions; (3) improved understanding of the pathways of interferon induction and resistance associated with hepatitis C virus infection; (4) development of animal models and elucidation of molecular pathways that are disturbed in fatty liver disease; (5) development of molecular assays for diagnosis of neonatal cholestatic syndromes; (6) demonstration that the availability of living donor liver transplantation improves survival of patients with end-stage liver disease; and (7) identification of genes associated with gallstone disease in humans—genes that are linked to cholesterol metabolism and open up new therapeutic possibilities.

This Progress Review will be used by the Institutes and Centers of the NIH, as well as other Federal Agencies and non-Federal entities involved with liver disease research, to plan initiatives for the year 2008 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 75-80% of persons with hepatitis C virus (HCV) genotypes 2 and 3, but in only 45-50% 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 II and III studies, and preliminary results indicate that combinations of these agents with peginterferon and ribavirin increase response rates to a degree. 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. Several small pilot studies have started on silymarin, and trials are being designed to test S-adenosylmethionine (SAME) and weight-loss agents, including the cannabinoid receptor 1 antagonist, rimonabant. Several of these approaches may also be applicable to alcoholic liver disease. Meanwhile, animal models of fatty liver disease are beginning to demonstrate the disturbances in intracellular signaling and metabolic pathways that lead to accumulation of fat in hepatocytes. These studies are likely to provide targets for better therapies of alcoholic and nonalcoholic fatty liver disease. Clearly, therapies for nonalcoholic steatohepatitis will likely be developed in the next few years; however, their degree of efficacy and general applicability will need 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 treating chronic hepatitis B in the United States and a seventh is likely to be approved in the next year. Licensed therapies include standard interferon alfa, 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 warrant careful prospective study. An NIH Consensus Development Conference on "Management of Hepatitis B" has been scheduled for October 2008. A long-term prospective database and resource to conduct clinical trials in hepatitis B has been planned for funding in 2008 (Hepatitis B Clinical Research Network), which will address several of the issues raised in the trans-NIH Action Plan for Liver Disease Research. Thus, there have been major advances in the therapy of hepatitis B and, with these additional research efforts, 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 currently is ultrasound-based transient elastography, which measures the degree of stiffness of the liver. Early, cross-sectional results of prospective studies have shown that elastography is reproducible and provides relatively reliable estimates of advanced fibrosis and cirrhosis. The presence of portal hypertension or varices is also predicted by elastography. The role of this technique in monitoring chronic liver disease and informing

decisions 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. Several genetic markers for gallstone development have been identified in mice, and homologues to these genes are now being identified in human populations. Most of the identified genes are involved in cholesterol absorption, metabolism, and secretion and, thus, may be 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 four ancillary studies directed at the etiology of biliary atresia, focusing on genetics, proteomics, gene expression arrays, and possible viral agents. The current understanding of etiology of biliary atresia was reviewed in an NIH research workshop on “Screening and Outcomes of Biliary Atresia,” a summary of which was published in 2007 (Sokol RJ. *Hepatology* 2007; 46: 566).

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 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 for nonalcoholic steatohepatitis, pediatric cholestatic syndromes, and drug-induced liver disease. For example, an international, NIH-sponsored workshop on “Drug-Induced Liver Injury: Standardization of Nomenclature and Causality Assessment” will be held December 1-2, 2008.

Goal 10. Decrease the mortality rate from liver disease. The ultimate goal of the trans-NIH Action Plan for Liver Disease Research is to decrease morbidity and mortality from liver and biliary disease in the United States. One difficulty in assessing this goal is the lack of consistently reliable data on the prevalence, incidence, and death rates from liver disease. A major source of information is the National Center for Health Statistics, CDC, and their yearly publication of Vital Statistics for the United States (available online at: <http://www.cdc.gov/nchs/deaths.htm>). Mortality rates in these reports are based upon death

records, which can be unreliable, but are consistent enough to measure trends. Another challenge is that Vital Statistics are published 2 to 3 years after data are collected, so that the most recent results currently available are preliminary data from 2005. 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 1, which includes the final data from 2004, but only the preliminary data for 2005.

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

Diagnosis	2000	2001	2002	2003	2004	2005*
Chronic Liver Disease and Cirrhosis	9.5	9.4	9.4	9.3	9.0	8.9
Viral Hepatitis	1.9	1.8	2.0	1.8	1.8	1.8
Malignant Neoplasms of the Liver and Intrahepatic Bile Ducts	4.7	4.7	5.0	4.9	5.1	5.2
Cholelithiasis and Disorders of the Gallbladder	1.0	1.0	1.0	1.0	1.0	1.0
Total: Liver and Biliary Disease	17.1	16.9	17.4	17.0	16.9	16.9
Total Number of Deaths	47,635	48,068	50,076	50,588	50,802	52,028

* Preliminary results.

Thus, the total numbers of deaths from liver and biliary disease in the United States has increased, but the age-adjusted death rate is stable. Death rates from chronic liver disease and cirrhosis are decreasing and are at an all-time low. However, death rates from cancers of the liver and bile ducts are increasing. Clear challenges are posed by these findings to pursue additional advances in liver disease research that lead to further decreases in death rates from chronic liver disease and cirrhosis, but, importantly, to also make inroads into the prevention, early identification, and therapy of hepatocellular carcinoma.

Appendix 1. Acronyms Used in This Progress Review

A2ALL	Adult-to-Adult Living Donor Liver Transplantation Cohort Study
AACTG	Adult AIDS Clinical Trials Group
AASLD	American Association for the Study of Liver Diseases
ABC	ATP-binding cassette
AFP	alpha-fetoprotein
AIP	acute intermittent porphyria
ALFSG	Acute Liver Failure Study Group
Anti-HBs	antibodies against hepatitis B surface antigen
apo	apolipoprotein
ARPKD	autosomal recessive polycystic kidney disease
ATP	adenosine triphosphate
BARC	Biliary Atresia Research Consortium
BMP	bone morphogenetic protein
BSEP	bile salt export pump
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
CYP	cytochrome P450
DCP	des-gamma-carboxy prothrombin
DDICC	Digestive Diseases Interagency Coordinating Committee
DILIN	Drug-Induced Liver Injury Network
EBV	Epstein-Barr virus
EDRN	Early Detection Research Network
EGF	epidermal growth factor
EPISOD	Evaluating Predictors and Interventions in Sphincter of Oddi Dysfunction
EPP	erythropoietic protoporphyria
ERK	extracellular signal-regulated kinase
FAH	fumarylacetoacetate hydrolase
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FXR	farnesoid X receptor
FY	fiscal year
GDF	growth differentiation factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GRP	gastrin-releasing peptide
HALT-C	Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial
HAV	hepatitis A virus
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBV-OLTS	Hepatitis B Orthotopic Liver Transplantation Study

HCC	hepatocellular carcinoma
HCIG	hepatitis C immune globulin
HCV	hepatitis C virus
HDV	hepatitis D virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HLPP	Human Liver Proteome Project
HNF	hepatocyte nuclear factor
HRSA	Health Resources and Services Administration
HURSO	High Dose Ursodiol for Primary Sclerosing Cholangitis study
HVPG	hepatic venous pressure gradient
IFN	interferon
IGF-I	insulin-like growth factor-I
IL	interleukin
ILK	integrin-linked kinase
INR	international normalized ratio
IP3	inositol 1,4,5-trisphosphate
IRG	initial review group
LABS	Longitudinal Assessment of Bariatric Surgery
LADR	Low Dose Accelerating Regimen study (of A2ALL)
Mcl-1	myeloid cell leukemia 1
MDR	multi-drug resistance protein
MELD	Model for End-stage Liver Disease
MRI	magnetic resonance imaging
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
ncRNA	non-coding RNA
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
NMR	nuclear magnetic resonance
NO	nitric oxide
NPC1L1	Niemann-Pick C1-Like 1 protein

NTCP	Na ⁺ /taurocholate co-transporting polypeptide
OATP	organic anion transporter
ODS	Office of Dietary Supplements
ORD	Office of Rare Diseases
ORWH	Office of Research on Women's Health
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
PDGF	platelet-derived growth factor
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
PGC	PPAR γ coactivator
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
RFA	request for applications
SAMe	S-adenosylmethionine
SBIR	Small Business Innovation Research Program
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
SOCS	suppressor of cytokine signaling
Sod	superoxide dismutase
SPLIT	Study of Pediatric Liver Transplantation
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
TLR	toll like receptor
TNF- α	tumor necrosis factor- α
TPN	total parenteral nutrition
VEGF	vascular endothelial growth factor
Virahep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C
VLDL	very low density lipoprotein