Drug-Induced Liver Injury Network (DILIN)

A Multi-Center, Longitudinal Study of Drug- and CAM-Induced Liver Injury

Protocol

Compiled by:

The DILIN Research Group

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Distributed by the DILIN Data Coordinating Center:

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PROTOCOL CHANGES FROM VERSION 2.6 TO VERSION 2.7

In this section, changes to Version 2.6 to Version 2.7 are described. This excludes corrections for simple typographical and grammatical errors.

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The title of Project Officer is corrected to Project Scientist and Program Official is added and is represented by Patricia Robuck. Huiman Barnhart, Ph.D., has replaced James Rochon, Ph.D., as the Principal Investigator at the Data Coordinating Center.

Table of Contents

The Table of Contents was update throughout in the removal of controls from the protocol.

Controls

The enrollment of controls in the protocol has been clarified. Any reference to the enrollment of controls patients in the protocol has been deleted. This involves the deletion of former Sections 5, 7.3, 10, 10.1, 15, 15.3, and 15.5 of the protocol. Former Sections 1.0, 7.4, 11.1, 12.2 have been revised accordingly because of the deletion of controls from the protocol.

Data Management Procedures

Section 1.0 under Data Management Procedures in the Executive Summary and Section 13.3.2 have been revised due to the conversion from a paper CRF to electronic case report forms. Data was migrated from the ClinTrial database system to Oracle instead, data is now entered into the InForm ™ eCRF by personnel at the clinical centers. Any out-of-range values and missing or inconsistent key variables can be addressed at the site in real time during the data entry process.

Statistical Considerations

Section 1.0 under Statistical Considerations in the Executive Summary and Section 14.1 and 14.2 have been revised as to population controls. Sample size and power calculations will be carried out based on the proposed genetic analyses using existing population control subjects.

Study Administration

Former section 15.1 on Cooperative Agreement Mechanism is removed and the new section 15.3 describes the role of Data and Safety Monitoring (DSMB) to indicate that data and safety will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of DILIN studies. The description of DSMB activities is included in the DSMB Charter.

PROTOCOL CHANGES FROM VERSION 2.5 TO VERSION 2.6

In this section, changes to Version 2.5 of the protocol to create Version 2.6 are described. This excludes corrections for simple typographical and grammatical errors.

Liver Biopsy:

Previous versions of the protocol were imprecise concerning liver biopsy. Liver biopsy is not mandated by this protocol and is not a required study procedure. Biopsy material is only captured opportunistically, i.e., if a participant has had a liver biopsy in the past, if one is electively planned for diagnostic purposes, or if an autopsy is performed. Section 11.4 further described the complications due to liver biopsy implying that this is an adverse event related to study procedures. This is incorrect.

The following sections in the protocol have been changed to make clear that biopsy is not a mandated DILIN procedure:

- Executive Summary (Section 1)
- Evaluations Performed at the Initial Study Visit (Section 8.2)
- Evaluations at the Twelve- and Twenty-Four Month-Follow-up (Section 9.3)
- Adverse Events Related to Study Procedures (Section 11.4)
- Schedule of Evaluations (Section 17.1)

In addition, the informed consent templates for cases have been changed to reflect this clarification.

Controls:

The protocol has been clarified regarding the enrollment of controls into the study. Controls for any specific drug may be identified from the general population, computerized databases with pharmacy records and ICD-9 diagnostic codes, general medical clinics, or subspecialty clinics located near the same DILIN clinical center as the case to minimize potential referral bias. The decision on what type of control to enroll is unclear at this point and will be determined by the DILIN Steering Committee.

The following paragraph has been added to Section 5:

> "Whether population controls or specific drug-treated controls will be required for each of the selected drugs is unclear at this time and is debated by experts. For example, most people in the US have probably been treated with a course of amoxicillin/clavulonate so it is logical that carefully selected population controls may be sufficient for genetic analyses. The DILIN Steering Committee will determine whether drug treated controls are appropriate for each drug."

Volume of Blood for During Follow-up Visits (Sections 9.1 and 9.3):

The volume of blood collected for diagnostic and standard blood studies has been shown explicitly in these sections.

> "Up to 75 ml of blood from adult cases and up to 30 ml of blood from pediatric cases will be required for these diagnostic and standard blood studies (depending on what tests had already been performed prior to this visit)."

In addition, the informed consent templates for cases have been changed to reflect this clarification.

PROTOCOL CHANGES FROM VERSION 2.4 TO VERSION 2.5

In this section, changes to Version 2.4 of the protocol to create Version 2.5 are described. This excludes corrections for simple typographical and grammatical errors.

Exclusion Criteria (Section 7.1):

- Allogeneic bone marrow transplant has been added as an exclusion criterion.

Evaluations During the Initial Study Visit (Section 8.2):

A number of changes have been made to the data collected during the initial study visit and include the following:

- For patients with suspected CAM hepatotoxicity, a limited quantity of each product will be stored at the initial study visit for future use.
- The dose and indication for medications other than the implicated medication will not be recorded. Only the start date and stop date of these medications will be captured. Similarly for CAM products such as herbal products and dietary supplements other than the implicated products.
- The section on risk factors for acquiring hepatitis and HIV disease has changed to the following:

  HIV / Hepatitis Risk Factors: risk factors that could have resulted in the subject acquiring HIV or hepatitis disease, e.g., animal contacts, travel history, eating habits, injectable drug use, transfusion, etc.
- Up to 4 values of ANA and ASMA diagnostic serologies drawn prior to the initial study visit will be collected.
- Retrospective data starting 8 weeks prior to starting the implicated DILI medication will be restricted to: AST, ALT, alkaline phosphatase, serum total bilirubin, INR, hemoglobin, WBC, %eosinophils, platelets, serum creatinine, albumin and serum direct bilirubin.
- Causality assessment will only be undertaken after the 6-month follow-up data are available. This has been removed from this section and moved to Section 9.2. Similarly, the description of the clinical narrative and the chronic DILI assessment have also been moved to Section 9.2.
- Medwatch reporting to the FDA will only occur after the data have been cleaned and the clinical narrative has been received. This has been moved to Section 9.2.

Blood Draw for Future Mechanistic Studies (Sections 8.2, 9.1, 9.3 and 11.1):

These sections have been changed as follows. For adult cases only, an additional 40 ml of whole blood will be drawn and sent to the Rutgers University Cell and DNA Repository (RUCDR) for future mechanistic studies. Samples will be collected at the initial study visit and follow-up visits at Months 6 and 12 (but not Month 24).

- The corresponding “Research Blood Samples” paragraphs in Sections 8.2, 9.1, and 9.3 as well as Section 11.1 have been revised accordingly.
- The Informed Consent and HIPAA Authorization templates for adult cases (Section 18.4) have been revised to reflect the new total blood volume.

Causality Assessment (Section 8.3):

- Procedures for causality assessment have been moved to Section 9.2.

Evaluations During the Month 6 Study Visit (Section 9.1):

- All medications, including prescription and OTC medications, and CAM products taken by the participant since the initial study visit will be recorded. However, the dose and indication will not be recorded. Only the start date and stop date of these medications will be captured.

- A limited quantity of the implicated CAM product will only be collected at the initial study visit.
- The results from all liver function tests performed since the initial study visit will be recorded including AST, ALT, alkaline phosphatase, serum total bilirubin, and INR.
- Diagnostic Studies: Anti-nuclear antibody and anti-smooth muscle antibody will be performed from the blood sample drawn from participants at the 6-month study visit.
- Risk factors for acquiring HIV disease including sexual contacts, injectable drug use, transfusion, hemophilia transfusions will not be collected and has been removed
- Causality assessment will only be undertaken after the 6-month follow-up data are available. This has been removed from this section and moved to Section 9.2. Similarly, the description of the clinical narrative and the chronic DILI assessment have also been moved to Section 9.2.

Causality Adjudication (Section 9.2):

- A new section has been created summarizing the causality process. It includes a description of the clinical narrative, chronic DILI assessment, the causality adjudication process, and Medwatch reporting.

Evaluations During the Month 12 and 24 Study Visits (Section 9.3):

- All medications, including prescription and OTC medications, and CAM products taken by the participant within 4 weeks of the visit will be recorded. However, the dose and indication will not be recorded. Only the start date and stop date of these medications will be captured.
- The results from all liver function tests performed since the previous study visit will be recorded including AST, ALT, alkaline phosphatase, serum total bilirubin, and INR.

Evaluations with Controls During the Initial Study Visit (Section 10.1):

- The section on risk factors for acquiring hepatitis and HIV disease has changed to the following:

  HIV / Hepatitis Risk Factors: risk factors that could have resulted in the subject acquiring HIV or hepatitis disease, e.g., animal contacts, travel history, eating habits, sexual contacts, injectable drug use, transfusion, etc.

Hardware and Software Configuration (Section 14.1):

- The InForm™ software will be used for web-based data entry.

Sources of Data (Section 14.2):

- Basic clinical information, e.g., demographic information, will be abstracted from the participant's medical records and charts. They will be entered into the database using the web-based data entry system.

Data Management Activities (Section 14.3):

- This section has been completely re-written to reflect the web-based data entry system.

Schedule of Evaluations (Section 18.1):

- Small changes have been made to this section to reflect accurately the timing of the data collected in this study.

PROTOCOL CHANGES FROM VERSION 2.3 TO VERSION 2.4

In this section, changes to Version 2.3 of the protocol are described. This excludes corrections for simple typographical and grammatical errors.

Section 8.2:

The following language has been inserted into the beginning of this section:

> "As far as possible, the following data will be collected from DILI cases during the initial study visit. It is recognized, however, that there will be some cases in which obtaining complete data is not possible."

The following changes were made to the diagnostic procedures:

- HCV RNA by PCR will not be restricted to cases in which the anti-HCV is negative and identified parenteral risk factor in 6 months prior to the date of onset of the DILI injury.
- anti CMV IgM has been added to the list.

Sections 8.2, 9.1, 9.3 and 11.1:

For adult cases only, an additional 34 ml of whole blood will be drawn into 4 x 8.5 ml ACD Vacutainer tubes. The blood tubes will be sent immediately at room temperature to the Rutgers University Cell and DNA Repository (RUCDR) for PBMC isolation for future mechanistic studies.

- The corresponding "Research Blood Samples" paragraphs in Sections 8.2, 9.1, and 9.3 as well as Section 11.1have been revised accordingly.
- The Informed Consent and HIPAA Authorization template for adult cases (Section 18.4) have been revised to reflect the new blood volume.

Section 14:

The electronic data capture (EDC) described in earlier versions of the protocol has been changed to a paper-based, case report form (CRF) approach.

- This section has been revised to reflect this new approach. The corresponding section in the Executive Summary has been revised accordingly.

Appendix 18.4 – Informed Consent and HIPAA Authorization Templates:

Clarifications have been made to these templates to reflect more accurately what biological samples are being shipped and stored at the different locations. Specifically, for adult cases, the language now reads:

> "First, your blood sample will be sent to Rutgers University Cell and DNA Repository where genetic information will be extracted. This genetic information will be stored for 20 years. Second, your urine, serum, and plasma samples as well as one part of your liver biopsy will be sent to Fisher BioServices (formally McKesson Health Solutions) where they will be stored for future use. The storage locations will be forwarded to a Data Coordinating Center at the Duke Clinical Research Institute."

Similar clarifications were made to the templates for adult controls as well as those for pediatric cases and controls.

PROTOCOL CHANGES FROM VERSION 2.2 TO VERSION 2.3

In this section, changes to Version 2.2 of the protocol are described. This excludes corrections for simple typographical and grammatical errors.

Section 7.2:

- A formal definition of the "date of onset" has been added. It is defined as follows: "The date of onset is defined as the date of the first qualifying lab value(s) on or after the date on which participant started taking the implicated DILI medication."

Section 8.2:

This section describes the evaluations performed during the initial study visit.

- Under the Medical History, prior surgical history will be captured within the past 5 years (unless the implicated DILI medication is a general anesthetic).
- Under Diagnostic Blood Studies, AMA is collected for cholestatic and mixed-pattern cases only.
- Under Research Blood Samples, the NIDDK Biosample Repository is located at Fisher BioServices (formerly called McKesson BioServices)

Section 9.1:

This section describes the evaluations performed at the 6-month follow-up visit.

- For patients who become HIV+ since the baseline visit, a detailed log of all antiretroviral medications will be captured from the date of the initial study visit (only).
- Under Research Blood Samples, the NIDDK Biosample Repository is located at Fisher BioServices (formerly called McKesson BioServices)

Section 9.3:

This section describes the evaluations performed with chronic DILI patients at Months 12 and 24.

- Follow-up is restricted to these two time points only.
- This section has been re-written to make it more consistent with the evaluations performed at the 6-Month visit. This includes changes and clarifications to the following: interval medication history, dose, duration, and indication for CAM products, physical exam, alcohol use questionnaire, smoking history, standard blood studies, imaging studies, and the data collected for HIV+ patients.
- Under Research Blood Samples, the NIDDK Biosample Repository is located at Fisher BioServices (formerly called McKesson BioServices)

Section 10.1:

This section describes the evaluations performed with DILI controls.

- Under Research Blood Samples, the NIDDK Biosample Repository is located at Fisher BioServices (formerly called McKesson BioServices)

Section 11.2:

This section describes the DNA and plasma extraction activities

- Under Plasma Separation, the NIDDK Biosample Repository is located at Fisher BioServices (formerly called McKesson BioServices)

Section 18.4:

This section provides template ICFs for all patient categories in this trial.

- With Fisher BioServices (formally called\ McKesson BioServices/Health Solutions) (pages 61, 69, 75, 89)

PROTOCOL CHANGES FROM VERSION 2.1 TO VERSION 2.2

In this section, changes to Version 2.1 of the protocol are described. This excludes corrections for simple typographical and grammatical errors.

General:

There was some ambiguity concerning the word "baseline" in this study. The name for the "baseline" study visit has been changed to the "initial study visit" throughout. "Baseline" now refers to the 12-month period prior to starting the implicated DILI medication.

Section 2.7:

- The study will include HIV-positive subjects. This new section has been added providing the background and significance of hepatotoxicity in this disease group.

Section 3 and Section 8.1:

- The timing of entry into the study has been changed from 6 months following presentation to a health care professional to within 6 months of the date of onset of the liver injury.

Section 4.2:

A new section has been added providing a formal definition for chronic drug-induced liver injury.

Section 8.2:

A number of clarifications, additions, and deletions have been made to the data collected from cases during the initial study visit.

- Demographics: include country of birth and remove employment status.
- Implicated DILI Medication: A careful assessment of adherence to medication regimens in general and for the implicated DILI medication in particular will be collected.
- Implicated CAM Product: if a CAM product is implicated as causing the liver injury, then detailed medication history of this product will be collected.
- Other Medication History: will be collected starting 8 weeks prior to starting the implicated DILI product and proceeding to the date of the initial study visit.
- CAM products: The dose, duration, and indication for a limited number of herbal/ CAM medications will be queried starting 8 weeks prior to starting the implicated DILI product and proceeding to the date of the initial study visit.
- Medical history: medical history will be reviewed over the lifetime of the subject including major medical illnesses and personal history of allergies to other medications, systemic autoimmune disorders (e.g., lupus, arthritis); diabetes / endocrine disorders; infectious diseases; heart disease and congestive heart failure; hypotension and hypertension; renal, pulmonary and gastrointestinal diseases; prior surgical history; prior history of liver problems; and, a previously experienced drug-induced liver injury. Liver-specific diseases over the lifetime of the subject, e.g., HCV, HBV, alcohol-related liver disease, non-alcoholic fatty liver disease, Wilson disease, hemochromatosis, Gilbert's syndrome, cirrhosis, ischemic hepatitis, organ transplantation.
- Family History: Vital status and demographics of all first-degree relatives including biological parents, all siblings, and biological children, together with a history of liver reactions to drugs as appropriate; for twins and multiple births, further information concerning zygosity.
- Smoking history during the 5 years prior to starting the DILI medication.
- Alcohol history: during the 5 years prior to starting the DILI medication
- Diagnostic blood studies: the following tests have been added: Anti-HBc IgM, anti-HBs, HBeAg, Anti-HBe, HBV-DNA, and Anti-HDV. All tests must have been performed in the 6 months prior to the date of onset of the DILI injury.

- HIV / Hepatitis Risk Factors: any risk factors that could have resulted in the subject acquiring HIV disease including sexual contacts, injectable drug use, transfusion, hemophilia transfusions, etc.
- Hepatitis B Patients: the following information will also be obtained starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study visit: HBV DNA by quantitative PCR, HBeAg, anti-HBe, anti-HDV; and, and a detailed log of all hepatitis medications.
- Hepatitis C Patients: the following information will also be obtained starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study visit: a quantitative HCV RNA level; and, and a detailed log of all hepatitis medications.
- HIV-Positive Patients: for patients chronically infected with HIV, additional data will be obtained including the presence of CMV, herpes simplex, syphilis, and MAI co-infections; detailed summary of serum lactate, amylase, lipases and CPK levels; HIV RNA and CD4 counts that will assist causality assessment; and a detailed log of all antiretroviral medications starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study.
- Blood Tests: Serum iron, serum transferrin, serum ferritin, serum alpha-1 antitrypsin, serum protein electrophoresis; serum IgM, IgG and IgA will be recorded if available but will not be specifically drawn for research purposes.
- Standard blood studies: This section has been revised as follows:

  The results from two sets of blood tests will be obtained. The first set consists of retrospective data starting 8 weeks prior to starting the implicated DILI medication and proceeding up to but excluding the date of the initial study visit. Results from the following tests will be obtained: complete blood count with platelets and manual differential, blood urea nitrogen, serum creatinine, sodium, potassium, serum total protein, serum albumin, AST, ALT, alkaline phosphatase, serum total bilirubin, INR and prothrombin time, total cholesterol and triglycerides.

  The second set will be derived from a blood sample drawn from participants at the initial study visit. These samples should be drawn in the fasted state whenever possible and indicate if fasting or fed on the data collection form. The following tests will be performed: complete blood count with platelets and manual differential; blood urea nitrogen and serum creatinine; sodium, potassium, serum total protein and serum albumin; AST, ALT, alkaline phosphatase, serum total bilirubin and serum direct bilirubin, INR and prothrombin time; total cholesterol, triglycerides, serum amylase, lipase, CPK, GGTP and LDH; and a urinalysis.

  Up to 75 ml of blood from adult cases and up to 30 ml of blood from pediatric cases will be required for these diagnostic and standard blood studies (depending on what tests had already been performed prior to the initial study visit).

- Research blood samples: The amount of blood drawn from cases for research purposes has been increased from 37 ml to 47 ml. This paragraph now reads as follows:

  "Research blood samples: In addition to these amounts, 47 ml of whole blood will be drawn in a fasted state from adult and pediatric cases for research purposes: 37 ml of whole blood will be obtained for DNA isolation, plasma, and PBMC cryopreservation for future genetic studies as described in Section 10.1 below; and, 10 ml of blood will be collected using a red topped plastic tube for serum storage. The serum isolated from this blood draw will be centrifuged, aliquoted into cryovials, and frozen at the clinical site. They will be shipped in bulk on dry ice to the NIDDK Biosample Repository at McKesson BioServices for future use."

- Signs and Symptoms at Onset: This section has been added and includes: jaundice, nausea, anorexia, dark urine, fever, abdominal pain vomiting, rash, itching, change in mental status, ascites, edema, hepatomegaly, splenomegaly, and lymphadenopathy.
- Imaging studies: Data to be captured from the liver imaging studies has been revised as follows: the presence of biliary dilatation, ascites, liver mass, gallstones, nodular contour of the liv-

er, intra-abdominal varices, splenomegaly, and hepatomegaly via yes / no response. The maximal spleen diameter in cm will also be recorded if available. Data regarding the morphology and contour of the liver surface or liver parenchyma will not be recorded.

- Quality of life form: the Rand 36-Item Health Survey will be self-administered to all adult subjects; the PedsQL will be used for children.
- History of the Liver Injury: section has been added, including the following: seen by a gastroenterologist / hepatologist; pregnant during the event, extrahepatic manifestations, hospitalized, rechallenged, liver transplantation, biopsy, received prednisone or other corticosteroids; and, how long the patient was sick with the liver injury, and how long was the disruption in daily living.
- Clinical narrative: The narrative will include the following information: details surrounding the presentation, names of the implicated products, other medications, past medical history, pertinent family and social history, physical exam, laboratory studies, diagnostic studies, and a summary of any clinical events following presentation.

Sections 9.1 and 9.3

Similar changes have been made to the data collection protocol at Months 6, 12 and annually thereafter. In addition, we record whether a liver transplantation was performed, whether the subject died, and the dates of these events.

Section 10:

- A new section has been added to the beginning of this section clarifying how data will be collected from the control subjects. The following paragraphs have been added:

  "As described in Section 5, up to three age-matched controls will be individually matched to each standard DILI case. Controls will not be sought for CAM cases or for cases with chronic liver disease as defined in Section 7.3. Each control must have an exposure to the implicated DILI drug for as long or longer as that for the case patient, but with no evidence of severe liver injury during this exposure interval.

  "Because of the individual matching, the time frames for data collection for any control mimic those of the case to which s/he is matched. That is, we collect exposure information for cases during the "pre-drug interval" leading up to the start of the DILI medication. The "pre-drug interval" for the matched control is an interval of the same length of time leading to when s/he started taking the DILI medication. Moreover, the "post-drug exposure interval" for any case is the elapsed time from starting the DILI mediation until the onset of the liver injury. The "post-drug exposure interval" for the control is the same duration of time following the date when s/he started the DILI medication. Thus, the lengths of the two exposure intervals for the control are the same as those of the case to which s/he is matched."

Section 10.1:

Changes similar to those described above for the data collection protocol for the cases at the initial visit have been made to the data collection protocol for the controls. In particular, the following change has been made:

- The amount of blood drawn from controls for research purposes has been increased from 37 ml to 47 ml. This paragraph now reads as follows:

  "Research blood samples: In addition to these amounts, 47 ml of whole blood will be drawn in a fasted state from adult and pediatric cases for research purposes: 37 ml of whole blood will be obtained for DNA isolation, plasma, and PBMC cryopreservation for future genetic studies as described in Section 11.1 below; and, 10 ml of blood will be collected using a red topped plastic tube for serum storage. The serum isolated from this blood draw will be centrifuged, aliquoted into cryovials, and frozen at the clinical site. They will be shipped in bulk on dry ice to the NIDDK Biosample Repository at McKesson BioServices for future use."

Section 11.1:

- Provision has been for an additional blood draw if the DNA yield from the initial blood draw is less than 50ug DNA/ml blood. The second paragraph has been added as follows:

  "Specifically, two 10ml NaEDTA (lavender top) and two 8.5ml ACD (yellow top) tubes will be drawn. If the initial DNA yield from the submitted whole blood sample is less than 50μg DNA/ml blood, the participant will be requested to return for a repeat blood draw. At that time, two additional 10 ml NaEDTA (lavender top) and two additional 8.5ml ACD (yellow top) tubes will be drawn. These samples will be sent directly to Rutgers without refrigerating, freezing, or delay for immediate DNA extraction and in cases of low DNA yield for cell line immortalization."

Appendix 18.4 – Informed Consent for Adult Cases:

- In the section entitled, "What will happen if you take part in this study?," paragraph No. 4 has been updated to reflect the increase in the amount of blood and the possibility of a subsequent draw when the amount of DNA extracted is inadequate. The is paragraph has been revised as follows:

  "4. You will have blood drawn and a urine sample collected for standard laboratory tests and for research purposes. Blood will be drawn by a qualified person who will obtain a little more than 8 tablespoons of blood (122 ml) from a vein in your arm. Very rarely, we may need to contact you for a second (subsequent) blood draw. In this case, an additional 3 tablespoons (37 ml) of blood will be drawn from a vein in your arm."

- In the section entitled, "How will your privacy be protected?" the phrase, "… all steps allowable by law …," conflicts with the purpose of the Certificate of Confidentiality. The sentences that read:

  "If your research record is reviewed by any of these groups, they may also need to review your entire medical record. There may also be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, [Institution] will take all steps allowable by law to protect the privacy of personal information."

  have been changed to:

  "If your research record is reviewed by any of these groups, they will take will take every precaution or protect your privacy."

Appendix 18.4: HIPAA Authorization Templates for Adult Cases:

- In the HIPAA Authorization for use of Protected Health Information, the paragraph indicating that biological samples will be sent to the NIDDK Biosample Repository has been expanded as follows:

  "As part of this study, your biological samples (i.e., the blood, urine, and liver samples) will be sent to the NIDDK Central Repositories, a research resource supported by the National Institutes of Health. The Repository collects, stores, and distributes biological samples and associated data from people with many kinds of disorders, unaffected family members, and other healthy people. The purpose of this collection is to make samples available for use in research of the genetic and clinical factors related to your injury after the current study is completed. Sending samples to the Repository may give scientists valuable research material that can help them to develop new diagnostic tests, new treatments, and new ways to prevent disease.

  "First, your blood sample will be sent to Rutgers University Cell and DNA Repository where genetic information will be extracted. This genetic information will be stored for 20 years. Second, your urine sample and one part of your liver biopsy will be sent to McKesson Health Solutions where they will be stored for future use. The storage locations will be forwarded to a Data Coordinating Center at the Duke Clinical Research Institute.

"Another part of your liver biopsy will be sent to a laboratory at the U.S. National Institutes of Health. This laboratory is separate from the NIDDK Central Repositories."

Appendix 18.5, 18.6, and 18.7 - Informed Consent and HIPAA Authorization Templates for Adult Controls, Pediatric Cases and Pediatric Controls:

Similar changes have been made to these templates.

1. EXECUTIVE SUMMARY

Background and Rationale [Section 2]: Liver injury due to prescription and non-prescription medication use is a medical, scientific, and public health problem of increasing frequency and importance in the United States. Indeed, drug-induced liver injury (DILI) is the most common reason for non-approval, withdrawal, limitation in use, and clinical monitoring by the Food and Drug Administration (FDA). However, detection of signals for liver injury frequently relies upon the reporting of cases by practitioners to health authorities in post-marketing surveillance. Underreporting of cases, lack of mandatory reporting systems, and difficulties in establishing a diagnosis make the current system sub-optimal. Moreover, with the growing use of complementary and alternative medications (CAM), there have also been increasing reports of liver toxicity due to various non-prescription herbal, dietary, and food additive supplements. Because the manufacturing, dispensing, and testing of these products is not regulated, the hepatotoxic potential of these formulations is poorly characterized or completely unknown.

As a result, there is a great need to develop an improved means of detecting, defining, and studying DILI in the United States. The DILIN prospective study is a multi-center study designed to gather clinical information and biological specimens on cases of suspected liver injury due to drugs and CAM. The goals of this study include the earlier recognition of DILI, especially due to newer drugs, development of standardized instruments and terminology to help identify cases of DILI, investigating clinical and genetic risk factors that predict DILI, and performing a careful longitudinal follow-up of DILI subjects. The biological samples collected will be used in future studies of the mechanisms and genetics of DILI.

Specific Aims and Objectives [Section 3]: The primary objective of this study is to prospectively identify *bona fide* cases of liver injury due to drugs and complementary and alternative medications within 6 months of the date of onset of the liver injury. Secondary objectives include collecting clinical data and biological specimens including blood, DNA, urine, and liver tissue from affected patients and matched controls for future mechanistic and genetic studies. The natural history of drug- and CAM-induced DILI will be tracked for at least 6 months following enrollment, with longer follow-up for those in whom there is evidence of chronic liver injury at 6 months. We will also develop and test causality assessment instruments for drug and CAM-induced liver injury that are sensitive, specific, and reproducible.

Basic Study Design [Section 5]: The DILIN Prospective Study is a multi-center, prospective, registry study. Patients who are referred to one of the DILIN clinical sites and who, in the opinion of a gastroenterologist / hepatologist, experienced a drug-induced liver injury will be enrolled. Detailed clinical data and biological specimens will be collected. Clinical data will be reviewed by the DILIN Causality Committee, and it will make the final determination of whether the subject qualifies as a *bona fide* DILI case. DILI cases (only) will be followed for at least 6 months to derive the longitudinal profile of drug- and CAM-induced liver injury. Detailed clinical data and biological specimens will be collected at this time point. Patients who satisfy the definition of chronic DILI will be evaluated at 12 months and yearly thereafter.

Pilot Testing [Section 6]: Pilot testing of the proposed methodology will be conducted before the full-scale implementation of this protocol. The primary purpose of this phase is to determine how difficult it will be to identify and recruit DILI patients, to determine whether the set of evaluations scheduled for the initial study visit is practicable, and to fine-tune and streamline the data collection forms. Up to three DILI cases will be enrolled at each of the participating clinical sites. All subjects will have suffered a drug-induced liver injury and satisfy the inclusion and exclusion criteria. The pilot phase will be restricted to the screening and initial study activities, and all the data and biological specimens will be collected. Any changes to the protocol will be documented and tracked, and revised applications reflecting these changes will be made to the IRBs at the participating clinical sites.

Study Populations [Sections 7.1 & 7.2]: Consecutive patients who are referred to one of the DILIN clinical sites and appear to have suffered a drug-induced liver injury will be considered for inclusion in the study. Subjects must be > 2 years at the time of enrollment; have evidence of liver injury that is known or suspected to be related to consumption of a drug or CAM product in the 6-month period

prior to enrollment; and, have documented clinically important DILI defined in terms of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (Alk Phos) as described below. Subjects will be excluded if there is acetaminophen hepatotoxicity, a competing cause of acute liver injury, or liver transplant prior to the development of drug- or CAM-induced liver injury.

Recruitment & Retention [Section 7.4]: Patients with potential DILI will be recruited from the nine DILIN clinical centers. Each of the clinical centers is a tertiary-care, hepatology unit and anticipates seeing a reasonable number of patients with potential DILI. Additionally, each center will undertake an aggressive strategy to recruit cases from local collaborators, outreach and affiliated community practices. DILIN will also undertake nationwide efforts to enhance its visibility and thereby promote more referrals from physicians not directly involved in the DILIN.

Data Collection Protocol [Sections 8, 9, 11, and 18.4]: The "initial study visit" is the first in-person visit by the participant to the DILIN clinical site. A number of evaluations will be performed at that time including a detailed medication history of the implicated DILI drug plus any drugs from the same class of medications; the dose, duration, and indication for all medications including prescription, OTC and herbal medications taken starting 8 weeks prior to starting the implicated DILI product; medical history; family history of drug allergies/ hepatotoxicity to the implicated drug or its class of drugs, and so on. Serological tests will be performed to exclude competing causes of acute liver disease; additionally, a complete blood count, kidney and liver function tests, total cholesterol, triglycerides, and a urinalysis will be performed. A blood sample will be drawn for DNA isolation, plasma, PBMC cryopreservation and serum storage. A voided urine sample will be collected, aliquoted into cryovials at the clinical site and frozen. If the subject had not been previously imaged, a screening liver ultrasound will be obtained. Follow-up evaluations will only be undertaken for DILI cases, and a subset of the tests as described in Section 9 will be repeated then. A schedule of evaluations is provided in Section 17.1.

Informed Consent Procedures [Sections 11.2 and 17.4]: Study personnel will provide a description of the study's purpose and procedures, and the participant will be provided an information packet including the informed consent document, HIPAA authorization and release of medical record forms. Informed consent will be undertaken by study personnel in person with the subject. Because a primary purpose of this study is to investigate the genetic determinants of the DILI event, the informed consent process will specifically include consent to participate in the genetics component of this study. An assent form will be used for pediatric subjects who are unable to understand the main consent, based on their parent's decision.

Adverse Events Related to Study Procedures [Section 11.4]: Protecting subjects from untoward risk related to study procedures is of paramount concern in this study. Because DILI cases are seriously ill, the vast majority of adverse events (AEs) will be due to their underlying illness. For this reason, only adverse events specifically related to study procedures will be recorded and reported. The study procedure most likely to result in adverse events is venipuncture for drawing blood. A formal definition of a "serious" adverse event (e.g., death, hospitalization, persistent or significant disability, etc.) will be adopted for this study. Timelines will be established for reporting serious AEs to the Data Coordinating Center and the DILIN Steering Committee.

Data Management Procedures [Section 13]: Electronic Case Report Forms (eCRFs) are designed specifically for the needs of this study. The eCRF will be partitioned into sections according to the type of data captured. Data will be entered into the InForm™ eCRF by personnel at the clinical centers. Any out-of-range values and missing or inconsistent key variables are flagged and addressed/answered at the site in real time during the data entry process. The Data Coordinating Center will perform internal database quality-control checks, and data audits throughout the course of the study.

Quality Control Procedures [Section 12]: A Manual of Procedures (MoP) will be written to elaborate all study procedures. It will form the basis for a training session to be conducted immediately prior to enrolling participants. An initiation visit will be performed prior to starting recruitment and enrollment. This is designed to ensure that facilities are adequate, personnel are trained and ready to recruit subjects, and that appropriate regulatory documents have been filed. A subsequent site visit will be performed approximately halfway through the study. Its purpose is to ensure a high level of fidelity to the protocol and consistency across the clinical sites.

Statistical Considerations [Section 14]: Without drug-matched controls, population controls are used to carry out genetic analyses to determine DILI-associated genetic risk factors. Sample size and power calculations are carried out based on the proposed genetic analyses using existing population control subjects. Tests for association for individual variants included in a GWAS, or in whole-genome or whole-exome sequencing studies, will be performed using logistic regression, including quantitative measures of genetically-inferred ancestry as covariates. Assuming 5,000 available population controls, sample size estimates between 1,000 patients and 25 patients (the sample size of many drug-specific or class-specific analyses) give varying levels of detectable effect sizes. For genetic variants that predispose to DILI generally (i.e. that are not drug-specific) with genotype relative risk greater than approximately 1.8 should be detectable with 80% power across the common variant frequency spectrum. For drug-specific risk variants the minimum detectable effect sizes are on the order of RR > 4 (for studies including n = 50 cases) to RR > 6 (for studies including n = 25 cases).

Study Administration [Section 15]: The Steering Committee is the main governing body of the project. It is composed of the Principal Investigators of the clinical centers, the Principal Investigator of the Data Coordinating Center, and the NIDDK Project Scientist. The clinical centers, the Data Coordinating Center and the NIDDK each have one vote on the Steering Committee. All decisions are determined by majority vote. In addition, a number of subcommittees have been established and report to the main Steering Committee. Data and safety will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the DILIN studies. The description of DSMB activities is included in the DSMB Charter.

2. BACKGROUND AND RATIONALE

2.1 Overview

Liver injury due to prescription and non-prescription medication use is a medical, scientific, and public health problem of increasing frequency and importance in the United States. Drug-induced liver injury (DILI) is the most common reason for nonapproval, withdrawal, limitation in use, and clinical monitoring by the Food and Drug Administration (FDA). Unfortunately, early toxicology and animal testing does not reliably identify agents that may lead to DILI. In addition, only a limited number of highly selected patients (i.e. 5,000 to 10,000) receive medication during the clinical testing of new therapeutic agents. As a result, it is not uncommon that clinically significant adverse drug reactions (ADR) such as DILI are not detected until the drug is used by a large number of patients in the general population.

Establishing a diagnosis of DILI is problematic due to the presence of other potential causes of liver injury, a lack of standardized, objective, and reproducible diagnostic criteria, and the need for drug discontinuation and observation. In addition, most instances of DILI are not dose dependent, quantifiable, or predictable. Furthermore, the clinical manifestations of DILI markedly vary from asymptomatic laboratory abnormalities to life-threatening acute liver failure (ALF). Because of the need for a high index of suspicion and the lack of reliable diagnostic criteria, a diagnosis of DILI is frequently delayed or occasionally missed. With the widespread and growing use of complementary and alternative medications (CAM), there have also been increasing reports of liver toxicity due to various non-prescription herbal, dietary, and food additive supplements. Because the manufacturing, dispensing, and testing of these products is not regulated, the hepatotoxic potential of these formulations is poorly characterized or completely unknown. Clinical studies of drug and CAM mediated liver injury have largely been limited to small, uncontrolled retrospective case series of patients with severe disease at a single center. There have been few prospective studies of DILI that have provided meaningful data on risk factors, characteristic profiles, and possible mechanisms of hepatotoxicity.

2.2 Clinical Presentation

Drug-induced liver injury can mimic nearly all forms of acute and chronic liver disease. Although most drugs have a characteristic clinical “signature,” these signatures are not specific, and a multitude of alternative potential causes must be excluded. In addition, some drugs such as carbamaz-

epine can lead to a range of clinicopathological states varying from acute hepatitis to granulomatous hepatitis and cholangitis [2]. Because the liver is the principal organ involved in the metabolism, bioactivation, detoxification, and excretion of most xenobiotics including drugs, it is not surprising that nearly all cell types in the liver including hepatocytes, cholangiocytes, endothelial cells, and stellate cells can be adversely affected by drugs. As a result, a plethora of histopathological abnormalities have been reported with DILI.

Table 2.1: Examples of Different Liver Injury Patterns and Associated Mechanisms

Liver Injury Pattern	Mechanism(s)	Example(s)
Acute hepatitis	Parent drug or drug metabolite mediated toxicity Parent drug or metabolite-mediated immunoallergy and/ or auto-immunity	Troglitazone Isoniazid Dihydralazine, tienilllic acid
Acute cholestasis	Inhibition of biliary secretion	17-Alkyl steroids clavulanic acid / amoxicillin, erythromycin
Macrovesicular steatosis	Decreased secretion of lipoproteins	Corticosteroids, asparaginase; Tamoxifen
Microvesicular steatosis	Inhibition of fatty acid miotchondrial B-oxidation	Aspirin, valproic acid
Phospholipidosis	Inhibition of lysomal phospholipases	Amiodarone
Chronic hepatitis	Metabolite-mediated immune reaction	Nitrofurantoin, methyldopa
Vanishing bile duct syndrome	Autoimmune destruction of small bile ducts ? Abnormal PGP	Chlorpromazine
Sclerosing cholangitis	Biliary ischemia caused by arterial lesions	FUDR
Veno-occlusive disease	Metabolite-mediated endothelial lesions	Pyrrizolidine alkaloids, Vinca alkaloids, cyclophosphamide, busulfan
Perisinusoidal fibrosis	Stellate cell activation	Vitamin A
Fibrosis/ cirrhosis	Stellate cell activation	Methotrexate

DILI has been biochemically classified as “hepatocellular” when ALT is > 2 X upper limit of normal or ALT/ Alkaline phosphatase ratio is > 5 [3]. A “cholestatic” liver injury pattern is defined by an alkaline phosphatase level that is > 2 X ULN or ALT/ Alkaline phosphatase ratio < 2. A “Mixed” injury pattern is characterized by ALT/ Alkaline phosphatase ratio of 2 to 5 and individual values both being > 2 X ULN. Most patients with clinically manifest DILI present with an acute hepatitic-like illness characterized by malaise, nausea, and abdominal pain of varying severity which generally resolves as the patient improves. However, some patients may have no symptoms and minimal or non-specific histopathological changes such as cytoplasmic expansion and glycogenated nuclei. As many as 1% of patients with cholestatic DILI may develop progressive chronic liver disease with loss of intrahepatic bile ducts and prolonged jaundice [4]. It is estimated that < 10% of all idiosyncratic DILI cases lead to encephalopathy or coagulopathy [1]. However, if acute liver failure develops, the likelihood of spontaneous recovery is very low (i.e. < 20%) [5].

DILI can be broadly classified into 2 mechanistic subgroups: Immunoallergic and idiosyncratic. With hypersensitivity reactions, it is thought that toxic metabolites are generated which can covalently bind to intracellular proteins or be expressed as haptens and lead to inadvertent host immune response. Patients with immunoallergic drug reactions frequently have a short latency period between ingestion and presentation (1 to 28 days) and have an even shorter latency upon rechallenge. Features such as fever, rash, lymphocytosis or eosinophilia, and non-hepatic internal organ involvement may be seen in patients with sulfa and sulfonamide related DILI [6]. Phenytoin related DILI may present with fever, lymphadenopathy, and circulating au-

toantibodies [7]. Serum aminotransferase, alkaline phosphatase, and bilirubin levels are variably elevated. Liver biopsy typically reveals an infiltrate of lymphocytes and eosinophils while a minority of patients may have granulomas [8]. The majority of patients with an immunoallergic reaction will recover following discontinuation of the drug without the need for glucocorticoids. However, some patients with severe manifestations have been treated with glucocorticoids with apparent benefit. Others may go on to develop severe hepatic necrosis leading to liver transplantation or rarely death.

In the majority of patients with DILI, there is no evidence of allergy or hypersensitivity. In these cases, aberrant host metabolism of the drug or a subsequent intermediate is believed to mediate cell damage [9]. Unfortunately, the majority of cases of metabolic idiosyncrasy do not have a predictable time course or latency from exposure with cases occurring 1 year or more after initiation of exposure having been reported [10]. In addition, there is variable elevation in serum aminotransferase, alkaline phosphatase, and bilirubin levels and a myriad of histopathological findings with varying degrees of lobular necrosis and inflammation. Non-specific hepatitis symptoms such as nausea, vomiting, abdominal pain, and fatigue may occur early on and generally improve with clinical recovery. Although most patients with idiosyncratic DILI will improve with medication discontinuation and supportive care, some individuals may go on to develop progressive liver failure as has been seen in older individuals with isoniazid hepatotoxicity [11] or in patients of all ages who took troglitazone [12]. Currently, there are no reliable clinical and laboratory features that help identify individuals at risk for poor outcomes with DILI. However, it is estimated that 10% of individuals who are hospitalized with jaundice due to severe hepatocellular injury from DILI will die of liver failure [1].

2.3 Causality Assessment / Diagnosis

Diagnostic instruments are used to objectively identify individuals with a diagnosis or disease of interest. Two diagnostic instruments for DILI have been developed in Europe [13 14]. In 1989 under the auspices of The Council for International Organizations of Medical Sciences (CIOMS) a conference was held on DILI and the principles developed by this group were later incorporated into a scoring system termed the Roussel Uclaf Causality Assessment Method (RUCAM) [13]. The RUCAM provides a semi-quantitative assessment of causality by assigning an arbitrary number of points to medical history features in 6 domains. The domains include time of onset from drug exposure and improvement in liver injury with drug withdrawal. The instrument also collects information on concomitant therapy, exclusion of non-drug related causes, rechallenge, and prior reports of liver injury associated with the suspect agent [15-17]. A score with a maximal range from -3 to + 3 is assigned to each domain. The total score is divided into categories of likelihood that the drug was the cause of injury: definite or highly probable (score>8), probable (score 6-8), possible (score 3-5), unlikely (score 1-2), excluded (score <0). A limitation of the RUCAM includes the rigidity applied to the temporal association between drug exposure and liver injury (i.e. < 90 days). Drugs that may cause liver injury months after discontinuation, such as clavuainic acid / amoxicillin may not be accurately categorized by RUCAM. In addition, rechallenge is heavily weighted in the RUCAM which may be inappropriate since most patients are not intentionally rechallenged after a severe adverse event due to safety and ethical concerns. An additional limitation is that the exclusion of other causes of liver disease is limited to viral hepatitis, hypotension, alcohol, and gallstones or biliary tract disease [18]. Testing for other less common causes of chronic liver disease, such as alpha-1-antitrypsin deficiency, Wilson's Disease, and hemochromatosis are not required but may be appropriate in certain circumstances. Finally, the RUCAM is limited in the type of information it collects on risk factors [18]. Although data on alcohol use and age are collected, information on other potential risk factors, such as HIV infection, are not captured.

Maria and Victorino developed the Clinical Diagnostic Scale (CDS) for DILI with the goal of improving upon the RUCAM [14]. This scale includes five distinct domains: temporal association, exclusion of alternative causes, extrahepatic manifestations, rechallenge, and previous reports of DILI with the suspect drug. The domain scores can range from -3 to +3 depending on the category. Compared to the RUCAM, the CDS is more likely to capture drugs with immunoallergic manifestations because it incorporates extrahepatic manifestations, including rash, fever, arthralgias, eosinophilia (>6%) and cytopenia. Similar to RUCAM, the final score is

divided into categories of likelihood that the drug was the cause of liver injury: definite DILI (score>17), probable DILI (score 14-17), possible DILI (score 10-13), unlikely DILI (score 6-9), and excluded (score<6). Limitations of the CDS include it s poorer sensitivity for idiosyncratic and delayed onset drug reactions. The RUCAM and CDS were compared in a study of 215 cases of DILI with the opinion of three experts serving as the gold standard [19]. The concordance for identifying DILI cases was disappointingly low at 18% between the two instruments. Overall the RUCAM performed better than CDS when compared to the expert panel. Although CDS had a specificity of 100% for DILI, its sensitivity for DILI was only 37% [19]. The CDS appears to be better for cases of immunoallergic DILI while the RUCAM is better for DILI cases without systemic manifestations.

One of the goals of the DILIN network is to develop and validate a sensitive, specific, and reproducible diagnostic instrument for DILI. A simple instrument that reliably categorizes DILI cases and that can be used by practicing physicians will be developed. Despite the limitations of RUCAM and CDS, components of each instrument will likely be incorporated into the DILIN diagnostic instrument. Possible improvements in the DILIN instrument will include expanding the criteria for the temporal association between drug exposure and liver injury. The DILIN instrument will be developed in a cohort of patients who have been diagnosed with DILI. The instrument will be prospectively validated in DILI cases collected by the DILIN. The gold standard to which the DILIN instrument will be validated will be the expert opinion of the causality committee, comprised of experienced hepatologists from the clinical sites, the data coordinating center, and the NIDDK.

2.4 Risk factors

2.4.1 Genetic Risk Factors

A genetic basis for idiosyncratic DILI is supported by its rare occurrence and largely unpredictable nature. However, genetic studies of DILI are challenged by its low incidence, difficulty in applying family studies, heterogeneous clinical manifestations, and variable penetrance. Polymorphisms in drug metabolism or cytokine expression may affect the risk of developing DILI. However identifying genetic markers is not the only necessary step due to the wide spectrum of phenotypes seen with specific genetic mutations. Environmental factors, such as diet and comorbidities may also influence the phenotypic expression of genetic variations in drug metabolism.

Specific examples of the association between polymorphisms in drug metabolizing enzymes and drug-induced liver injury exist for a number of drugs. Most of the studies on the association between drug metabolizing enzymes and DILI have been case-control studies. Phenytoin-induced liver injury has been reported in patients who are unable to detoxify arene oxide, an intermediate toxic metabolite, presumably due to a defect in the activity of epoxide hydrolase. Arene oxide may behave as a hapten resulting in immunological reaction and hepatocellular injury [22]. Deficiency in cytochrome P450 enzymes has also been associated with DILI. For example, CYP 2D6 deficiency has been associated with DILI from perhexiline. Similarly, deficiency in N-acetyltransferase has been associated with DILI from sulfonamides [22].

A prospective study of the association between CYP 2E1 polymorphisms and DILI from isoniazid (INH) was conducted in 318 Taiwanese patients receiving antituberculous therapy [23]. The investigators hypothesized that individuals with wild type CYP2E1 (c1/c1) may generate more hepatotoxic metabolites from INH compared to those with mutant CYP 2E1 allele (c1/c2 or c2/c2). A total of 185 (58%) patients were genotyped as CYP2E1 c1/c1, 118 (37%) patients as CYP2E1 c1/c2, and 15 (4.7%) patients as CYP2E1 c2/c2. The risk of hepatotoxicity was also higher in patients homozygous for wild type allele, c1/c1 compared to patients with mutant allele c1/c2 or c2/2, 20% vs. 9%, respectively, $p = 0.009$. The risk of hepatotoxicity was higher in slow acetylators compared to rapid acetylators, 24.7% vs. 12.4%, respectively, $p = 0.011$. Individuals who were slow acetylators and homozygous for the wild type allele were 7.4 times more likely to develop hepatotoxicity compared to rapid acetylators with a mutant allele. However, in multivariate analysis only CYP 2E1 genotype was independently associated with INH hepatotoxicity.

Identifying genetic polymorphisms that increase an individual's risk for DILI is a major goal of DILIN. The study on INH discussed above demonstrates how testing for polymorphisms in drug metabolizing enzymes can potentially become a clinically useful tool. DILIN will collect DNA from subjects who develop DILI to test hypothesis-driven associations between specific polymorphisms and risk of DILI. In addition, by using high throughput technology such as single nucleotide polymorphism approaches, these samples can be used to search for as yet unknown polymorphisms or genetic markers associated with DILI. Results of gene analysis from high throughput genetic techniques can be potentially validated in prospective studies that determine the rates of hepatotoxicity in individuals with a genetic polymorphism of interest compared to those with wild type.

2.5 Natural History

Data are scarce on the natural history of DILI with most studies on the long term follow up of DILI being case reports. In addition, follow-up liver biopsies are usually not performed.

It was generally thought that patients who experienced an acute liver injury from a drug recovered without consequences or permanent liver injury. However, rare cases of chronic liver injury with ductopenia have been reported following exposure to amoxicillin, other b-lactam antibiotics, chlorpromazine, and other phenothiazines. Furthermore, methotrexate and amiodarone are known to lead to chronic liver injury. Lastly, aldomet and nitrofurantoin may lead to chronic active hepatitis. The notion that nearly all DILI cases are acute and/or self-limited was challenged by Aithal and Day who reported results on the natural history of DILI in 44 patients over an 18 year period [24]. Antibiotics and anti-inflammatory drugs accounted for 24 (54%) of the cases. Liver biopsy showed acute hepatitis in 6 patients, chronic hepatitis in 20 patients, and cholestasis in 18 patients at presentation. During a median follow-up of 5 years after initial diagnosis (range 1-19 years), 4 patients died, and 7 patients were lost to follow-up leaving 33 patients for review. Eight patients had abnormal liver tests with or without abnormal imaging and 5 patients had normal liver tests with abnormal liver imaging. Five of the eight patients with persistently abnormal liver tests underwent liver biopsy. Three liver biopsies showed either chronic hepatitis, fibrosis, or ductopenia, but in no case had the histology worsened from the initial biopsy. Fibrosis on the initial biopsy and continued exposure to the offending drug (> 6 months) were associated with persistent liver enzyme elevations.

Although there were only five patients with follow-up liver biopsies, this study raised doubts about the prevailing belief that liver injury from drugs either led to acute death or liver transplantation or resolved without long-term sequelae. However, prospective data in a larger number of patients with bona fide DILI are needed to better examine this important area. DILIN is uniquely positioned to prospectively follow a large group of US patients with *bona fide* DILI to better define the natural history of this rare form of liver disease.

2.6 Hepatotoxicity and HIV

Over the last decade, advances in drug therapy for human immunodeficiency virus (HIV) infection have translated into dramatic improvements in patient survival. Over 15 drugs belonging to 3 major classes – nucleoside/nucleotide analog reverse transcriptase inhibitors, protease inhibitors, and non-nucleoside analog reverse transcriptase inhibitors – are currently approved for treatment of HIV infection. Highly active anti-retroviral therapy (HAART) is the term used to describe a regimen that combines at least three agents from two or more of these classes. Although use of HAART significantly decreases morbidity associated with HIV infection and increases patient survival, it is also associated with significant adverse events including hepatotoxicity [25]. Indeed, drugs from all three classes of antiretroviral agents have been associated with liver injury, particularly in patients with hepatitis B and/or C coinfection.

Nucleoside analogs (NAs) are prodrugs that become active only after phosphorylation by cellular kinases. As triphosphate nucleotides, they competitively inhibit the HIV reverse transcriptase and also result in DNA chain termination after incorporation into DNA. Significant liver injury (i.e., ALT or AST $\geq$ 5 x ULN) has been reported with the early NAs, zidovudine (AZT, Retrovir), didanosine (DDI, Videx), and stavudine (d4T, Zerit), but less frequently with the newer NAs lamivudine (3TC, Epivir), abacavir (ABC, Ziagen), and the nucleotide analog, tenofovir

(TFV, Viread). Abacavir toxicity has been associated with a rapid-onset, potentially life-threatening hypersensitivity reaction that may include signs of liver injury although overt liver failure appears to be rare [26]. Significantly increased ($\geq$ 5 x ULN) but generally asymptomatic aminotransferase levels have been reported in approximately 5% of subjects during cohort studies of AZT, ddI, and d4T [25]. Symptomatic and often life-threatening NA-associated liver injury is characterized by hepatomegaly, liver dysfunction and lactic acidosis – the so-called lactic acidosis syndrome – has been reported much less commonly (1-15 cases per 1000 person-years) but carries a mortality rate of >60% in severe cases [27]. It is characterized by marked hepatic steatosis, typically occurs after 3-12 months of drug exposure, and may be associated with neuropathy, myopathy, and pancreatitis. Inhibition of mitochondrial DNA polymerase appears to play a major role in the pathogenesis of the syndrome [28]. This syndrome has been reported most commonly with d4T, and some studies suggest the combination of d4T and ddI carries the highest risk [27].

Protease inhibitors (PIs) – including Saquinavir (Fortovase), Ritonavir (Norvir), Indinavir (Crixivan), Nelfinavir (Viracept), Amprevavir (Agenerase), and Lopinavir-Ritonavir (Kalebra) - target the active site of HIV aspartyl protease and thereby prevent processing of the viral gal-pol polyprotein and viral maturation. Moderate to severe elevations of aminotransferases have been reported in 3-10% of patients treated with PIs in phase I/II trials, but symptomatic liver injury is much less common [25]. Ritonavir is the only PI independently associated with severe HAART-related liver injury independent of viral hepatitis serostatus [29]. The mechanism of PI-associated liver injury is unknown and the histological findings are variable and nonspecific.

The non-nucleoside analog reverse transcriptase inhibitors (NNRTIs) – Nevirapine (NVP, Viramune), Delavirdine (DLV, Rescriptor), and Efavirenz (EFV, Sustiva) - are non-competitive inhibitors of HIV reverse transcriptase. Of the three NNRTIs, NVP has most often been associated with significant liver injury, including aminotransferase elevations $\geq$ 5 x ULN in 1-15% of patients in post-marketing studies and rare reports of fulminant liver failure [30]. Two distinct clinical patterns of NVP-associated liver injury have been described. The first, early form occurs within 6 weeks of initial exposure and is clinically indistinguishable from ABC-associated liver injury and is associated with fever, rash, arthralgias, and eosinophilia. The second more common pattern, typically occurring 2-3 months after initiation of NVP, is not associated with extra-hepatic findings and appears carry a better prognosis than the early form [31].

Attribution of causality in the setting of HAART-related liver injury can often be challenging for a number of reasons. Most importantly, patients with possible HAART-related toxicity are, by definition, on multiple potentially hepatotoxic medications and assigning blame to a particular drug may be difficult. Furthermore, many patients with chronic HIV infection are co-infected with chronic viral hepatitis B and C, and data support the notion that immune reconstitution as a result of HAART can cause a flare in viral hepatitis [32]. In addition, patients with HIV infection can develop relatively unique liver conditions that can also contribute to diagnostic uncertainty including opportunistic infections (e.g*., Mycobacterium avium-intracellulare* (MAI), cytomegalovirus (CMV), adenovirus) and malignancies (e.g., Kaposi's sarcoma, lymphoma). Finally, HIV-infected patients are frequently exposed to a host of non-antiviral medications (e.g., sulfonamides) that may result in liver injury, and use of non-prescription, complementary alternative medications, and illicit drugs (e.g., cocaine) in these patients is also common.

Thus, in a patient on HAART the differential diagnosis of severe liver injury might include a flare of underlying hepatitis (if present), and DILI directly related to HAART or other medications. Certain information should be captured in order to optimize causality assessment in these cases. For example, viral titers (i.e., HCV RNA and HBV DNA by quantitative assays) should be recorded; if there is little or no evidence of viremia, a hepatitis flare is less likely. If, on the other hand, the titers are high, and particularly if they are rising, a flare would be a strong competing cause of liver injury. In order to address the issue of immune reconstitution, CD4 counts, and HIV viral loads should be recorded, ideally both before HAART and during the course of anti-viral therapy. If the CD4 count and/or HIV viral load have changed little compared with the baseline values, and immune reconstitution event would appear to be a less likely cause of liver injury. On the contrary, if they respond appropriately to HAART, then immune reconstitution would be a strong competing cause. Finally, lactic acid levels and drug

levels obtained during an episode of liver injury should be recorded if available, as they may be useful in causality assessment and defining the mechanism of liver injury.

2.7 CAM Hepatotoxicity

Over the last several decades, use of complementary and alternative medicines has been growing in popularity in Western countries. Indeed, it has been estimated that 3% of adults in the United States routinely use some herbal product, and sale of complementary and alternative medication (CAM) products currently represents a multi-billion dollar industry [33]. This growing popularity is despite the fact that herbal products are generally not subjected to scientifically rigorous efficacy and safety testing. Because they are labeled as "natural" products, they are often assumed to be inherently safe by the general public. However, there are numerous reports of hepatotoxicity from herbal and other complementary preparations, in both animal models as well as humans [34]. Examples of CAM products that have well-established potential to cause liver injury include pyrrolizidine alkaloids (Comfrey), chaparral leaf, germander, pennyroyal (squawmint oil), mistletoe, kava, and weight loss preparations containing usnic acid [35].

There are no specific diagnostic tests for hepatotoxicity from CAM and the diagnosis thus relies on careful assessment of the temporal relationship between the CAM use and liver injury, exclusion of other competing causes, dechallenge, and rarely rechallenge. Although this approach is not unique to liver injury from CAM, studying liver injury from these agents poses a number of challenges. First, herbal products often have multiple, poorly characterized ingredients including several different plants and herbs. , One active ingredient may induce hepatic metabolism of another component or otherwise alter the pharmokinetics and pharmacodynamics of another component. Furthermore, herbal products may be adulterated with various non-herbal chemicals and prescription drugs, and it is rare that a single CAM product is taken in isolation. Second, like prescription medications, CAM hepatotoxicity can clinically and histologically mimic many other types of acute and chronic liver disease. However, uncommon patterns of liver injury, such as zonal necrosis, necrotic lesions with steatosis or bile duct injury, and veno-occlusive disease (VOD), should raise suspicion of CAM hepatotoxicity [36]. Third, despite presenting with liver injury, patients may not readily admit that they are taking herbal products both because of fear of physician disapproval or because they believe that such products are safe and unrelated to the acute illness. For example, in one academic liver clinic, it was estimated that no more than a third of patients taking CAM products disclosed use of herbal remedies [37]. Finally, patients with liver disease, particularly chronic hepatitis C, commonly use CAM, making it difficult to exclude underlying liver disease as an explanation for abnormal liver biochemistries. Likewise, obese and overweight patients, who are at risk for having non-alcoholic steatohepatitis, frequently use herbal medications touted as effective weight reduction agents. For all of these reasons, we should anticipate that causally linking a particular herbal compound with liver injury, a necessary first step in deciphering the pathogenic mechanisms of CAM-related hepatotoxicity, will be very challenging.

In order to assess exposure to CAM products, one can ask patients for a self-reported history. Alternatively, one may provide a list of commonly used CAM products and see if this prompts improved recall. Since there are over 30,000 CAM products available in the US, we will use a list of the more commonly encountered CAM products.

2.8 Mechanisms of Drug-Induced Liver Injury

A phenotype of DILI results from an imbalance between damage and repair to the liver. The pathogenesis of drug and toxin liver injury is believed to involve the generation of toxic metabolites or intermediates which lead to cell death via the extrinsic immune system or intracellular stress which can result in apoptosis or necrosis. Drug metabolites can undergo or promote a number of chemical reactions including covalent binding, depletion of reduced glutathione, or oxidative stress resulting in lipid peroxidation, protein thiol oxidation, and DNA oxidation. These chemical reactions can directly affect intracellular organelles and either trigger apoptosis or necrosis or lead to sensitization to the lethal action of intrahepatic cytokines.

Genetic tools to investigate the pathogenesis of drug hepatotoxicity are more plentiful and more precise than they have ever been, and this in itself makes a powerful case for the inclusion of a pharmacogenetic component to this protocol. The majority of the enzymes that carry out hepatic drug metabolism have been shown to be genetically polymorphic [38], as have a large number of important hepatic drug transporters, including the p-glycoprotein drug transporter [39]. These enzymes and transporters control access of drugs and toxins to the liver and their metabolism to more or less toxic metabolites. They help to determine the toxin load that the liver is called upon to dispose of. It seems likely that polymorphic variants in the genes that code for these proteins influence the hepatic exposure to drugs toxins. An example of this paradigm can be illustrated by the recent study which revealed a genetic basis for the hepatotoxicity induced by isoniazid (INH) [40]. As Cytochrome P450 2E1 (CYP2E1) is involved in the metabolism of INH and the generation of potential hepatotoxins, the authors hypothesized that polymorphisms in CYP2E1 gene may increase the susceptibility to INH hepatotoxicity. A total of 318 tuberculosis patients who received anti-TB treatment were followed prospectively and 49 of them (15.4%) had drug-induced hepatotoxicity. Patients with homozygous wild genotype *CYP2E1* c1/c1 had a higher risk of hepatotoxicity (20.0%; odds ratio [OR], 2.52) than those with mutant allele c2 (*CYP2E1* c1/c2 or c2/c2, 9.0%, p = .009). Furthermore, polymorphism in the N-acetyltransferase gene (slow acetylators) had an additive effect to CYP2E1 polymorphism for the risk of INH hepatotoxicity.

It is possible that inherent compromise of the systems involved in hepatic regeneration, either alone or in combination with other possible genetic defects described above, might predispose to damage from multiple drugs. Some examples that are suitable for study include the candidate genes in the IL-6 and TNFα pathways that have documented genetic variants and likely functional consequences. These paracrine pathways are involved in the initiation of hepatic regeneration, and act in concert with growth factors such as hepatocytes growth factor (HGF) to bring about regeneration [41]. Since HGF itself is required for normal liver development, and defects in it are likely fatal and are not likely to play a role in the phenotypic expression of drug hepatotoxicity. In contrast, IL-6 variants appear to contribute to changes in the plasma concentrations of inflammatory mediators such as C-reactive protein in response to stress [42] and result in real functional consequences such as changes in bone densitometry [43]. Genetic variants in TNFα have been tested in the pathogenesis of various liver diseases such as hepatitis C, alcoholic liver disease, and nonalcoholic steatohepatitis [44], and the approach of using a series of genetic tests for variants in inflammatory mediators such as these has been successfully applied [45] to the study of the severity, progression, and regression of coronary atherosclerosis.

Thus, DILI may be due not only to the liver being flooded with a drug-derived toxin, but also to a genetically-determined defect in hepatic systems of defense. If this is true, one might expect evidence of such heritable frailty to be present in the literature, and although this question has not been systemically investigated until now, suggestions are present. Indeed, although the discipline of pharmacogenomics is still in its infancy, several genetic factors have been identified as modulating DILI as summarized in Table 2.2.

Table 2.2: Selected Genetic Factors Identified as Modulating DILI

Factor	Drug	HLA Types	Drug
Deficiency in CYP 2D6	Perhexiline	A11	Halothane, Diclofenac
Deficiency in CYP 2C19	Atrium, Troglitazone (?)	DR6 and DR2	Nitrofurantoin
Deficiency in NAT2	Sulfonamides	A8	Clometacin
Deficiency in Sulfoxidation	Chlorpromazine (?)	A11	Tricyclics
Deficiency in Glutathione Synthetase	Acetaminophen	DR6	Chlorpromazine
Deficiency in GST type T	Tacrine (?)	DRB1 1501	Clavulanic acid / Amoxicillin

CYP 2E1 polymorphism	INH	RQB1 0602	

Modified from Larrey D. *Seminars in Liver Disease* 2002:22:152

The genetic basis for drug hepatotoxicity can be approached either by evaluating the associations between hypotheses-driven candidate genes (e.g., transporter gene mutations in drug-induced cholestasis or NAT/CYP2E1 genes for INH toxicity) or by exploratory pan-genomic SNP analyses. The relative merit of one approach over another is debatable but they both can be complementary. Our understanding of the genetic basis for drug hepatotoxicity is largely limited by the lack of adequate number of DNA samples from persons who have sustained drug-induced liver injury and controls. This deficiency serves as the major impetus for the current proposal, an important aim of which is to collect genetic material from adequate number of well-characterized individuals with DILI and suitable controls.

2.9 Study Rationale

Liver injury is a rare but increasingly recognized adverse effect of medications associated with variable outcomes. Although many patients improve with withdrawal of the offending agent, some patients go on to develop severe liver injury resulting in liver failure, liver transplantation, or even death. Over the past 20 years, idiosyncratic DILI has been the most common reason by regulatory authorities for non-approval, withdrawal, or limitation in usage of drugs, and mandates for special monitoring of patients receiving these drugs. Despite extensive preclinical *in vitro* and animal toxicology testing during drug development, most hepatotoxic drugs are not identified until they have been used in large numbers of patients. Although patients enrolled in clinical trials are prospectively monitored for adverse events, the low incidence of liver injury (typically < 1 in 10,000) frequently precludes the reliable detection of a liver injury signal. In addition, since most cases of DILI are due to presumed metabolic idiosyncrasy which is independent of dose and exposure, it has been exceedingly difficult for clinicians and scientists to reliably identify high risk individuals. Furthermore, the lack of standardized definitions and causality assessment instruments has further hampered our ability to study the incidence, risk factors, and clinical impact of DILI. Lastly, existing postmarketing surveillance for hepatotoxicity is sub-optimal due to the underreporting of cases, lack of a mandatory system, and a delay in pattern recognition. As a result, there is a great need to develop an improved means of detecting, defining, and studying DILI in the United States to safeguard the health of the nation. The DILIN is a multi-center study designed to gather clinical information and biological specimens on cases of suspected liver injury due to drugs and CAM. Goals of the network include the earlier recognition of DILI, especially due to newer drugs, development of standardized instruments and terminology to help identify cases of DILI, and organized and careful longitudinal follow-up of such subjects. The biological samples collected will be used in future studies of the mechanisms and genetics of DILI. The network will also serve as a regional and national clinical resource for practicing physicians and consumers.

3. SPECIFIC AIMS AND OBJECTIVES

Primary objectives:

1. To prospectively identify *bona fide* cases of liver injury due to drugs and complementary and alternative medications within 6 months of the date of onset of the liver injury and to collect clinical data, blood, DNA, urine, and liver tissue samples from affected patients and controls for future mechanistic and genetic studies.

Secondary objectives:

2. To identify genetic risk factors that may help explain variability in susceptibility and outcome of drug and CAM-induced liver injury.
3. To characterize the natural history of drug- and CAM-induced DILI for at least 6 months following enrollment. For those in whom there is evidence of on-going liver injury at 6 months, to determine the natural history of their disease at 12 and 24 months from the date of onset of the liver injury.

4. To develop terminology and standardized definitions for DILI and to test causality assessment instruments for drug and CAM-induced liver injury that are sensitive, specific, and reproducible.

4. DEFINITIONS / TERMINOLOGY

The following definitions are applied in this document.

4.1 Standard Definitions

- Acute liver failure (ALF): Acute liver failure is the development of coagulopathy (INR > 1.5) and encephalopathy within 8 weeks of presentation in a patient without known underlying liver disease.
- Cholestatic DILI: Liver injury is designated as “cholestatic” if the alkaline phosphatase is elevated and the normalized ALT/ alkaline phosphatase ratio is < 2.
- Chronic hepatitis B: A form of chronic liver disease due to infection with the hepatitis B virus that is defined by the persistence of detectable hepatitis B surface antigen in blood for at least 6 months.
- Chronic hepatitis C: A form of chronic liver disease due to infection with the hepatitis C virus that is defined by the persistence of detectable hepatitis C virus RNA in blood for at least 6 months.
- Complementary and alternative medicine (CAM): Herbal, vitamin, or natural remedies administered for the purpose of healing or treating a health condition or to maintain well being.
- Drug: Drugs are defined as products that treat, cure, prevent, mitigate, or diagnose a disease. Routes of exposure include oral, transdermal, rectal, intravenous, intramuscular, and implantable.
- Drug-induced liver injury (DILI): Hepatocellular, cholestatic or mixed liver injury that is caused by a drug or complementary alternative medicine. For the purposes of this protocol only severe cases of DILI will be collected as defined in this document.
- Genotype: The genetic composition of the individual determined by the arrangement of bases in DNA.
- Hepatocellular DILI: Liver injury is designated as “hepatocellular” if ALT is elevated and the normalized ALT/ Alkaline phosphatase ratio is $\geq$ 5.
- Index case: The individual identified with DILI.
- Mixed DILI: Liver injury is designated as “mixed” when both ALT and alkaline phosphatase are elevated and the normalized ALT/Alkaline phosphatase ratio is between 2 and 5.
- Pharmacogenetics: The study of the relationship between genetic variation and the therapeutic and adverse effects of drugs.
- Phenotype: The clinical expression of drug-induced liver injury, including symptoms, physical findings, and laboratory tests, including protein expression (proteomics) and metabolite formation (metabonomics).
- Polymorphism: A stable genetic variant that is present in at least 1% of the general population.
- Severe liver injury: Severe liver injury is defined if one or more of the following are present: 1) Jaundice with total bilirubin > 2.5 mg/dL in the absence of hemolysis, Gilbert's Syndrome; 2) Prothrombin time prolongation (INR > 1.5 x ULN) in the absence of coumadin therapy or known vitamin K deficiency 3) Hepatic encephalopathy.
- Subacute liver failure: SLF is the development of coagulopathy (INR > 1.5) and encephalopathy within 8 to 24 weeks of presentation in a patient without known underlying liver disease.
- Xenobiotic: Any natural or synthetic substance made outside the body that can be taken by an individual.

4.2 Chronic DILI

The following criteria will be applied to assess whether a case is considered to have chronic drug-induced liver injury.

Inclusion Criteria:

A subject will be considered to have chronic DILI and be eligible for participation in the chronic DILI follow-up protocol if any of the following are present:

1. A persistently elevated (i.e. above the upper limit of normal) serum AST, ALT, alkaline phosphatase, INR (in the absence of coumadin therapy or vitamin K deficiency) or total bilirubin level (in the absence of hemolysis or known Gilbert's syndrome) measured on 2 separate occasions at least 6 months after the date of onset of the liver injury in a patient with normal or unknown baseline values prior to initiation of suspect medication
2. A serum AST, ALT, alkaline phosphatase, INR (in the absence of coumadin therapy or vitamin K deficiency), or total bilirubin level (in the absence of hemolysis or known Gilbert's syndrome) measured on at least 2 separate occasions at least 6 months after the date of onset of the liver injury that exceeds 1.25 times the baseline value in a patient with abnormal baseline values prior to initiation of suspect medication
3. Clinical evidence of portal hypertension at least 6 months after the date of onset of the liver injury such as ascites (fluid in the abdomen by imaging), esophageal or gastric varices on endoscopy, or hepatic encephalopathy.
4. Histological evidence of liver injury on a liver biopsy obtained at least 6 months after the date of onset of the liver injury (i.e. laboratory onset of DILI)
5. Radiological evidence of chronic liver disease such as ascites, hepatomegaly, splenomegaly, nodular contour of the liver, or intra-abdominal varices obtained at least 6 months after the date of onset of the liver injury (i.e. laboratory onset of DILI).

Exclusion criteria:

1. Subjects with known chronic HBV or chronic HCV prior to initiation of the suspect medication will by definition have chronic viral hepatitis and not be eligible for participation in the chronic DILI follow-up protocol.
2. Subjects who have undergone liver transplantation by the 6 month study visit will not be eligible for the long-term chronic DILI follow-up protocol.
3. Subjects with biopsy proven cirrhosis or clinical evidence of portal hypertension due to any chronic liver disease (i.e. ascites, esophageal or gastric varices, hepatic encephalopathy) prior to initiation of the suspect medication.

5. BASIC STUDY DESIGN

The DILIN Prospective Study is a multi-center, prospective, epidemiological study. Consecutive patients who are referred to one of the DILIN clinical sites and appear to have suffered a drug-induced liver injury will be considered for inclusion in the study. Eligibility criteria described in Section 6 will be applied, and those who, in the opinion of a gastroenterologist / hepatologist, experienced a drug-induced liver injury will be enrolled. Detailed clinical data and blood and urine samples will be collected. These data will be reviewed by the DILIN Causality Committee, and it will make the final determination of whether the subject qualifies as a *bona fide* DILI case.

All DILI and CAM cases will be followed longitudinally for at least 6 months to derive the longitudinal profile of liver injury following enrollment. Detailed clinical data will be collected at this time point. Chronic DILI patients will be followed at 12 months and annually thereafter.

6. PILOT TESTING

Pilot testing of the proposed methodology will be conducted before the full-scale implementation of this protocol. The primary purpose of this phase is to determine how difficult it will be to identify and recruit DILI patients, to determine whether the set of evaluations scheduled for the

initial study visit is practicable, and to fine-tune and streamline the data collection forms. If necessary, modifications will be made to the study protocol to insure that the study can be conducted effectively and efficiently.

During the pilot phase, up to three DILI cases will be enrolled at each of the participating clinical sites. If possible, subjects will be recruited from patients who already have an established relationship with one of the clinical center investigators. A DILIN investigator will contact these patients directly and determine if they are interested in participating. Local collaborators, outreach and affiliated community practices will also be contacted, and asked to refer patients who might be eligible to participate. For all subjects, the study will be described and informed consent obtained.

To provide a realistic assessment of the burden of the study procedures, all subjects will have suffered a drug-induced liver injury and satisfy the inclusion and exclusion criteria as described in Section 7.1 below. Because the data collected from controls are a subset of the data for DILI cases, controls will not be recruited for the pilot phase. Similarly, the data scheduled for the follow-up visits are also a subset of the initial study visit data, and the follow-up visits will be excluded as well.

Thus, the pilot phase will be restricted to the screening and initial study visit activities, and all the data and biological specimens described in Section 8.2 will be collected.

It is possible that as a result of this pilot testing, significant changes will be made to the study protocol. If so, these changes will be documented and tracked, and revised applications reflecting these changes will be made to the Institutional Review Boards at the participating clinical sites. It will likely be desirable to continue pilot phase participants in the main study. If so, any changes to the protocol will be explained to these subjects, and informed consent will be re-administered as appropriate.

7. PARTICIPANT ELIGIBILITY, SCREENING, AND RECRUITMENT

7.1 Standard DILI Case Definition

Consecutive patients who are referred to one of the DILIN clinical sites with suspected drug-induced liver injury will be considered for inclusion in the study. The following inclusion and exclusion criteria will be applied at the time of the initial screening.

Inclusion Criteria:

- Age > 2 years at enrollment into the study.
- Evidence of liver injury that is known or suspected to be related to consumption of a drug or CAM product in the 6-month period prior to enrollment.
- Written Informed consent from the patient or the patient's legal guardian.
- Documented clinically important DILI, defined as any of the following:
    1. ALT or AST >5 x ULN or A P'ase >2 x ULN observed on at least 2 consecutive blood draws in patients with previously normal values.
    2. If baseline (BL) ALT, AST or A P'ase are known to be elevated, then ALT or AST >5 x BL or A P'ase >2 x BL on at least 2 consecutive blood draws. "Baseline" is defined as the average of at least 2 measurements performed during the 12-month period prior to starting the DILI medication.
    3. Any elevation of ALT, A P'ase, or AST, associated with (a) increased total bilirubin [≥ 2.5 mg/dL], in the absence of prior diagnosis of liver disease, Gilbert's syndrome, or evidence of hemolysis or (b) coagulopathy with INR > 1.5 in absence of coumadin therapy or known vitamin K deficiency.

Exclusion Criteria:

Patients with any of the following will not be eligible for participation:

- Competing cause of acute liver injury such as hepatic ischemia that is felt by the investigator to be the primary reason for observed liver injury and supported by laboratory tests, serologies, liver biopsy, or radiology.
- Known, pre-existing autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, or other chronic biliary tract disease which may confound the ability to make a diagnosis of DILI.
- Acetaminophen hepatotoxicity
- Liver transplant or allogeneic bone marrow transplant prior to the development of drug- or CAM-induced liver injury.

7.2 Date of Onset

The date of onset is defined as the date of the first qualifying lab value(s) on or after the date on which participant started taking the implicated DILI medication. For example, under Criterion No.1 above, it is the first date when ALT or AST >5 x ULN or A P'ase >2 x ULN. For Criterion No. 3, it is the date when there was an elevation in ALT, A P'ase, or AST, coincident with total bilirubin ≥ 2.5 mg/dL. For Criteria Nos. 1 and 2, there must be two consecutive dates when this elevation was observed. It is recognized that in some cases, the confirmatory elevation may not have occurred on the date of the immediately following liver tests. In this case, the only requirement is that there be two consecutive dates at some point after starting the implicated DILI medication when the required elevations were observed.

7.3 Liver Disease DILI Case Definition

There are multiple reports of DILI arising in patients with underlying chronic liver disease such as chronic hepatitis B and chronic hepatitis C. For example, DILI due to isoniazid and HAART therapy appear to be more common and severe amongst patients with underlying chronic hepatitis B and C [20, 21]. To improve our understanding of the risk factors and outcomes of patients with suspected DILI in the setting of chronic viral hepatitis, we will recruit patients with suspected DILI and known, pre-existing chronic hepatitis B or hepatitis C.

Inclusion Criteria:

- Age > 2 years at enrollment into the study.
- Evidence of liver injury that is known or suspected to be related to consumption of a drug or CAM product in the 6-month period prior to enrollment.
- Known chronic hepatitis B or C infection defined by detectable HBsAg or HCV RNA respectively for at least 6 months prior to DILI onset
- Written Informed consent from the patient or the patient's legal guardian.
- Documented clinically important DILI, defined as any of the following:
    1. ALT or AST >5 x ULN or A P'ase >2 x ULN confirmed on at least 2 consecutive blood draws in patients with previously normal values.
    2. If baseline (BL) ALT, AST or A P'ase are known to be elevated, then ALT or AST >5 x BL or A P'ase >2 x BL on at least 2 consecutive blood draws. "Baseline" is defined as the average of at least 2 measurements performed during the 12-month period prior to staring the DILI medication.
    3. Any elevation of ALT, A P'ase, or AST, associated with (a) increased total bilirubin [≥ 2.5 mg/dL], in absence of prior diagnosis of liver disease, Gilbert's syndrome, or evidence of hemolysis or (b) coagulopathy with INR > 1.5 in absence of coumadin therapy or known vitamin K deficiency.

Exclusion Criteria:

Patients with any of the following will not be eligible for participation:

- Competing cause of acute liver injury such as hepatic ischemia that is felt by the investigator to be the primary reason for observed liver injury and supported by laboratory tests, serologies, liver biopsy, or radiology.

- Known, pre-existing autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, or other chronic biliary tract disease which may confound the ability to make a diagnosis of DILI.
- Acetaminophen hepatotoxicity.
- Liver transplant prior to the development of drug- or CAM-induced liver injury.

7.4 Recruitment and Retention Procedures

The aim of this network is to identify a large number of DILI cases that will contribute data and biological specimens to a repository so that scientific investigations into the causes, risk factors, and natural history of this phenomenon can be undertaken. Patients with potential DILI (and corresponding controls) will be recruited from the five DILIN clinical centers. Each of the clinical centers is a tertiary-care, hepatology unit and anticipates seeing a reasonable number of patients with potential DILI. Additionally, each center will undertake an aggressive strategy to recruit cases from local collaborators, outreach, and affiliated community practices. These individuals will be asked to refer their patients to one of the DILIN clinical sites. Other recruitment mechanisms that are unique to individual clinical centers may also be considered.

In addition to these local efforts, DILIN will undertake nationwide efforts to enhance its visibility and thus enhance the possibility of more referrals from physicians who are not directly involved in the DILIN. Some of these nationwide efforts may include (but not be limited to):

- Advertising: Advertisements of the DILIN in gastroenterology, hepatology, and general medical journals, requesting referrals of patients with potential DILI.
- Letter-writing: Writing letters to physicians (at local and national level through various organizations) to disseminate information about DILIN and to seek referral of patients with potential DILI.
- Website: a DILIN dedicated website will be developed by the DCC and will include general information on DILI, associated internet resources, and contact information for the clinical centers including phone numbers and URL links.
- Educational programs and written materials: Investigators will provide lectures and teaching materials to physicians and other health care providers at national meetings to enhance awareness and to seek referral of patients with potential DILI.
- National Scientific meetings: The investigators, DCC, and NIH will make a concerted effort to hold conferences and symposia at large national scientific meetings, e.g., DDW, AASLD, and ACG meetings to inform practicing physicians of the study. Outreach efforts to large bodies of primary care physicians such as the AMA, ACP, and American Academy of Family Physicians will also be undertaken.

8. INITIAL STUDY PROCEDURES

8.1 Initial Study Visit

The participant will be considered “enrolled” in this study when s/he signs the informed consent document. The initial study visit is the date of the first in-person visit by the participant to the DILIN clinical site. This may occur at the same time as, or shortly after, the time of enrollment into the study. In any event, the initial study visit must occur within 6 months of the date of onset of the liver injury as defined in Section 7.1 above.

8.2 Evaluations Performed at the Initial Study Visit

As far as possible, the following data will be collected from DILI cases during the initial study visit. It is recognized, however, that there will be some cases in which obtaining complete data is not possible.

- Demographics: age, sex, self-reported race and ethnicity, country of birth, geographic area of residence (county and zipcode), along with measures of socio-economic status, e.g., education level, marital status and health insurance status.

- Implicated DILI Medication: detailed medication history of the implicated DILI drug plus any drugs from the same class of medications over the lifetime of the patient that will include the dose, duration, indication. A careful assessment of adherence to medication regimens in general and for the implicated DILI medication in particular will be collected.
- Implicated CAM Product: if a CAM product is implicated as causing the liver injury, then detailed medication history of this product will be collected including the dose, duration, and indication. The name, manufacturer, and list of ingredients will be collected as far as possible. For patients with suspected CAM hepatotoxicity, a limited quantity of each product will be stored for future use.
- Other Medication History: All medications, including prescription and OTC medications, taken by the participant starting 8 weeks prior to starting the implicated DILI product and proceeding to the date of the initial study visit will be recorded. To verify the name and dose of ingested medications, the participants will be asked to bring in the original medication vials and bottles for review.
- CAM products: All herbal/ CAM medications will be queried starting 8 weeks prior to starting the implicated DILI product and proceeding to the date of the initial study visit will be recorded. To verify the name and dose of CAM medications, the participants will be asked to bring in the original bottles in for review.
- Pharmacy data: a questionnaire assessing the types of pharmacy used by cases to fill prescriptions in the past 12 months will be administered.
- Medical history: medical history will be reviewed over the lifetime of the subject including major medical illnesses and personal history of allergies to other medications, systemic autoimmune disorders (e.g., lupus, arthritis); diabetes / endocrine disorders; infectious diseases; heart disease and congestive heart failure; hypotension and hypertension; renal, pulmonary and gastrointestinal diseases; prior surgical history within the past 5 years (unless the implicated DILI medication is a general anesthetic); prior history of liver problems; and, a previously experienced drug-induced liver injury.

  Liver-specific diseases over the lifetime of the subject, e.g., HCV, HBV, alcohol-related liver disease, non-alcoholic fatty liver disease, Wilson disease, hemochromatosis, Gilbert's syndrome, cirrhosis, ischemic hepatitis, organ transplantation.
- Family History: Vital status and demographics of all first-degree relatives including biological parents, all siblings, and biological children, together with a history of liver reactions to drugs as appropriate; for twins and multiple births, further information concerning zygosity.
- Physical exam: height, weight (to derive BMI), vital signs; examination of the liver, abdomen, spleen, skin, etc.
- Smoking history during the 5 years prior to starting the DILI medication [46]
- Alcohol history: semi-quantitative estimate of alcohol consumption during the 5 years prior to starting the DILI medication using the interviewer administered Skinner questionnaire [47].
- Diagnostic blood studies: the following serological tests are required for all study participants to exclude competing causes of acute liver disease. These tests must be obtained after the onset of the DILI episode and should be ordered at the initial study visit if not previously done: IgM anti-HAV, anti-HCV, HCV RNA by PCR, HBsAg, anti-HBc, Anti-HBc IgM, anti-HBs, HBeAg, Anti-HBe, HBV-DNA, Anti-HDV; anti-nuclear antibody, anti-smooth muscle antibody (up to 4 values for each test), AMA (for cholestatic and mixed-pattern cases only), heterophile antibody (monospot), anti CMV IgM, and HIV antibody. For subjects < 50 years of age a serum ceruloplasmin will be obtained. For subjects with a mixed or cholestatic liver injury pattern, an anti-mitochondrial antibody will also be obtained.
- HIV / Hepatitis Risk Factors: risk factors that could have resulted in the subject acquiring HIV or hepatitis disease, e.g., animal contacts, travel history, eating habits, injectable drug use, transfusion, etc.

- Hepatitis B Patients: For patients with known, pre-existing chronic hepatitis B and suspected DILI, the following information will also be obtained starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study visit: HBV DNA by quantitative PCR, HBeAg, anti-HBe, anti-HDV; and, and a detailed log of all hepatitis medications.
- Hepatitis C Patients: For patients with known, pre-existing chronic hepatitis C and suspected DILI, the following information will also be obtained starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study visit: a quantitative HCV RNA level; and, and a detailed log of all hepatitis medications.
- HIV-Positive Patients: for patients chronically infected with HIV, additional data will be obtained including the presence of CMV, herpes simplex, syphilis, and MAI co-infections; detailed summary of serum lactate, amylase, lipases and CPK levels; HIV RNA and CD4 counts that will assist causality assessment; and a detailed log of all antiretroviral medications starting 5 years prior to date of onset of the DILI event and continuing to the date of the initial study.
- Blood Tests: Serum iron, serum transferrin, serum ferritin, serum alpha-1 antitrypsin, serum protein electrophresis; serum IgM, IgG and IgA will be recorded if available but will not be specifically drawn for research purposes.
- Standard blood studies: The results from two sets of blood tests will be obtained. The first set consists of retrospective data starting 8 weeks prior to starting the implicated DILI medication and proceeding up to but excluding the date of the initial study visit. Results from the following tests will be obtained: AST, ALT, alkaline phosphatase, serum total bilirubin, INR, hemoglobin, WBC, %eosinophils, platelets, serum creatinine, albumin and serum direct bilirubin.

  The second set will be derived from a blood sample drawn from participants at the initial study visit. These samples should be drawn in the fasted state whenever possible and indicate if fasting or fed on the data collection form. The following tests will be performed: complete blood count with platelets and manual differential; blood urea nitrogen and serum creatinine; sodium, potassium, serum total protein and serum albumin; AST, ALT, alkaline phosphatase, serum total bilirubin and serum direct bilirubin, INR and prothrombin time; total cholesterol, triglycerides, serum amylase, lipase, CPK, GGTP and LDH; and a urinalysis.

  Up to 75 ml of blood from adult cases and up to 30 ml of blood from pediatric cases will be required for these diagnostic and standard blood studies (depending on what tests had already been performed prior to the initial study visit).
- Research blood samples: In addition to these amounts, 47 ml of whole blood will be drawn in a fasted state from adult and pediatric cases for research purposes: 37 ml of whole blood will be obtained for DNA isolation, plasma, and PBMC cryopreservation for future genetic studies as described in Section 10.1 below; and, 10 ml of blood will be collected using a red topped plastic tube for serum storage. The serum isolated from this blood draw will be centrifuged, aliquoted into cryovials, and frozen at the clinical site. They will be shipped in bulk on dry ice to the NIDDK Biosample Repository at Fisher BioServices (formally McKesson BioServices) for future use. For adults only, an additional 40 ml of whole blood will be drawn and sent immediately to the Rutgers University Cell and DNA Repository (RUCDR) for future mechanistic studies.
- Urine Sample: 50 ml of voided urine will be collected and aliquoted into cryovials at the clinical site and frozen. Frozen samples will be shipped in bulk on dry ice for storage at the NIDDK Biosample Repository at McKesson BioServices.
- Signs and Symptoms at Onset: jaundice, nausea, anorexia, dark urine, fever, abdominal pain vomiting, rash, itching, change in mental status, ascites, edema, hepatomegaly, splenomegaly, and lymphadenopathy.
- Imaging studies: we anticipate that most patients will have undergone an imaging study of the liver (e.g., a liver ultrasound, abdominal CT scan, or abdominal MRI) during evaluation of DILI episode. If the patient has not been previously imaged, a screening liver ultrasound will be obtained for research purposes. Data to be captured from the liver imaging studies

include the presence of biliary dilatation, ascites, liver mass, gallstones, nodular contour of the liver, intra-abdominal varices, splenomegaly and hepatomegaly via yes/ no response. The maximal spleen diameter in cm will also be recorded if available.

- Liver pathology: a liver biopsy may have been obtained for clinical purposes in cases of diagnostic uncertainty or for prognostic purposes. For example, if a patient fails to have improvement in liver biochemistries within 1 month of stopping the suspect drug or if there is a clinical suspicion of autoimmune hepatitis, a liver biopsy may be recommended for diagnostic purposes. In addition, patients with persistently abnormal liver biochemistries 12 months following the onset of DILI event as defined in Section 7.1 may be recommended to undergo liver biopsy for prognostic assessment. If a participant has ever had a liver biopsy in the past, a release form will be signed so that the specimen can be reviewed by the study pathologist for comparative purposes. For patients in whom a liver biopsy is electively planned for diagnostic purposes, a small sample of liver tissue (e.g., 5 mm) will be flash frozen in liquid nitrogen (RNA later) and a 5 mm sample, if available, will also be placed in glutaraldehyde for future electron microscopy studies. If a patient proceeds to liver transplantation or dies during follow-up and an autopsy is performed, formalin fixed liver tissue will be retrieved for study purposes. A minimum of 5 unstained tissue sections and preferably 10 will be sent to Dr. David Kleiner of NIH for histopathological review and interpretation. Criteria outlined in Appendix 18.2 will be applied to grade and stage liver biopsies. The following data will be captured on all liver biopsy specimens: Biopsy demographics (date of biopsy, type of biopsy, total number of portal tracts, liver weight if explant or autopsy, stains available) and histological features using predetermined criteria for inflammation type and severity, hepatic fibrosis staging, steatosis, cholestasis, hepatocellular injury, vascular changes, and miscellaneous changes.
- Quality of life form: the Rand 36-Item Health Survey will be self-administered to all adult subjects; the PedsQL will be used for children.
- Symptom score (visual analogue): patients will be given a self-administered form to assess the following symptoms over the prior week on a visual analogue scale ranging from none to worst ever: fatigue, nausea, pain over the liver area, poor appetite, fever/chills, muscle/ joint aches or pains, weakness in the arms and legs, itchiness, rash, and depressed /sadness.
- History of the Liver Injury: seen by a gastroenterologist / hepatologist; pregnant during the event, extrahepatic manifestations, hospitalized, rechallenged, liver transplantation after starting the implicated drug, biopsy, received prednisone or other corticosteroids; and, how long the patient was sick with the liver injury, and how long was the disruption in daily living.

9. PARTICIPANT FOLLOW-UP

9.1 Evaluations Performed at the Six-Month Follow-up

The date and time of the 6-month study visit will be arranged at the end of the initial study visit. It will be scheduled for 6 months after the initial study visit. The following data will be collected.

- Interval Medication History: All medications, including prescription and OTC medications, taken by the participant since the initial study visit will be recorded. To verify the name and dose of ingested medications, the participants will be asked to bring in the original medication vials and bottles for review.
- CAM products: All herbal/ CAM medications since the initial study visit will be recorded. To verify the name and dose of CAM medications, the participants will be asked to bring in the original containers for review.
- Interval medical history: a medical history and review of systems to capture all changes in health status since the initial study visit including major medical illnesses and personal history of allergies to other medications, systemic autoimmune disorders, (e.g., lupus, arthritis); diabetes / endocrine disorders; infectious diseases; heart disease and congestive heart

failure; hypotension and hypertension; renal, pulmonary and gastrointestinal diseases; interval allergy and surgical history.

Liver-specific diseases since the initial study visit, e.g., HCV, HBV, alcohol-related liver disease, non-alcoholic fatty liver disease, Wilson disease, hemochromatosis, Gilbert's syndrome, cirrhosis, ischemic hepatitis, organ transplantation.

Medical release forms obtained at the initial study visit will be used to obtain medical records of all ER visits, hospitalizations, outpatient visits, and diagnostic tests obtained since the initial study visit. The total number of days hospitalized, number of doctor visits, number of emergency room visits, and number of diagnostic tests and whether they were related to the DILI episode or for other medical diagnoses will be recorded.

Whether a liver transplantation was performed, whether the subject died, and the dates of these events.

- Physical exam: height, weight (to derive BMI), vital signs; examination of the liver, abdomen, spleen, skin, etc.
- Alcohol use questionnaire: semi-quantitative estimate of alcohol consumption since the initial study visit.
- Smoking history since the initial study visit.
- Standard blood studies: The results from two sets of blood tests will be obtained. First, the results from all liver function tests performed since the initial study visit will be recorded including AST, ALT, alkaline phosphatase, serum total bilirubin, and INR.

  The second set will be derived from a blood sample drawn from participants at the 6-month study visit. These samples should be drawn in the fasted state whenever possible and indicated if fasting or fed on the data collection form. The following tests will be performed: complete blood count with platelets and manual differential; blood urea nitrogen and creatinine; sodium, potassium, serum total protein and serum albumin; AST, ALT, alkaline phosphatase, serum total bilirubin and serum direct bilirubin, INR and prothrombin time; total cholesterol, triglycerides, serum amylase, lipase, CPK, GGTP and LDH.

  Up to 75 ml of blood from adult cases and up to 30 ml of blood from pediatric cases will be required for these diagnostic and standard blood studies (depending on what tests had already been performed prior to this visit).
- Diagnostic Studies: Anti-nuclear antibody and anti-smooth muscle antibody will be performed from the blood sample drawn from participants at the 6-month study visit.
- Hepatitis B Patients: For patients with known, pre-existing chronic hepatitis B and suspected DILI, the following information will also be obtained since the initial study visit: HBV DNA by quantitative PCR, HBeAg, anti-HBe; and a detailed log of all hepatitis medications.
- Hepatitis C Patients: For patients with known, pre-existing chronic hepatitis C and suspected DILI, the following information will also be obtained since the initial study visit: a quantitative HCV RNA level; and a detailed log of all hepatitis medications.
- HIV-Positive Patients: for patients chronically infected with HIV, additional data will be obtained since the initial study visit: the presence of CMV, herpes simplex, syphilis, and MAI co-infections; detailed summary of serum lactate, amylase, lipases and CPK levels; HIV RNA and CD4 counts; and a detailed log of all antiretroviral medications.
- Research blood samples: 20 ml of whole blood will be obtained in a fasted state for research purposes. 10 ml of blood will be collected using a red topped plastic tube for serum storage. The serum isolated from this blood draw will be centrifuged, aliquoted into cryovials, and frozen at the clinical site. Similarly, a 10 ml tube of whole blood collected in a blue (citrated) tube will be processed into plasma, aliquoted into cryovials, and frozen at the clinical site. Both sets of cryovials will be shipped in bulk on dry ice to the NIDDK Biosample Repository at Fisher BioServices (formally McKesson BioServices) for future use. For adults only, an additional 40 ml of whole blood will be drawn and sent immediately to the Rutgers University Cell and DNA Repository (RUCDR) for future mechanistic studies.

- Urine sample: 50 ml of voided urine will be collected and aliquoted into cryovials at the clinical site and frozen. Frozen samples will be shipped in bulk on dry ice for storage at the NIDDK Biosample Repository at McKesson BioServices.
- Liver radiology: a liver ultrasound will only be obtained in patients who have persistently elevated AST, ALT, alkaline phosphatase, or bilirubin levels or other clinical evidence of chronic liver disease at the 6 month visit. Data to be captured include the presence of biliary dilatation, ascites, liver mass, gallstones, nodular contour of the liver, intra-abdominal varices, splenomegaly and hepatomegaly via yes/ no response. The maximal spleen diameter in cm will also be recorded if available.
- Quality of life form: the Rand 36-Item Health Survey will be self-administered to all adult subjects. The PedsQL will be used for children.
- Symptom score (visual analogue): patients will be given a self-administered form to assess the following symptoms over the prior week on a visual analogue scale ranging from none to worst ever: fatigue, nausea, pain over the liver area, poor appetite, fever/chills, muscle/ joint aches or pains, weakness in the arms and legs, itchiness, rash, and depressed /sadness.

9.2 Causality Adjudication

Clinical Narrative: After interviewing and examining the patient, the investigator will provide a brief written narrative summarizing the case. The narrative will provide a summary of details not easily captured in the data forms including the presentation, names of the implicated products, other medications, past medical history, pertinent family and social history, physical exam, laboratory studies, diagnostic studies, and a summary of any clinical events following presentation. (See Appendix 17.3 for an example).

Chronic DILI assessment: At the Month-6 visit, the investigator will review all available laboratory, radiological, and clinical information and evaluate whether the patient satisfies the definition of chronic DILI as outlined in Section 4.2.

Causality Adjudication: Using data collected at the baseline and Month-6 visits, as well as the clinical narrative outlined above, a determination concerning causation will be made by the DILIN Causality Committee. If the Causality Committee does not consider the case as clearly due to the drug, it will decide whether the patient can be included as a control, or whether the patient's data cannot be used for this study. Additionally, the committee will review the information provided concerning chronic DILI status, and provide a determination on whether the patient qualifies as a chronic DILI patient. Patients who meet criteria for chronic DILI at 6 months will have a follow-up visit at 12 and 24 months following the baseline visit.

MEDWATCH reporting: After the data have been cleaned and the clinical narrative has been received, a standard MEDWATCH Form 3500 will be completed and forwarded to the Food & Drug Administration.

9.3 Evaluations at the Twelve- and Twenty-Four Month-Follow-up

The date and time of the 12-month study visit will be arranged at the end of the 6-month study visit. Only DILI cases who meet criteria for a definition of chronic DILI will be followed, and it will be scheduled for 12 and 24 months after the initial study visit. The following data will be collected at these visits

- Interval Medication History: All medications, including prescription and OTC medications, currently being taken by the participant within 4 weeks of the follow-up visit will be recorded. To verify the name and dose of ingested medications, the participants will be asked to bring in the original medication vials and bottles for review.
- CAM products: All herbal/ CAM medications currently being taken by the participant within 4 weeks of the follow-up visit will be recorded. To verify the name and dose of CAM medications, the participants will be asked to bring in the original containers for review.
- Interval medical history: a medical history and review of systems to capture all changes in health status since the previous visit will be conducted including major medical illnesses

and personal history of allergies to other medications, systemic autoimmune disorders, (e.g., lupus, arthritis); diabetes / endocrine disorders; infectious diseases; heart disease and congestive heart failure; hypotension and hypertension; renal, pulmonary and gastrointestinal diseases; interval allergy and surgical history.

Liver-specific diseases since the previous study visit, e.g., HCV, HBV, alcohol-related liver disease, non-alcoholic fatty liver disease, Wilson disease, hemochromatosis, Gilbert's syndrome, cirrhosis, ischemic hepatitis, organ transplantation

Medical release forms obtained at the initial study visit will be used to obtain medical records of all ER visits, hospitalizations, outpatient visits, and diagnostic tests obtained since the previous study visit. The total number of days hospitalized, number of doctor visits, number of emergency room visits, and number of diagnostic tests and whether they were related to the DILI episode or for other medical diagnoses will be recorded.

Whether a liver transplantation was performed, whether the patient died, and the dates of these events.

- Physical exam: height, weight (to derive BMI), vital signs; examination of the liver, abdomen, spleen, skin, etc.
- Alcohol use questionnaire: semi-quantitative estimate of alcohol consumption since the previous study visit.
- Smoking history since the previous study visit.
- Symptom score (visual analogue): patients will be given a self-administered form to assess the following symptoms over the prior week on a visual analogue scale ranging from none to worst ever: fatigue, nausea, pain over the liver area, poor appetite, fever/chills, muscle/joint aches or pains, weakness in the arms and legs, itchiness, rash, and depressed /sadness.
- Quality of life form: the Rand 36-Item Health Survey will be self-administered to all adult subjects. The PedsQL will be used for children.
- Standard blood studies: The results from two sets of blood tests will be obtained. First, the results from all liver function tests performed since the previous study visit will be recorded including AST, ALT, alkaline phosphatase, serum total bilirubin, and INR.

  The second set will be derived from a blood sample drawn from all chronic DILI patients at each follow-up visit. The sample should be drawn in the fasted state whenever possible and indicated if fasting or fed on the data collection form. The following tests will be performed: complete blood count with platelets and manual differential; blood urea nitrogen and creatinine; sodium, potassium, serum total protein and serum albumin; AST, ALT, alkaline phosphatase, serum total bilirubin and serum direct bilirubin, INR and prothrombin time; total cholesterol, triglycerides, serum amylase, lipase, CPK, GGTP and LDH.

  Up to 75 ml of blood from adult cases and up to 30 ml of blood from pediatric cases will be required for these diagnostic and standard blood studies (depending on what tests had already been performed prior to this visit).
- Research blood draw: 20 ml of whole blood will be obtained in a fasted state for research purposes. 10 ml of blood will be collected using a red topped plastic tube for serum storage. The serum isolated from this blood draw will be centrifuged, aliquoted into cryovials, and frozen at the clinical site. Similarly, a 10 ml tube of whole blood collected in a blue (citrated) tube will be processed into plasma, aliquoted into cryovials, and frozen at the clinical site. Both sets of cryovials will be shipped in bulk on dry ice to the NIDDK Biosample Repository at Fisher BioServices (formally McKesson BioServices) for future use. For adults only, an additional 40 ml of whole blood will be drawn (at Month 12 only) and sent immediately to the Rutgers University Cell and DNA Repository (RUCDR) for future mechanistic studies.
- Urine sample: 50 ml of voided urine will be collected and aliquoted into cryovials at the clinical site and frozen. Frozen samples will be shipped in bulk on dry ice for storage at the NIDDK Biosample Repository at McKesson BioServices.

- Imaging studies: The results for all imaging studies of the liver (e.g., a liver ultrasound, abdominal CT scan, or abdominal MRI) performed since the previous study visit. Data to be captured include the presence of biliary dilatation, ascites, liver mass, gallstones, nodular contour of the liver, intra-abdominal varices, splenomegaly and hepatomegaly via yes/ no response. The maximal spleen diameter in cm will also be recorded if available.
- Liver pathology: In patients with suspected chronic DILI (based on laboratory, clinical or imaging criteria), a liver biopsy will be recommended for clinical purposes at 12-month visit according to the local standard of care. The recommendation to undergo liver biopsy will depend on the clinical reasons, rather than to support this research project. The results of this liver biopsy will be compared to prior specimens if available. For patients in whom a liver biopsy is electively planned, a small sample of liver tissue (i.e. 5 mm) will be flash frozen in liquid nitrogen (RNA later) and a 5 mm sample, if available, will also be placed in glutaraldehyde for future electron microscopy studies. If a patient proceeds to liver transplantation or dies during follow-up and an autopsy is performed, formalin fixed liver tissue will be retrieved for study purposes. A minimum of 5 unstained tissue sections and preferably 10 will be sent to Dr. David Kleiner of NIH for histopathological review and interpretation. The following data will be captured on all liver biopsy specimens: Biopsy demographics (date of biopsy, type of biopsy, total # of portal tracts, liver weight if explant or autopsy, stains available) and histological features using predetermined criteria for inflammation type and severity, hepatic fibrosis staging, steatosis, cholestasis, hepatocellular injury, vascular changes, and miscellaneous changes. In addition, each biopsy sample will have the pattern of injury categorized into one of the following using predetermined criteria: Acute hepatitic, chronic hepatitic, acute cholestatic, chronic cholestatic, combined hepatitic/ cholestatic, granulomatous, steatotic-macrovesicular, steatotic- microvesicular, steatohepatitic, coagulative/confluent necrosis-zonal, coagulative/confluent necrosis-nonzonal, vascular, hepatocellular alteration, nodular regenerative changes, mild non-specific changes, mixed or unclassifiable patterns, or normal.
- HIV-Positive Patients: for patients chronically infected with HIV, additional data will be obtained since the previous study visit: including the presence of CMV, herpes simplex, syphilis, and MAI co-infections; detailed summary of serum lactate, amylase, lipases and CPK levels; HIV RNA and CD4 counts; and a detailed log of all antiretroviral medications.

10. RESEARCH BLOOD SAMPLES AND REPOSITORY ACTIVITIES

10.1 Collection and Submission of Blood and Urine Samples

After informed consent has been obtained from each patient, staff at each clinical center will draw blood. In addition to the standard blood studies described above, blood will be drawn for the research purposes of DNA storage, PBMC cryopreservation, and serum and plasma storage in the central repository. Cryopreservation of PBMC's (buffy coat) from ACD Vacutainer blood tubes, as well as DNA extraction, plasma separation and aliquoting of plasma from NaEDTA Vacutainer blood tubes will be performed by the Rutgers University Cell and DNA Repository (RUCDR) in collaboration with the clinical centers.

Specifically, two 10ml NaEDTA (lavender top) and two 8.5ml ACD (yellow top) tubes will be drawn. If the initial DNA yield from the submitted whole blood sample is less than 50μg DNA/ml blood, the participant will be requested to return for a repeat blood draw. At that time, two additional 10 ml NaEDTA (lavender top) and two additional 8.5ml ACD (yellow top) tubes will be drawn. These samples will be sent directly to Rutgers without refrigerating, freezing, or delay for immediate DNA extraction and in cases of low DNA yield for cell line immortalization.

For adults only, an additional 40 ml of whole blood will be drawn and sent immediately to the Rutgers University Cell and DNA Repository (RUCDR) for future mechanistic studies.

As well, a free catch urine specimen will be collected into a wide mouth container with a volume of 90 ml or greater. 50 ml of voided urine will be collected and aliquoted into cryovials at the clinical site and frozen. Frozen samples will be shipped in bulk on dry ice for storage at the NIDDK Biosample Repository at McKesson BioServices.

10.2 DNA and Plasma Extraction

DNA Extraction from Whole Blood: Upon sample receipt, tubes will be logged in and labeled with a barcode bearing the DILIN number (e.g. Kxxxxx). The RUCDR sample number (Kxxxxx) and the DILIN ID number will be entered into the RUCDR database. Genomic DNA will be extracted using an automated system (Gentra, Autopure LS). The extracted DNA storage tube and 30μg aliquots at a concentration of 0.2μg/μl (up to six in number) will be stored at –70°C.

Plasma Separation: Upon sample receipt, tubes will be logged in and labeled with a barcode bearing the DILIN number (e.g. Kxxxxx). This RUCDR sample number (Kxxxxx) and the DILIN number will be entered into the RUCDR database. The plasma portion of the blood sample (from the NaEDTA Vacutainer tube) will be isolated by centrifugation. The plasma will be aliquoted into plastic cryovials at one ml volumes up to a total of ten aliquots. The plasma aliquots will be placed in temporary storage at –70°C. Plasma specimens will be shipped in batch to the NIDDK Biosample repository at Fisher BioServices (formally McKesson BioServices) for long term storage and subsequent distribution.

10.3 DNA Quality Control

DNA sample identity and integrity are assured by: (a) individual logging of samples on receipt and contemporaneous establishment of computer records; (b) application of barcodes with ID numbers when samples are logged in, and to all tubes used to process each sample; (c) checking the identification material on each tube before processing; (d) application of analytical procedures to check DNA integrity, absence of cross-contamination, quantity, and quality; (e) careful attention to detail during the entire extraction process; and (f) multiple quality assurance check points at each step of the above procedures and described in detail in the Manual of Procedures.

10.4 Establishment of Cryopreserved PBMC's and EBV Transformed Lymphocyte Cell Lines (LCLs)

Cryopreservation of PBMC's (peripheral blood mononuclear cells)**:** PBMC's will be isolated from ACD Vacutainer tubes as described in the Manual of Procedures. In brief, whole blood is centrifuged in Vacutainer tubes; the buffy coat is removed and resuspended in RPMI-1640. The lymphocytes from this mixture are then separated by centrifugation on a gradient of Lymphoprep. The lymphocyte layer at the Lymphoprep/RPMI-1640 interface is removed and washed twice with RPMI-1640 followed by centrifugation. After the final wash the lymphocytes are resuspended in Freeze media composed of RPMI-1640/DMSO (Dimethyl Sulfoxide)/fetal calf serum. The isolated lymphocytes are then cryopreserved using a computerized control rate freezer and placed into storage for EBV transformation at a later date, should the DILIN Steering Committee warrant it.

EBV Transformed Lymphocyte Cell Lines (LCLs). If requested at a later date, cryopreserved lymphocytes may be immortalized and transformed by RUCDR, as indicated above. Briefly, a series of steps as described in the Manual of Procedures will allow this and include the following: (a) culture initiation and cell line establishment, (b) culture expansion for DNA extraction, (c) cryopreservation of EBV transformed LCLs, (d) freezing of additional transformed cell lines in the event of depletion of stocks. Quality control steps at each stage to insure adequate yields, prevention of contamination, storage, viability of the transformed lines, and back up procedures.

10.5 Distribution of DNA, Serum, Plasma, Urine and Cryopreserved Transformed Lymphocyte Cell Lines

Distribution of Plasma Samples: Plasma aliquots will be shipped on dry ice from the RUCDR to the NIDDK Biosample repository maintained at the McKesson Bioservices in Rockville, MD, on a monthly basis. The applicable data files for cross-referencing the original sample ID number with the RUCDR ID number will be sent electronically. These samples will then be available for distribution for future ancillary studies approved by the DILIN Steering Committee.

Distribution of DNA Samples: DNA samples will be shipped within 2 weeks of approval by the DILIN Steering Committee.

Distribution of Cryopreserved Transformed Lymphocyte Cell Lines: Approved requests for these live transformed cell lines will require an NIDDK approved MTA for the distribution of all biomaterials. The applicable data files for cross-referencing the original sample ID number with the RUCDR ID number will be available to investigators electronically.

11. PATIENT RIGHTS, SAFETY AND CONFIDENTIALITY

11.1 Confidentiality and HIPAA Considerations

Participant confidentiality will be protected throughout the study. All participant data will be kept strictly confidential, and no subject-identifying information will be released to anyone outside the project. Confidentiality will be through several mechanisms. First, each participant will be assigned an anonymous study ID, which will then be used on all study forms. Secondly, any study forms, blood samples, and paper records that contain participant information (e.g., address lists, phone lists) will be kept at the clinical sites in secured, locked areas, coded by number. Once blood is collected, there will be no subject identifiers placed on blood samples, only the study ID number and the date of sample collection. Third, access to all participant data and information, including laboratory specimens, will be restricted to authorized personnel. In the case of computerized data, this restricted access will be assured through user logon IDs and password protection.

At the Data Coordinating Center, only authorized personnel will have access to the data files containing study data. Security will be assured through user logon IDs, passwords and appropriate access privileges. All study participants will be identified only by their DILIN ID number, and no personal identifying information, such as name, address, social security number, etc., will be entered into the Coordinating Center database. Any participant-specific data reported to the Steering Committee or will be identified only by the DILIN ID number.

Finally, participants will not identified by name in any reports or publications, nor will the data presented in such a way that the identity of individual participants can be inferred. Analysis files created for further study by the scientific community will have no participant identifiers. These data files will be created in accordance with the Ancillary Studies and Publication Policy of the DILIN network.

11.2 Informed Consent Procedures

All DILIN participants will provide written informed consent using procedures reviewed and approved by each clinical center's Institutional Review Board (IRB). Study personnel will provide a description of the study's purpose and procedures, and the participant will be provided an information packet including the informed consent document, HIPAA authorization and release of medical record forms. Informed consent will be undertaken by study personnel in person with the subject. The subject has the option of declining further participation in the study at that point. No further study procedures, including the personal interview, retrieval of medical records and blood draw, will be conducted until the signed documents have been provided to the DILIN clinical site.

An assent form will be used for pediatric subjects who are unable to understand the main consent, based on their parent's decision. For older children, a similar consent form as for adults will be used but modified to include references to parent/guardians as necessary. Minor subjects will be reconsented with new forms as the study proceeds and they reach adulthood.

Because a primary purpose of this study is to investigate the genetic determinants of the DILI event, the informed consent process will specifically include consent to participate in the genetics component of this study. This component will be described, and indicate that the participant or members of his/her family may be contacted by one the DILIN clinical sites for a period of 20 years afterwards for subsequent studies. This informed consent document does not oblige the patient to participate in any subsequent study. It only provides the patient's consent

to be contacted. At that point, the patient will be given the option of participating in any subsequent study.

Sample informed consent documents are provided in Appendices 17.4 – 17.8 but will be modified according to the specific needs of the IRB at each participating clinical site.

11.3 Institutional Review Boards

Before initiating this study, the protocol, site-specific informed consent forms, HIPAA forms, recruitment materials, and other relevant information will be reviewed by a properly constituted Institutional Review Board (IRB) at each participating clinical site. A copy of the signed and dated IRB approval at each clinical site will retrieved during the site initiation visit and archived at the Data Coordinating Center. Any amendments to the protocol, other than simple administrative and typographical changes, must be approved by each IRB before they are implemented. The sites will seek annual renewals of their IRB approvals in accordance with local procedures.

11.4 Adverse Events Related to Study Procedures

11.4.1 Definition of an Adverse Event

According to guidelines from the International Conference on Harmonisation (ICH), an adverse event (AE) is any "untoward medical occurrence" to a study participant. It can consist of an unfavorable or unintended sign, symptom, or disease. In controlled clinical trials, especially in a regulatory environment, there are detailed procedures for identifying and reporting AEs to Institutional Review Boards, monitoring bodies, and regulatory authorities. It is noted, however, that this study is an observational, epidemiological study and not a clinical trial. No therapeutic intervention is being delivered to participants under this protocol. To be sure, DILI cases are seriously ill and are expected to experience many "adverse events" during the observational period of this study. However, the vast majority of these AEs will be due to their underlying illness. For this reason, only adverse events specifically related to study procedures will be recorded and reported.

The study procedure most likely to result in adverse events is venipuncture for drawing blood. The corresponding risks include discomfort from the blood draw, including bruising and/or tenderness at the site where the blood is taken, infection and fainting or feeling faint are possible

11.4.2 Serious Adverse Events Related to Study Procedures

It is unlikely that procedures directly related to this study will result in a serious adverse event (SAE). By definition, a serious adverse event (SAE) is any adverse event which (21 CRF §312.32):

- results in death (including suicide);
- is life-threatening;
- requires in-patient hospitalization, or prolongation of an existing hospitalization;
- results in persistent or significant disability or incapacity;
- results in a congenital anomaly / birth defect; or
- is an otherwise important medical event.

"Life-threatening" means that, in the view of the Investigator, the participant was placed at immediate risk of death from the event as it occurred. A "disability" is defined as a "… substantial disruption of a person's ability to conduct normal life functions." An "important medical event" is any other event that jeopardized the health of the participant and required medical or surgical intervention to prevent one of the other outcomes listed above from occurring. ICH guidelines also distinguish between "expected" and "unexpected" adverse events. However, this distinction will not be made in this study – any SAE related to study procedures will be considered "unexpected" and reported.

A "Serious Adverse Event Form" will be used specifically to capture information for each distinct occurrence. Events will be characterized according to severity (e.g., Mild / Moderate /

Severe); frequency (e.g., Single Occurrence / Intermittent / Continuous); resolution (e.g., Completely Resolved / Improved / Unchanged / Worsened); relationship to study procedures (e.g., Possibly / Probably / Definitely); and, action taken with respect to participation in this study (e.g., Unchanged / Temporarily suspended / Participant declined further participation in the study / Investigator removed participant from the study). A narrative describing the circumstances and sequelae surrounding the SAE will be provided.

Following ICH guidelines, an initial report describing the SAE will be reported promptly to the Data Coordinating Center. The DCC will forward this initial report to the Steering Committee (and its own IRB) within 7 calendar days of the clinical site becoming aware of the event. A detailed report will be prepared by the clinical site and sent to the Data Coordinating Center. It will forward this report to the Steering Committee (and its own IRB) within 15 calendar days of the clinical site becoming aware of the event. At the first appropriate moment, the SAE will be reviewed by the Steering Committee and remedial action will be taken as appropriate. Each Principal Investigator will inform his own IRB of the SAE according to local IRB requirements.

Finally, enrollment in the study will be monitored and tracked. The rates and reasons for drop-out and withdrawal from the study will be captured, and periodic reports will be made to the DILIN Steering Committee.

12. QUALITY CONTROL PROCEDURES

12.1 Manual of Procedures

A Manual of Procedures (MoP) will be written to elaborate all study procedures. It is the primary method for standardizing operations and maintaining consistency across the clinical sites and over time. It will contain specific instructions on how the study will be conducted, and what procedures will be performed, in what order, by whom and under what circumstances, and so on.

12.2 Training Sessions and Certification Procedures

Training is an important method for ensuring that all study procedures are performed consistently, accurately and reliably [48]. Site training will be specifically provided on the MoP, data collection activities and for data management activities as described above.

The Manual of Procedures will form the basis for the training session. Sessions will be organized around its chapters, and instructors will be identified from the subcommittees compiling these chapters. Staff will be certified only if they can perform these exercises satisfactorily, and the DCC will maintain a list of study personnel certified to perform various functions.

Training and (re)certification sessions will be repeated periodically as required over the course of the study.

12.3 Site Visits

Site visits will be conducted during this study. They ensure a high level of consistency across the clinical sites [49], and an opportunity to observe whether new study procedures are needed**.** In general, the following checks and procedures will be performed during these sites visits:

1. Tour of Facilities: Evaluate the adequacy of space, equipment and other resources being used for the project; ensure that all equipment required for the study is in place and working properly.
2. Protocol Compliance: Ensure that informed consent has been obtained for 100% of study subjects; screening criteria have been satisfied by 100% of study subjects; all evaluations mandated by the Protocol are accounted for.
3. Data Completeness: Electronic Case Report forms (eCRFs) are sensitive and specific, e.g., all evaluations recorded on the eCRFs are substantiated by information in source documents, and conversely all information in source documents is recorded on the eCRFs; ensure that eCRFs are completed according to Good Clinical Practice (GCP) guidelines.
4. Data Accuracy: Data values entered on the eCRFs were transcribed accurately from source documents.

The DCC will also conduct site visits to each of the CCs. First, a study initiation visit will be performed prior to initiating the study. This will ensure that facilities are adequate, personnel are trained and ready to recruit subjects, and that appropriate regulatory documents have been filed. A subsequent visit will be performed approximately halfway through the study. This is designed to capture protocol deviations and misunderstandings. Corrective action will be taken as appropriate. Training and recertification will be made available to redress deficiencies and misunderstandings. A final close-out visit will be conducted towards the end to verify the completeness of the site file and assist with any final questions regarding the data collected from the site.

On-site visits will be supplemented with in-house coordination (activities that can be handled via phone/ fax communication) to reduce project expenditures and to ensure that trials are conducted according to GCP guidelines. The CCs will be contacted on a routine basis to verify enrollment, review study progress, follow up outstanding queries, and answer any questions.

13. DATA MANAGEMENT PROCEDURES

13.1 Hardware and Software Configuration

Hardware and Database Software: Data will be stored in an Oracle database system. Oracle has advantages of processing efficiency and smooth linkage with other software systems. The application and database will be hosted on Solaris UNIX servers at the DCC. InForm™ will be used for web-based data entry.

Statistical Software: The Statistical Analysis System (SAS) will be used as the principal application for the management of analysis data files and statistical computations. S-Plus and STATA will be used to provide supplementary procedures as needed.

Access Control and Confidentiality Procedures: Access to databases will be controlled centrally by the DCC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage.

Security: Database and web servers will be secured by a firewall and through controlled physical access. Oracle has many security features to ensure that any staff member accessing the database has the proper authority to perform the functions s/he requests of the system. Within the secondary SAS databases, UNIX group-access control maintains similar security. The Sun workstation login is secured by extensive user-password facilities under UNIX.

Back-up Procedures: Database back-up will be performed automatically every day, and standard DCC policies and procedures will be applied to dictate tape rotation and retention practices.

Virus Protection: All disk drives that provide network services, and all user computers, will be protected using virus scanning software. Standard DCC policies will be applied to update these protection systems periodically through the study.

13.2 Sources of Data

Data will be captured and forwarded to the DCC from a variety of sources. First, basic clinical information, e.g., demographic information, will be abstracted from the participant's medical records and charts. They will be entered into the database using the InForm™ web-based data entry system. Additionally, a blood sample will be drawn from study participants and sent to the RUCDR. Tracking information for this sample will be recorded. Cataloguing information from the RUCDR concerning the biological samples will be forwarded to the DCC and merged with the clinical database. A final determination of causality will be provided by the DILIN Causality committee. The results of its determinations will be merged with the clinical database.

13.3 Data Management Activities

13.3.1 Overview

A web-based data management approach will be used in this study. In general, the following activities will be performed.

1. For every record type, the data dictionary (i.e., the database "schema") will identify key fields (e.g., the participant's ID and date of evaluation). As well, for each field, the field type (e.g., numeric, character, checklist, or date) and ranges for impossible and improbable values will be defined.
2. Each data field will be classified as normal or critical. The critical variables are those that must be present and clean for the purpose of data analysis. All data fields requiring queries will be identified, each variable classified, and the query-rule text written. Data entry will be tested and must be functional by the time the first patient is enrolled.
3. A database will be created on the DCRI computer network specifically for this study. As described above, the database will be designed in Oracle and will apply standard security procedures to restrict access to authorized staff.
4. Data-entry screens will be created using the web-based system. The Data Coordinating Center (DCC) will ensure that all data entry screens meet specifications. It will confirm entry screen functionality by entering sample data during beta-testing. It will also enter sample data to ensure that queries are invoked or not invoked as appropriate.
5. Designated personnel at the sites will be trained on the web-based system during an initial training session or during teleconference meetings.
6. Data "workbooks" will also be created to mimic the data entry screens. These paper-based forms serve only as a convenient device for collecting and organizing data extracted from source documents so that they can be entered expeditiously into the web-based system. They have no official status as they typically do for clinical trials in a regulatory environment, and will be by-passed completely during the data audit process.
7. Clinical Center staff will extract the requisite data from source documents and write them in the data workbooks. They will then key-enter these data using the web-based system. Pre-programmed range checks and consistency checks will be implemented as data are entered. Consistency checks will be reviewed by the DCC and queries will be sent to the sites for information. The sites will send the queries back to the DCC with the accurate response.
8. The DCC will run reports periodically to address data quality. These reports will check for consistency of data and will identify highly queried variables. Information derived from these reports will be communicated to the sites through the site management team for resolution and correction in the database. Data collection will be monitored to ensure that data is entered and cleaned in a timely and efficient manner.

13.3.2 Data Management, Quality Control Procedures and Data Audits

Several levels of database quality control will be performed. Any out-of-range values and missing or inconsistent key variables are flagged and addressed/answered at the site in real time during the data entry process. When a query is generated on a particular variable, a flag is set in a field in the database enabling the system to track the queries and produce reports of outstanding queries.

Queries can also be generated from manual review of the data forms. These queries will be entered into the database and tracked in the same manner as the computer generated queries. At regular intervals, all data will be transferred from InForm™ to SAS for statistical summarization, data description, and data analysis. Further crosschecking of the data are performed in SAS, and discrepant observations flagged and appropriately resolved through a data query system. The Data Coordinating Center will perform internal database quality-control checks, and data audits throughout the course of the trial. general, the following issues will be addressed.

1. Data Completeness: Completion by the clinical centers of all evaluations mandated by the protocol is checked.
2. Procedural Errors: Errors in performing study procedures, e.g., taking the blood samples.

Remedial action will be taken as appropriate; otherwise, the Protocol and Manual of Procedures may be revised as appropriate. Training and recertification will be made available to redress deficiencies and misunderstandings.

13.3.3 Data Management Reports

A variety of progress reports will be prepared during the course of a trial and include:

- Data Status Reports: Lag in entering data into eCRFs, missing visits, missing pages, listing of outstanding queries and summary of totals of outstanding queries.
- Quality Control Reports: Duplicates, Missing from Table, blanks.
- Data Surveillance Reports: Query Frequencies, perfect data.
- Recruitment Reports: Numbers of participants screened and enrolled (by age, race, gender, and clinical site); reasons for screening failure (as much as they are available).
- Protocol Deviation Reports: Numbers of ineligible participants enrolled in the study.
- Participant Characteristics: Summary of the demographic and other characteristics of study participants.

Reports will be prepared for the periodic meetings of the Steering Committee and the Data Safety and Monitoring Board. Some reports, such as the recruitment report, may be generated more frequently as required.

14. STATISTICAL CONSIDERATIONS

14.1 Power Calculations and Analyses for Genetic/Genomic Studies

The primary goals of this study are to create a clinical database consisting of individuals who have experienced a drug-induced liver injury, to create a bank of biological specimens obtained from these individuals so that genomic DNA as well as EBV-transformed, immortalized lymphoblasts are available for continuing functional or genetic studies, and to follow prospectively DILI cases to observe the natural history of their disease.

The DILIN Prospective Study is designed as a multi-center, prospective, cohort study. That is, DILI cases will be identified and enrolled by specialists at each participating clinical site. Detailed exposure information prior to the DILI event will be collected using a telephone / personal interview with the participants as well as from patient charts and medical records. Moreover, DILI cases will also be followed prospectively so that the course of the illness, including results of follow-up physical examinations, liver tests, quality of life, other clinical, laboratory, and imaging features following the DILI event, can be observed.

Although we would like to include many thousands of DILI cases in the study, the study is limited by the network's capacity to find and enroll DILI cases over the timeframe of this study. Genetic studies are one of the main focuses in DILIN. For genetics analyses, drug matched controls may not be necessary. This is because DILI is extremely rare (ranging from 1:500 to fewer than 1:10,000 prescriptions, depending on the precipitant drug) so there is a very low probability of misclassification, i.e., DILI cases contaminating the control series. Without drug-matched controls, we plan to use population controls to carry out genetic analyses to determine DILI-associated genetic risk factors. Sample size and power calculations are carried out based on the proposed genetic analyses using existing population control subjects.

Genetic studies will utilize existing population control subjects with available genomic data in case-control analyses. For genome-wide association studies (GWAS), the control cohort includes approximately 6,000 unrelated individuals of primarily European ancestry enrolled in the 1958 British Birth Cohort or the United Kingdom National Blood Service control cohort and genotyped as part of the Wellcome Trust Case Control Consortium (http://www.wtccc.org.uk/ccc2/). Additional controls for GWAS and whole-genome or whole-exome sequencing have been made available by the Duke Center for Human Genome Variation (serving as the genomic center for DILIN). The use of population control subjects is far less susceptible to confounding in genetic analyses in DILI than in traditional epidemiological studies. Because DILI is extremely rare (ranging from 1:500 to fewer than 1:10,000 prescriptions,

depending on the precipitant drug), there is a very low probability of misclassification, i.e., DILI cases contaminating the control series. As proof of principle, the two most credible and successful genetic discovery efforts in DILI to date [89], [90] were GWAS that employed unselected population control samples.

Tests for association for individual variants included in a GWAS, or in whole-genome or whole-exome sequencing studies, will be performed using logistic regression, including quantitative measures of genetically-inferred ancestry as covariates. Due to the large number of tests performed in genome-scale studies, appropriate attention must be paid to the multiple testing problem in order to limit false discoveries. Assuming approximately 1,000,000 tests performed in a complete genomic study of DILI (including both common variants included in a GWAS, and rarer functional variants identified through whole-genome and/or whole-exome sequencing), and targeting an experiment-wide type I error rate of 0.01, a conservative, Bonferroni-adjusted significance threshold is $p < 10^{-8}$ (i.e. 0.01/1,000,000). It should be noted that this threshold is an approximation (benchmark) and thus individual variants yielding p-values just below this threshold may still require additional scrutiny and follow-up (i.e., replication studies). It is hoped, however, that some number of DILI risk variants will be found that clearly surpass this threshold, as has already been observed for the top-associated risk variants from GWAS of flucloxacillin-DILI ($p \sim 10^{-32}$) and amoxicillin/clavulanate-DILI ($p \sim 10^{-14}$).

In a case-control genetic study, power is specified by the following variables: disease prevalence, sample size of both cases and controls, the minor allele frequency (MAF) for variants predisposing to disease risk, the effect size of the genetic risk allele (i.e., genotype relative risk (RR), roughly equivalent to the genotypic odds ratio for rare traits such as DILI), the genetic model, and the specified type I error rate (Menashel et al., BMC Genetics, 9:36, 2008). To simplify this, we have estimated power over a range of effect sizes, minor allele frequencies and sample sizes holding the other variables constant as follows: prevalence of DILI, 1:10,000; additive genetic model; type I error (α) = 0.01 with 1,000,000 effective degrees of freedom (EDF), i.e. $p < 10^{-8}$ for any single variant as described above.

For relatively common genetic risk alleles (i.e. those with a minor allele frequency in healthy individuals of greater than 5%, which is the set of variants typically interrogated in a GWAS), power to detect their effects is fairly constant across the range of allele frequencies as depicted in Figure 14.1. Assuming 5,000 available population controls, sample size estimates between 1,000 patients (roughly the number of DNA samples available for study) and 25 patients (the sample size of many drug-specific or class-specific analyses) give varying levels of detectable effect sizes. Thus, for genetic variants that predispose to DILI generally (i.e. that are not drug-specific) with genotype relative risk greater than approximately 1.8 should be detectable with similar (80%) power across the common variant frequency spectrum. For drug-specific risk variants the minimum detectable effect sizes are on the order of RR > 4 (for studies including n = 50 cases) to RR > 6 (for studies including n = 25 cases).

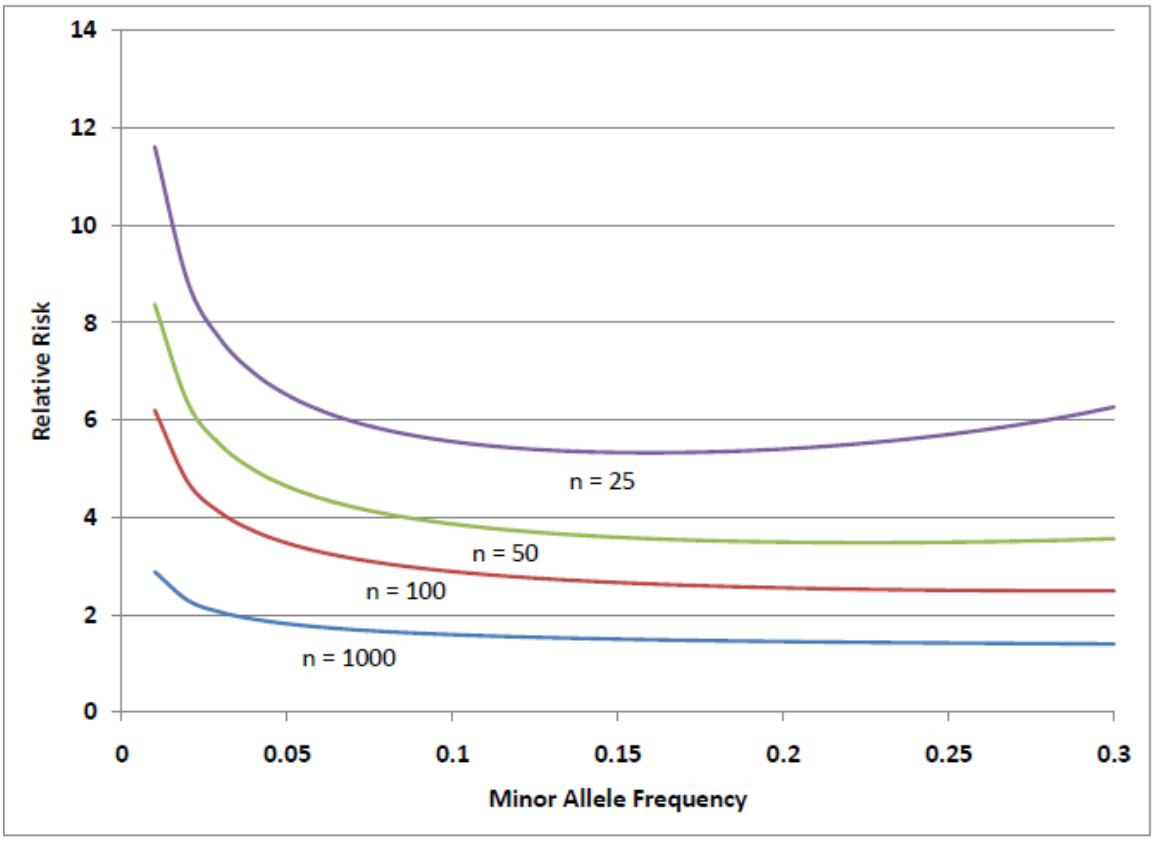

Figure 14.1. Range of detectable genetic risk markers for DILI in a genome-wide association study (GWAS). Assuming 5,000 available controls, a population prevalence of DILI of 1:10,000, co-dominant 1-degree-of-freedom genetic model, and an uncorrected p-value threshold of 10^{-8} we estimate the minimum effect size of a true DILI risk variant over a range of minor allele frequencies with at least 80% power. The different curves represent the minimum detectable relative risk vs risk allele frequency for different sample sizes (n).

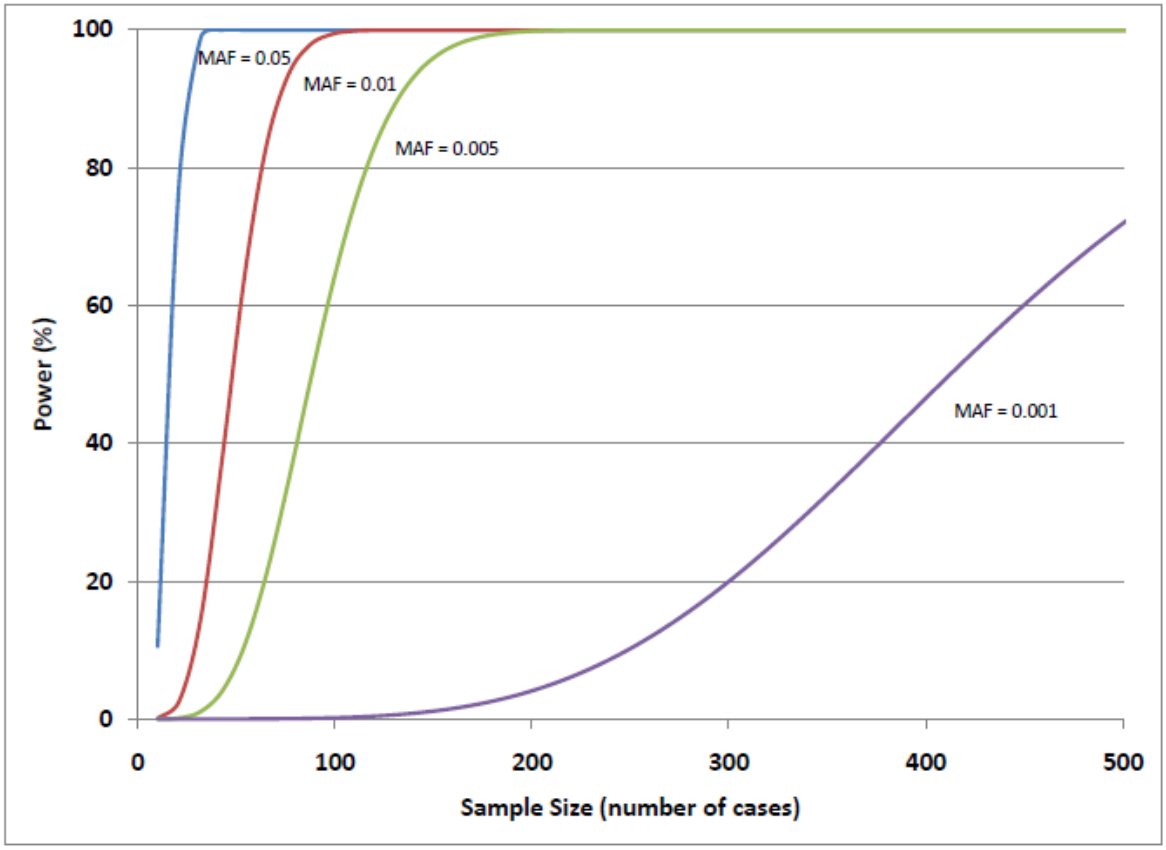

Figure 14.2. Relationship between power and sample size for low-frequency variants.

Assuming a ratio of population controls to cases of 10:1, a population prevalence of DILI of 1:10,000, co-dominant 1-degree-of-freedom genetic model, and an uncorrected p-value threshold of 10^{-8} we estimate power over a range of sample sizes. The different curves represent power vs sample size for risk factors with different minor allele frequencies (MAF).

For variants at the lower end of the frequency spectrum (MAF < 0.05), which includes the set of rare, putatively functional variants interrogated in a whole-genome or whole-exome sequencing scan, power is much more dependent on the risk allele frequency. However, it is anticipated that unlike GWAS of common variants, rare variant discovery through comprehensive sequencing may identify causal variants with much larger effect sizes than have been observed for common risk markers. Figure 14.2 shows the relationship between power and sample size for variants with a genotype relative risk of 10, over a range of different risk allele frequencies, showing that variants with population risk allele frequencies on the order of 0.005 (i.e., 0.5%) or higher should largely be detectable given the sample sizes currently estimated for inclusion in whole-genome/whole-exome sequencing projects. For variants with RR greater than 20, variants with risk allele frequencies down to 0.1% in the general population should largely be appreciable given reasonable sample sizes.

Due to the heterogeneity inherent in a collection of DILI cases due to any drug or combination, statistical analyses have thus far been performed separately for each drug. To date, most of the validated DILI-associated genetic risk factors to date have been drug-specific, providing strong support to this approach. Thus for the power estimates provided above, a focal point should be in the sample size range of 25 to 100, which would represent the available cohort of samples due to the most represented drugs or drug classes in the DILIN network currently. However, it might also be of interest to perform exploratory analysis pooled across all DILI cases and compared with available population controls. This would allow the potential discovery, for example, of associated variants in genes that may be generally involved in immune function, or in liver regeneration or recovery. Power to detect such general risk factors for DILI will be considerably higher than for drug-specific risk factors (see for example, the power associated with sample sizes of 1,000 cases vs that associated with sample sizes of 100 cases or fewer, in Figure 14.1).

Moreover, it may be desirable to consider all patients who develop chronic DILI from the various agents to see if they have unique polymorphisms in fibrosis genes compared to all other patients whose episode resolved in 6 months. Such analyses may be performed on a post-hoc basis.

In addition to combining analyses of all DILI cases across drugs, one might similarly imagine combining analysis of multiple functional variants within each gene, and using the gene as the unit of analysis rather than individual genetic variants (which may in isolation show little evidence of association, e.g. due to allelic heterogeneity). This type of "collapsing" analysis will also be employed using the whole-genome and whole-exome sequence data, with the hope that the reduction in the number of effective degrees of freedom may improve power over single-variant association tests as has been suggested [58].

14.2 Analyses in Case Series and Longitudinal Studies

The DILIN data base provides unique opportunity to determine the DILI-signature of various drugs and/CAM products. Case series publications based on DILIN data are expected where analyses with descriptive statistics are employed. The profile of drug specific DILI-signature will include but not limited to signs and symptoms, time from drug start to onset, time from exposure to enzyme peak, time from enzyme peak to normalization, and causality score. Demographic and clinical features may also be compared between pre-specified subgroups, e.g., by category of causality score.

The natural history component of this study is to derive the longitudinal profile of drug- and CAM-induced DILI for at least 6 months following enrollment. Specific analyses carried out will include but not limited to the following investigations (1) if there are differences between the chronic and non-chronic DILI cases using data up to the 6 month visit; (2) the long-term consequences of chronic DILI patients up to 24 month visit. Chi-square test for categorical data and t-test or non-parametric test will be used for comparisons of groups. Survival analysis will be employed for time to event data such as time to death or time to liver transplant. More generally, longitudinal regression models [50] will be applied to characterize trends in outcome variables over time. Covariates known or suspected to be related to the outcome variable will be included to increase precision so that all effects will be adjusted for these variables. A stepwise regression approach may be adopted to derive a parsimonious list of covariates. For continuous variables, e.g., the results from the liver function tests, the mixed-effects model of Laird and Ware [51] and the model with structured covariance matrices [52] will be considered. For categorical data, e.g., the presence of abnormalities in the physical examination or the results from liver radiology, the generalized estimating equation (GEE) approach of Liang and Zeger [53 54] will be adopted.

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15. STUDY ADMINISTRATION

15.1 Steering Committee

The Steering Committee is the main governing body of the project. It is composed of the Principal Investigators of the clinical centers, the Principal Investigator of the Data Coordinating Center, and the NIDDK Project Scientist. The clinical centers, the Data Coordinating Center and the NIDDK each have one vote on the Steering Committee. All decisions are determined by majority vote.

All major scientific decisions are determined by the Steering Committee. It assumes overall responsibility for the design and conduct of the trial. It appoints (and disbands) subcommittees as the need arises; designs, approves and implements the study protocols; over-

sees the development of the Manual of Procedures; monitors participant recruitment and treatment delivery; evaluates data collection and management; oversees quality assurance procedures; and, implements changes and enhancements to the study as required. It also has primary responsibility for facilitating the conduct of the trials and reporting the project's results.

15.2 Subcommittee Structure

Executive Subcommittee: Although the Steering Committee is the decision and policy-making body of the study, an Executive Subcommittee has been appointed to address the day-to-day activities of the trial and provide overall direction. This group consists of the chair of the Steering Committee, the NIDDK project officer and the Principal Investigator of the Data Coordinating Center. Executive Committee meetings are held regularly by telephone conference.

Retrospective Protocol Subcommittee: This subcommittee has been charged with developing the design of the retrospective protocol. Indeed, this protocol was written as a collaborative effort by this group. The overall basic design, characteristics of the study population, study procedures, data collection, and statistical issues for this design are addressed by this group. This subcommittee will also be responsible for monitoring progress with this study and making changes and additions as required.

Prospective Protocol Subcommittee: This subcommittee has been charged with developing the design of the prospective protocol. The overall basic design, characteristics of the study population, study procedures, data collection, follow-up activities, and statistical issues for this design are addressed by this group. This subcommittee will also be responsible for monitoring progress with this study and making changes and additions as required.

Causality Subcommittee: This subcommittee is responsible for making the causal determination between taking the implicated DILI medication and the observed liver injury. This subcommittee will review the relevant data provided by study participants and abstracted from patient charts and reach a conclusion. It will consider the temporal association between "cause" and "effect" and make sure there are no other competing explanations for the observed association.

Website / Recruitment / Education Subcommittee: Recruitment is always a challenge in randomized clinical trials. The role of this subcommittee is to document and describe effective methods for recruiting participants into the DILIN studies. This subcommittee also has responsibility for overseeing the development of a DILIN website and for links to educate health care professional in the diagnosis and treatment of DILI.

15.3 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the DILIN studies. The description of DSMB activities is included in the DSMB Charter.

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17. APPENDICES

17.1 Schedule of Evaluations

Study Evaluation	Source	Screening	Initial Study Visit	Follow-up: (All DILI Cases)[1]		
				6 mo.	12 mo.[1]	24 mo.[1]
Written Informed Consent	Participant	×				
Screening for Standard DILI Cases: age, drug, liver tests; no competing cause, pre-existing disease, acetaminophen or liver transplant	Clinic Visit	×				
Screening for Liver Disease DILI Cases: age, chronic hepatitis B or C infection, drug, liver tests; no competing cause, pre-existing disease, acetaminophen or liver transplant	Clinic Visit	×				
Screening for controls: age, drug, no evidence of liver injury; matched for clinical center, duration of exposure	Clinic Visit	×				
Demographic Information	Interview		×			
Implicated DILI Medication / CAM History	Interview / Records		×			
Other Medication / CAM Products History	Interview / Records		×	×	×	×
Pharmacy Questionnaire	Interview		×			
Medical History	Interview / Records		×	×	×	×
Family History	Interview		×			
Physical Exam	Clinic Visit		×	×	×	×
Smoking and Alcohol Use	Interview		×	×	×	×
History of liver injury; signs and symptoms at onset	Clinic Visit		×			
Diagnostic Blood Studies	Clinic Visit		×[2]	×		
For hepatitis patients: diagnostic blood studies, hepatitis medications[3]	Clinic Visit		×	×		
For HIV+ patients: diagnostic blood studies, HIV medications	Clinic Visit		×	×	×	×
Standard Blood Studies, Research Blood Sample, Urine Sample	Clinic Visit		×	×	×	×
Imaging Studies (cases only)	Clinic Visit		×[4]	×[5]	×	×
Quality of Life (36-Item Health Survey, PedsQL, VAS Symptom Score)	Interview		×	×	×	×

1. Follow-up is performed for all DILI cases including standard DILI cases defined in Section 7.1 and liver disease DILI cases defined in Section 7.3. The latter group is only followed up to 6 months.
2. Performed only for DILI cases after the onset of the DILI episode. If not previously performed, they should be ordered at the initial study visit.
3. Only for DILI patients with known, pre-existing chronic hepatitis B or C.
4. If the patient has not been previously imaged, an liver ultrasound will be obtained at the initial study visit.
5. A liver ultrasound will only be obtained in cases who have persistently elevated liver tests or other evidence of clinical chronic liver disease at the 6 month visit.

17.2 Classification of Histological Changes in Drug-induced Liver Injury – Patterns of Injury and Feature Scoring System

For protocol purposes, all pathology specimens will be reviewed by the central pathologist (David E. Kleiner, M.D., Ph.D.). An attempt will be made to review an H&E and Masson trichrome stained section on each biopsy, although in some cases only an H&E slide may be available for review. Other stains will be used as necessary and only if sufficient material is available for review.

17.2.1 Patterns of Injury

Each biopsy will be reviewed in the absence of clinical information and an attempt will be made to classify the overall pattern of injury into one of the following categories. The descriptions for each pattern are meant to be guidelines rather than absolute definitions to allow the pathologist some diagnostic flexibility. The categorization is based on pathological patterns previously described for drug induced liver injury [55].

1. **Acute Hepatitic** – predominantly lobular injury with variable infiltrate of lymphocytes and other cells, apoptotic hepatocytes, rosette formation, reticulin collapse, portal infiltrates and interface hepatitis. No cholestasis and little or no fibrosis. Pattern may resemble acute viral hepatitis or mononucleosis.
2. **Chronic Hepatitic** – predominantly portal/periportal inflammation with lymphocytes, plasma cells, macrophages. Spotty lobular inflammation, fibrosis may be seen. No cholestasis.
3. **Acute Cholestatic** – accumulation of bile in hepatocytes and canaliculi, typically in zone 3. May be associated with bile in main ducts, portal edema. Little to no inflammation.
4. **Chronic Cholestatic** – Bile duct injury, bile duct loss, cholatestasis, hepatocellular copper accumulation. May have features of chronic hepatitis. May have visible bile.
5. **Combined hepatitic/cholestatic** – Mixed pattern of lobular inflammation, apoptosis and bile accumulation. Essentially combines patterns #1 and #3 above.
6. **Granulomatous** – necrotizing or non-necrotizing granulomas as predominant form of inflammation.
7. **Steatotic, macrovesicular** – Macrovesicular steatosis alone or with small amounts of microvesicular steatosis. May have small amount of spotty inflammation.
8. **Steatotic, microvesicular** – Predominantly microvesicular steatosis with or without small amounts of macrovesicular steatosis and spotty inflammation.
9. **Steatohepatitic** – Steatosis, spotty lobular inflammation and zone 3 ballooning injury (not due to bile), with or without Mallory bodies and perisinusoidal fibrosis.
10. **Coagulative/Confluent necrosis, zonal** – Patches of hepatocyte necrosis following acinar architecture. Necrosis may be predominantly zone 1, zone 2 or zone 3. Varying amounts of inflammation, especially at edges of necrosis, and reticulin collapse may be present.
11. **Coagulative/Confluent necrosis, non-zonal** – Patches of hepatocyte necrosis showing no relationship to acinar architecture. Varied amounts of inflammation may be seen.
12. **Vascular** – Catch all category for a variety of vascular injuries, including veno-occlusive disease, peliosis, marked sinusoidal dilation, and portal venopathy.
13. **Hepatocellular alteration** – cytoplasmic inclusions, glycogenosis, phospholipidosis as only or dominant change.
14. **Nodular regenerative hyperplasis**
15. Mixed or unclassifiable patterns – Specify if multiple patterns present.
16. **Mild, nonspecific changes** – e.g. very mild steatosis, portal/lobular inflammation that is too mild to categorize with confidence.
17. **Absolutely normal** – no detectable abnormalities whatsoever.

17.2.2 Feature scoring

Because of the huge variety of patterns, there are potentially a large number of features that are of interest to track. Although all features will be recorded on each biopsy, most biopsies will only have positive values for a subset of the features, based on the pattern of injury present. To the extent that it was possible, the scores are based on validated systems or systems in use by other current multi-center clinical trials.

The features are organized into logical sections by type of change.

#	Feature	Values (Range)	Reference or Clinical Trial
1	**Biopsy Demographics**		
1A	Date of biopsy	Date	
1B	Date of central review	Date	
1C	Specimen Type	Needle, Wedge, Resection, Explant, Autopsy	
1D	Total Portal Areas	0-255	
1E	Liver weight (Explant or Autopsy only)	Weight in grams	
1F	Stains available (mark all that apply):	H&E, Masson, Iron, PAS with diastase, Copper, Other (list).	
2	**Inflammation**		
2A	Interface hepatitis	0-4	[56]
2B	Lobular inflammation	0-4	[56]
2C	Portal inflammation	0-4	[56]
2D	Confluent Necrosis	0-6	[56]
2E	Granulomas	0: None 1: Microgranulomas only 2: Non-necrotizing epithelioid granulomas 3: Necrotizing granulomas	
2F	Plasma cells	0: None to mild 1: Noticeably increased	
2G	Eosinophils	0: None to mild 1: Noticeably increased	
2H	Neutrophils	0: None to mild 1: Noticeably increased	
2I	Lymphoid aggregates	0: None 1: Lymphoid aggregates 2: Germinal centers	
2J	Bridging necrosis	0: None 1: Bridging necrosis 2: Multiacinar collapse	
2K	Lipogranulomas	0: Absent 1: Present	
	Fibrosis		
3A	Stage	0-6	[56]
3B	Perisinusoidal	0: None 1: Mild, requiring Masson to see 2: Moderate to Marked, visible on H&E	Adapted from ref. [57]
	Steatosis		

4A	Character	0: Absent 1: Predominantly Macrovesicular 2: Mixed Macro and Microvesicular 3: Predominantly Microvesicular	
4B	Location	N/A, Predominantly zone 3, Predominantly zone 1, Panacinar, Azonal	[57]
4C	Degree	0: None 1: <5% of hepatocytes 2: 5-33% of hepatocytes 3: 33-66% of hepatocytes 4: >66% of hepatocytes	HALT-C

	Cholestasis		
5A	Cholestasis, Degree	0: Absent 1: Cholestasis identified only after careful high magnification search 2: Cholestasis easily demonstrated at high magnification (40x), but not readily apparent at low magnification 3: Cholestasis readily apparent at low magnification	
5B	Hepatocellular	0: Absent 1: Present	
5C	Canalicular	0: Absent 1: Present	
5D	Cholangiolar	0: Absent 1: Present	
5E	Ductal	0: Absent 1: Present	
5F	Cholatestasis	0: Absent 1: Present	
5G	Ductular reaction	0: None to mild 1: Prominent	
5H	Duct Injury	0: None 1: Single duct 2: Multiple ducts	
5I	Duct Paucity	0: None discernible (ducts in > 75% of portal areas) 1: Mild duct paucity (ducts in 50-75% of portal areas) 2: Moderate to marked (ducts in <50% of portal areas)	
5J	Acute cholangitis	0: Absent 1: Present	

	Hepatocellular Injury		
6A	Ballooning degeneration	0-2	NASH CRN
6B	Apoptosis	0: None to rare 1: Mild (<1 per 40x hpf) 2: Moderate (1-3 per 40x hpf) 3: Marked (>3 per 40x hpf)	
6C	Coagulative necrosis location	0: None 1: zone 1 predominant 2: zone 2 predominant 3: zone 3 predominant 4: panacinar	

		5: azonal	
6D	Coagulative necrosis degree (fraction of parenchyma affected):	0: None 1: Minimal (<5% necrosis) 2: Mild (5-33%) 3: Moderate (33-66%) 4: Marked (>66%)	
6E	Hepatocyte Rosettes	0: None to rare 1: More than rare	
6F	Lobular Disarray	0: Absent 1: Present	

	Vascular		
7A	Veno-occlusive changes in central veins	0: Absent 1: Present	
7B	Central Vein Endophlebitis	0: Absent 1: Present	
7c	Portal venopathy	0: Absent 1: Present	
7D	Hemorrhage	0: Absent 1: Present	
7E	Sinusoidal Dilation	0: None to mild 1: Moderate to Marked 2: Peliotic	
7F	Nodular transformation	0: Absent 1: Present	

	Miscellaneous changes that don't require special stains		
8A	Hepatocyte ground glass change	0: None 1: Scattered cells 2: Diffuse	
8B	Hepatocyte globular cytoplasmic inclusions	0: Absent 1: Present	
8C	Mallory bodies	0: None to rare 1: Definitely present	NASH CRN
8D	Lipid-laden stellate cells	0: None to rare 1: Prominent	
8E	Hepatocellular glycogenosis	0: Absent 1: Present	
8F	Polarizable talc crystals	0: Absent 1: Present	

	Special stain evaluation (only performed if particular stain is available)		
9A	Iron stain - Hepatocellular iron	0-4	HALT-C
9B	Iron stain - Sinusoidal reticuloendothelial iron	0: Absent 1: Rare positive cells 2: Prominent	
9C	Iron stain - Portal reticuloendothelial iron	0: Absent 1: Present	
9D	Copper stain - Hepatocellular copper	0: None 1: Periportal hepatocytes positive in <50% of portal areas 2: Periportal hepatocytes positive in >50% of portal areas	
9E	PAS/diastase stain - PAS-	0: None 1: Scattered	

	positive macrophages	2: Clusters	
9F	PAS/diastase stain - PAS-positive hepatocellular inclusions:	0: Absent 1: Present	

17.2.3 Tissue Collection

Prospectively obtained biopsies: Liver biopsies should be processed as per protocol if tissue is to be set aside frozen or in RNA later. Note that since biochemical analysis may be important for evaluation of a drug induced liver injury, setting aside frozen tissue at the time of biopsy, while optional, may be considered part of the standard of care. The biopsy should then be placed as soon as possible into 10% buffered formalin. It is recommended that at least 1.5 cm of a liver needle core be processed for routine histopathological examination. For the purposes of protocol call review, 10 unstained slides cut from the formalin fixed block should be collected and sent _____.

Transplantation and Autopsy: It is possible that during follow-up some patients may die or undergo liver transplantation. In the event of death, an autopsy can be critical in the investigation of drug-induced injury. In both of these situations, liver tissue should be obtained as soon as possible after removal and fixed in 10% buffered formalin. Tissue should also be preserved in the other ways noted above. The pieces of formalin-fixed liver may be shipped in formalin to _____. One or more paraffin blocks can be made and used for the evaluation as well as archived for future studies.

Retrospectively obtained biopsies: If they exist, pre-event biopsies may be critical in determining the presence or absence of liver disease prior to the new injury. Previous biopsies may have been performed for evaluation of liver disease or may have been obtained as part of biopsies or resections of primary or metastatic lesions. If possible, 10 unstained recuts should be obtained as described above. If it is not possible to send unstained slides, permission should be obtained to release some of the original slides for review. If original slides are sent, then at least 1 H&E slide should be sent along with any special stains that were prepared in the initial evaluation.

17.3 Sample Clinical Narrative

A ___ y.o. ___ with a past medical history of x, y, and z presented on ___/ ___ to his local physician/ ER with symptoms of a,b,c. The patient had been receiving drug x,y, and z at e,f, and g doses for a,b,and c indications. Initial laboratory studies revealed a hemoglobin of ___, total WBC of ___ with x differential, serum AST ___, serum ALT ___, alkaline phosphatase ____, bilirubin ___ and INR of ____. Physical exam revealed ____, ____. Diagnostic studies to exclude other causes of liver injury included x, y and z serology and d imaging. A liver biopsy was/ was not performed. After the suspect agent, z, was discontinued on ___/ ____, his liver injury did/ did not improve. The patient was/ was not hospitalized. Social history was significant for x and y. At the time of the initial study DILIN study visit, the following additional information was obtained : ____, _____, _____.

17.4 Informed Consent and HIPAA Authorization for Adult Cases

17.5 Informed Consent for Adult Cases

Informed Consent for Adult Cases

[Name of Institution]

Consent to Participate in a Research Study

IRB Study #

Consent Form Version Date:

Title of Study: Drug-Induced Liver Injury Network: A Multi-Center, Longitudinal Study of Drug- and CAM-Induced liver Injury

Principal Investigator:

Phone number:

Sponsor: National Institutes of Diabetes and Digestive and Kidney Diseases

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You are being asked to take part in a research study. The investigators listed above are in charge of this protocol; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating in research. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your future care at this institution or your relationship with your doctor. If you are a patient with an illness, you do not have to participate in research in order to receive treatment.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this study is to try to understand the reasons why some people have unwanted liver reactions after taking certain drugs and other people do not.

You are being asked to participate in this research study because you have taken a drug or complementary and alternative medication (e.g., a non-prescription, herbal preparations), and you may have developed a liver reaction while taking the drug in question. **This study does not involve any treatment or intervention for any symptoms.**

How many other people will be participating in this study?

More than 1400 individuals will be participating in this study from more than 9 institutions. At this institution, you will be one of at least [fill in] individuals.

What will happen if you take part in this study?

If you choose to take part in this study, the following will occur:

There will be a Screening (visit/Initial Study Visit to the clinic. There will potentially be follow up visits at 6 months, 12 months, and 24 months. The following procedures will take place at one or more of the visits:

1. If you are agreeing to participate in the study, you will be asked to sign three forms at the Screening/Initial Study Visit: 1. the consent form (this document); 2. The HIPAA authorization form (to permit relevant health information to be obtained from medical records) and; 3. The medical release of information form. You will be able to keep a copy of all these forms.

2. Once you have signed the consent form, you will have a physical exam (initial study visit). You will be asked for your age, sex, and race, questions about medication history, medical history, family history, and exposures to a variety of potential risk factors including smoking and alcohol. You will be asked to complete some questionnaires that ask about your quality of life and current symptoms.

3. If you have had a liver biopsy in the past, you will be asked to sign a medical release of information form during the consenting process at screening so that the biopsy sample can be reviewed and a small amount of the sample used for this study

4. At each visit (initial through 24 month follow-up), you will have a standard blood drawn and urine sample collected. For the standard blood draw, up to 75 ml of blood may need to be collected. The urine sample will consist of 50 ml of urine being collected. Blood will be drawn by a qualified person.

5. At the time you have blood drawn and a urine sample collected for standard laboratory tests (at each visit), additional samples will be obtained for research purposes. Blood will be drawn by a qualified person. The additional research sample will amount to a little more than 10 tablespoons of blood (156 ml) from a vein in your arm. Very rarely, we may need to contact you for a second (subsequent) research blood draw. In this case, an additional 3 tablespoons (37 ml) of blood will be drawn from a vein in your arm.

6. Some of these biological samples (blood, urine, and liver biopsy if available) will be sent to research collaborators outside the hospital for research testing.

7. You will have a follow-up visit at 6 months where you will have another physical exam, be asked questions about your health and medical and medication histories, answer questionnaires on smoking and drinking history and provide a blood and a urine sample (see #4) Some of these blood and urine samples will be sent to qualified medical researchers outside the network for research testing. If your blood work suggests that your liver injury has not completely resolved, and you have not recently had a liver imaging procedure, you will be asked to undergo a liver ultrasound examination. The research study will cover the costs of this ultrasound examination. You will also be asked to return for repeat blood work in 6 additional months.

8. If your liver has not returned to normal at the 6 month visit, you will have another visit 6 months later. At this 12 month visit you will again have blood drawn (see #4) and be asked to provide a urine sample. During this visit, you will also have a liver ultrasound. The costs of the research ultrasound will be covered by the research study. If your evaluation suggests that your liver injury has not completely resolved, you will be asked to return for a final visit one year later (24 months from your initial visit). The blood work and ultrasound will be repeated.

9. Quality of Life measures (36-Item Health Survey and VAS Symptom Score) will be collected at the Initial Study Visit and all follow-up visits.

10. Medical history, medication history, smoking and alcohol history, and Complementary and Alternative Medications' history will be collected at the Initial Study Visit and all follow-up visits.

11. You will be contacted (usually by mail) each year for up to 20 years and asked to update your contact information.

12. Based on the information we obtain about you, the drug reaction you had or what is found in your blood, DNA, urine, and liver samples, you may be eligible to participate in future studies. You can decide at that time if you want to participate. **Signing this consent form does not mean you are automatically enrolled or are obligated to participate in these studies.**

13. You will be asked to provide your social security number to the study PI or associates. This is for the purpose of being compensated for study participation and the social security number is needed for tax purposes.

You will potentially be in the study for 24 months (initial study visit, 6 month follow up visit, 12 month follow up visit, and 24 month follow up visit). As noted above, you may be contacted for up to 20 years and asked to update your contact information.

What will happen to the biological samples and/or data?

Your blood will be separated into its different types of cells, stored, and used later for many different analyses to study why some people have unwanted liver reactions after taking certain drugs and other people do not. Genetic material called DNA that is obtained from the cells in your blood will be used to search for genes that may have increased your risk of having a liver injury from a drug. This is a type of genetic research. The research results of these studies will not be put in your medical records.

Some of your blood cells will be grown in the lab others will be stored and used later by approved researchers. The cells, along with the DNA they can provide, will be used in future research projects to understand why some people have reactions to drugs and others do not. Your biological samples (i.e., the blood, urine, and liver samples) and/or data will be stored with only an identification number and the date the sample was collected. Your name will not be kept with these biological samples or data. The "key" which links your identity and contact information to your biological samples and data will be kept secure and will not be given out to any researcher.

Biological specimens will be stored in two biobanks. Data, including medical record information, exposure and questionnaire information and information obtained from the biologic specimens will be collected and stored at the statistical center for the project. When specimens and/or data are shared with researchers, only your coded specimen or information will be given to researchers. Researchers will not obtain your name or other traditional ways to identify you.

Because this research is funded by the National Institutes of Health (NIH), data from this study will be shared with the NIH database, a centralized government funded database. However, only coded, de-identified data will be submitted to the database. We will NOT share your name, address, medical record number or any other traditional identifying information with the NIH database. Depositing data in the NIH database promotes widespread datasharing and allows approved investigators to more rapidly address health problems.

Any research records that identify you will be kept confidential as required by law. In order to meet federal and state research regulations your records may be reviewed by representatives from the National Institutes of Health, the Food and Drug Administration, the [Institution's] Institutional Review Board, the Data Coordinating Center at the Duke Clinical Research Institute, [add any others as appropriate]. If your research record is reviewed by any of these groups, they will take every precaution to protect your privacy.

Will you receive any results from the research on the biological samples?

You will not receive any results from research tests on your blood, DNA, urine, or liver tissue samples. However you can find out the types of studies that have been done with these samples and the overall results of these studies by asking the research staff or reading a future newsletter. Results of the research studies will not be placed in your medical record.

In contrast to research results, standard clinical test results and liver imaging results that may require further follow-up will be provided to you or your doctor. The results of such clinical tests, which are part of standard medical care for your condition, will become a part of your permanent medical record per hospital policy.

Subject Initials _____

Are there any reasons you should not participate?

You should not participate in this study if:

- You do not want your medical records to be shared with the research team.
- You do not want to give blood for study purposes.
- You do not want to participate in the genetic component of this study.
- You do not want the information and data from the current study placed in the NIH database for sharing with approved researchers worldwide.

What are the risks of participating in the study?

Participation in this study might involve the following risks and/or discomforts to you:

Physical Risk: Risk of blood drawing:

The risks of having blood drawn may include bleeding, pain, bruising, and/or tenderness at the site where the blood is taken, infection and feeling faint or fainting. Most of these risks are rare. Only qualified staff will be allowed to draw your blood. Whenever possible the blood for research will be drawn at the same time you have blood drawn for your clinical care.

Diagnostic ultrasound is a safe procedure that uses low power sound waves. There are no direct risks from ultrasound. Ultrasound does have its limitations in that it doesn't travel well through air or bone; therefore, it is not an effective tool for imaging parts of your body that have gas in them or are obscured by bone.

Non-Physical Risks: The greatest risk to you is the breach of your privacy or the confidentiality of your information.

What about your privacy and confidentiality?

The privacy and confidentiality of your information are very important to us and we will use many safety measures to protect you. However, in spite of all of the safety measures that we will use, we cannot guarantee that your identity will never become known or that confidential information will never be inadvertently released. Although the databases developed for this project or at NIH will NOT contain your name, address, telephone number, or medical record number, in the future, people may develop ways that would allow someone to link your genetic or medical information in the databases back to you. Since some genetic variations can help to predict future health problems for you and your blood relatives, this information might be of interest to health providers, life insurance companies, and others. As with any research study, there may be additional risks that are unknown or unexpected,

A new federal law, **called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information**. This applies to genetic information obtained in research or in clinical care. This new Federal law, however, does NOT protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Subject Initials _____

In addition to the federal law, GINA, we plan to greatly minimize risks to you by doing the following:

- Not disclosing any results of the genetic research to you or placing research results or other research information in your medical record.
- Coding your biologic specimens and information related to or derived from these specimens
- Researchers will receive only coded specimens or data
- Limiting access to the link between the code and your identity to only a few approved research staff at [institution].
- Protecting the security of this link by keeping this information in a password protected file on a password protected computer and only having the few approved research staff have access to the passwords
- [Institution] will not share your name or other identifiable information with any person or persons with which it collaborates, including the NIH database.
- No subjects will be identified in any report or publication about this study
- A Certificate of Confidentiality has been obtained to further protect your privacy.

Certificates of Confidentiality are issued by the National Institutes of Health (NIH). They are issued when sensitive information about clinical and genetic risk factors will be collected during the course of the study. The Certificate will help researchers avoid involuntary disclosure that could expose subjects or their families to adverse economic, legal, psychological, and social consequences. The Certificate does not protect you from being compelled to make disclosures that: 1. Have been consented to in writing by the research subject or the subject's legally authorized representative; 2 are required by the Federal Food, Drug, and Cosmetic Act or regulations issued under the Act; or 3. have been requested from a research project funded by NIH or DHHS by authorized representatives of those agencies for the purpose of audit or program review. The full protection afforded by this Certificate, especially in criminal cases, has not yet been tested. A Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research.

Your record may be reviewed in order to meet federal or state regulations. Reviewers may include auditors or representatives of the National Institutes of Health and/or the Duke Clinical Research Institute. If either of these groups reviews your research record, they may need to review your medical record.

Publication of Data

Please note that information obtained from you and others participating in this research study may be published in peer-reviewed medical journals. None of your personal-identifying information will be used in the publications.

What are the possible benefits?

There will be no direct benefit to you for participating in this study. However, if the research team can begin to identify factors that make people have certain drug reactions, in the future people may be able to be tested before receiving a drug to see if they are likely to having an unwanted reaction. In addition, once it is discovered why some people have these reactions, it may be possible to design drugs that will be safer.

What alternatives are there to participation in this study?

The alternative to participating in this study is to not participate and continue with your standard medical care.

What if we learn about new risks during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

What if you are injured as a result of being in this study?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all the safety measures, you might develop a reaction or injury from being in this study. In case of injury, please contact [INSERT DOCTORS NAME AND SITE CONTACT PHONE NUMBER (24 HOUR NUMBER, IF APPLICABLE)]. Immediate necessary care is available if you are injured as a result of taking part in this study. However, there is no provision for free medical care or for monetary compensation for such injury.](This last statement is to be included only if the sponsor has no provision to pay healthcare needed as a result of a study-related adverse event).

Will you be paid for participating?

You will receive [monetary amount] for participating in the study. [Each site needs to describe how and when payments will be made].

With every research study there is a possibility of commercialization of some product or test. If this happens you will NOT be compensated.

Will it cost you anything to participate?

There will be no cost to you for participating in the research study. Tests or procedures that are performed as part of your standard medical care will be your responsibility.

Who is sponsoring this study?

This research is funded by the U.S. National Institutes of Health. This means that the research team is being compensated by the U.S. government for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the outcome of the study.

What if you want to stop before your part in the study is complete?

You are free to leave the study at any time. If you leave the study before it is finished, there will be no penalty to you, and you will not lose any medical benefits to which you may otherwise be entitled. In addition, your participation in this study may be terminated, with or without your consent, by your physician if he/she believes it to be in your best interest or by the study sponsor. [Institution needs to insert how termination would affect payment/compensation to the research subject].

If in the future, should you decide that you no longer wish for the biological samples (i.e., the blood, DNA, urine and liver samples) to be stored and used for research, you may contact [Site PI] and/or his associates at [Institution] at [telephone number] or [Name of local IRB] at [telephone number]. Your request must be made in writing. Once your request is received the specimens that remain in the bank will be disposed of according to standard medical research procedures. This means that any specimens remaining in the biobank will no longer be used for research. Specimens that have already been distributed, however, will continue to be used. If you do not make such a request, the specimens will be stored up to 20 years. In addition, the specimens may be disposed of at any time at the discretion of the investigators.

Will biological specimens and/or information gathered for this study be used in future research studies?

By participating in this liver injury study, biologic specimens and data may be used in future research about liver injury. The specimens you contribute and associated information and study results can be very valuable for other types of research as well. You can decide whether or not you want the specimens and data to be used for future research in diseases or conditions other than liver injury, such as for research in heart disease, cancer, or mental illness. Some of these studies may involve genetic research. It is difficult to determine exactly what type of studies will be conducted in the future. You do not need to agree to the non-liver studies if you don't want to. It will not affect your participation in the liver injury studies. If you do decide to have the specimens and data used for other non-liver diseases in the future, we will take every precaution to ensure that all the policies and procedures described in this consent form are followed by future investigators. This includes having future researchers work with only coded samples or data and not having access to your identity.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions, or if a research-related injury occurs, you should call ***(PI's Name)*** at ***(PI's Number with Area Code)*** (24 HOUR NUMBER, IF APPLICABLE).

What if you have questions about your rights as a subject?

This research has been reviewed and approved by (Institutional Review Board Name) _____. If you have any questions or concerns regarding your rights as a research subject, you may contact *[the IRB name and contact number]*

Subject Initials _____

- -

Subject's Agreement:

"The purpose of this study, procedures to be followed, risks, and benefits have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to be in this study with the understanding that I may withdraw at any time. I understand that my decision to participate or not participate in this study will not affect my medical care. I have been told that I will be given a signed copy of this consent form." I voluntarily agree to participate in this study in the following way(s): (please circle your answer)

Yes No I agree to contribute biological samples and associated data and allow my medical information to be used in this study and for future research related to liver injury or liver disease. Some of these studies may include genetic research.

Yes No In addition to studies of liver injury or liver disease, I agree that the biological specimens and associated data collected for this study, can be used for future research for conditions, including but not limited to heart disease, cancer or mental illness. Some of these studies may include genetic research.

_____ _____

Signature of Research Subject Date

_____

Printed Name of Research Subject

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

17.6 ADDENDUM to Consent Form for Participating in a Research Study (HIPPA Authorization for use of Protected Health Information)

DRAFT: ADDENDUM TO CONSENT FORM FOR PARTICIPATING IN A RESEARCH STUDY (HIPAA Authorization for use of Protected Health Information)
[Name of Institution]

IRB Study Number:
Version Date of This Form:

Title of Study: Drug-Induced Liver Injury Network: A Multi-Center, Longitudinal Study of Drug- and CAM-Induced liver Injury

Principal Investigator:

Mailing Address:

Sponsor: **National Institutes of Diabetes and Digestive and Kidney Diseases**

What is the purpose of this form?
You are being asked to take part in a research study. The consent form for this study describes your participation, and that information still applies. This extra form is required by the federal "Health Insurance Portability and Accountability Act" (HIPAA). The purpose is to get your permission (authorization) to use health information about you that is created by or used in connection with the research. If you are signing on behalf of someone other than yourself, this permission applies to that person's health records.

What is Personal Health Information (PHI)?
Personal health information is information that is contained in your medical or health records. This can include information about your current condition, about your medical history, about the drugs you have taken in the past and how you have responded to those drugs and other health information that is unique to you.

What personal health information am I allowing to be used for this research study?
The information we might use includes:

- Information about your medical history (other medical conditions you have and medications you are/were taking)
- Information about the your liver (symptoms; results of tests, such as blood tests or imaging studies; results of other procedures, doctor's notes, laboratory evaluations)
- Contact information, including name, address, telephone number(s), e-mail addresses from you and close relatives; and date of birth.

What if I don't want my personal health information to be used in this research study?
You may refuse to give this permission. A decision not to sign this form will not change your ability to get health care outside of this research study. However, you may not be able to participate in this research study unless you sign this permission form. You should discuss this, and any other questions, with the investigators.

Subject Initials _____

Where will investigators go to find my personal health information?

We may ask to see your personal information in records at hospitals, clinics, or doctor's offices where you have received care in the past or are currently receiving care Based on what we know at this time, the places we will seek access to your records include:

- If you have been in the hospital for the problems with your liver, we will request records from that hospital
- Your local doctor if the liver problem was first identified there, or if you continue to see that doctor to manage your symptoms
- Records from [Institution] Hospitals if you are seen there.

Who will be allowed to use and disclose my personal health information for this research and why?

The investigators named above and their assistants will be allowed to see and to use your health information for this research study. We may use it to check on your progress during the study, or analyze it along with similar information from all other subjects. Sometimes research information is shared with collaborators at other institutions, or with laboratories running additional tests. Personal health information from all the individuals who participate in this study will be kept secure and stored at the Duke Clinical Research Institute.

This information may be shared with, used by, or seen by collaborating researchers, the sponsor of the research study, the sponsor's representatives, and government agencies (like the FDA or the National Institutes of Health) if needed to oversee the research study. Anybody who receives your information from us could share it with others without your permission and would not be protected by the HIPAA Privacy Rules. We can use or share your information in a way that nobody can tell it is your information.

What are the privacy protections for my health information used in this research study?

A federal law exists that protects the privacy of your personal health information (Health Insurance Portability and Accountability Act (HIPAA). The law prohibits the unauthorized or unapproved use of your personal health information (PHI). Therefore we are asking your permission (authorization) to use your PHI in our research. The federal privacy regulations (HIPAA) apply to personal health information in the records of health care providers, including doctors and hospitals and other groups that share such information. We are requesting your personal health information for research purposes. There are some differences in how these regulations apply to research, as opposed to regular health care. One difference is that you may not be able to look at your own records that relate to this research study, at least until the study is over. The HIPAA privacy protections may no longer apply, once your personal health information has been shared with others who may be involved in this research.

How long does this permission allow my personal health information to be used?

If you decide to be in this research study, your permission to access and use your health information in this study will not expire, unless you revoke or cancel it. Therefore, unless we hear from you in writing that you do not want your personal health information used in this research, we will continue to use it as long as is needed.

What if I change my mind after I give this permission?

You have the right to cancel this permission to use your personal health information for research. In this case, we will not get any more of your health information for use in this research. However, canceling this authorization will not reverse uses of your personal health information that have already happened, or uses that have already been promised and cannot reasonably be reversed. If you want to cancel this permission, you must put this in writing and send to [Site PI] at the mailing address listed at the top of this form. You should clearly state that you want to cancel this permission to use your personal health information in this particular research study.

Subject Initials _____

SUBJECT'S AUTHORIZATION

I have read the information provided above. By signing this form, I am giving permission for my personal health information to be used in research as described above. I will be given a copy of this authorization form after I have signed it.

_____	_____	_____
Printed Name of Research Subject (Or Authorized Representative*)	Signature	Date

_____	_____	_____

*Only if consent/authorization by someone other than immediate subject was approved by IRB. If used, also include description of Representative's relationship to subject, and their authority to act on subject's behalf (parent, legal guardian, etc.)

17.7 Minor Assent to Participate in a Research Study - CASES

[Name of Institution]

Minor Assent to Participate in a Research Study - CASES

IRB Study #

Assent Form Version Date:

Title of Study: Drug-Induced Liver Injury Network: A Multi-Center, Longitudinal Study of Drug- and CAM-Induced liver Injury

Principal Investigator:

Phone number:

Sponsor: National Institutes of Diabetes, Digestive and Kidney Diseases

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The researchers named above are doing a **research study**.

These are some things we want you to know about research studies:

You do not have to be in this study if you don't want to.

You may stop being in the study at any time. If you decide to stop, no one will be angry or upset with you.

Sometimes good things happen to people who take part in studies, and sometimes bad things happen. We will tell you more about these things below.

Why are you being asked to be in this research study?

In the human body, the liver is that part of the body that breaks down certain medicines that people take. In some people, their livers had a problem breaking down a medicine they were taking. In other people, their livers did not have any problem.

Why are they doing this research study?

The reason for doing this research is to find out why some people's livers have problems with certain drugs and other people do not.

How many other people will be participating in this study?

More than 1400 individuals will be participating in this study from more than 9 institutions. At this institution, you will be one of at least [fill in] individuals.

What will happen during this study?

This study will take place at [Institution] and will last up to 20 years.

During your first study visit, you will be asked for:

1. Records from your doctors about the problem you had with your liver (if any) and what they did to fix it. If you had to go to a hospital, this will include records from there. You will be asked a lot of questions about your family, medical history, medications you have used in the past, and any smoking or alcohol use in the past.
2. A physical exam (including height, weight, how fast your heart is beating).

3. A blood sample with a little more than 5 tablespoons (77 ml) of blood and a urine sample with 50 ml of urine.

4. You may have a liver ultrasound (picture of your liver) as part of this research study. If you had a prior liver biopsy (liver test), this information will be requested to be used in the study.

5. You will also have to answer a number of questions which look at your overall quality of life (PedsQL).

During your next study visit in 6 months, you will have:

1. Questions asked about any changes you have had with how you feel since your first study visit.

2. A physical exam (including height, weight, how fast your heart is beating).

3. A blood sample with nearly 4 tablespoons (50 ml) of blood and a urine sample (50 ml of urine).

4. Questions which ask about your overall quality of life (PedsQL).

5. You may have a liver ultrasound.

You may or may not have a 1 year and 2 year follow-up visit. If you have these visits, you will have:

1. Questions asked about any changes you have had with how you feel since your 6 month study visit.

2. A blood sample with nearly 4 tablespoons (50 ml) of blood and a urine sample (50 ml of urine)

3. Questions to answer which ask about your overall quality of life (PedsQL).

4. You will also undergo a liver ultrasound at your 1 year and 2 year visit and possibly be entered into an extended long-term follow-up. If you are one of these patients your study doctor or nurse will tell you at that time.

The researchers are collecting the same things from other people. If they find certain similarities in the blood or medical information, they may call you and your parents to see if you would like to be in another study. If this happens, someone will explain the new study and you will have a chance to ask questions and say yes or no to participating.

When you are 18 years old, you will be asked to sign a new form to make sure you still want to be in this study.

What are the bad things that might happen?

Sometimes things happen to people in research studies that may make them feel bad. These are called "risks." These are the risks of this study:

Having blood drawn may cause pain or a bruise on your arm however the blood taken for this study will be drawn at the same time that you are scheduled to have necessary blood tests done for your medical care. Therefore, you will not receive an extra needle stick to draw blood for this study.

The study will also require one or more liver ultrasounds. Ultrasound is a safe procedure that uses low power sound waves. There are no direct risks from ultrasound. It does have its limitations in that it doesn't travel well through air or bone.

Someone may find out you have donated your blood or provided your medical records.

Not all of these things may happen to you. None of them may happen. Or things may happen that the doctors don't know about yet.

What will happen to my samples and/or data?

Your blood will be separated into its different types of cells, stored, and later used for many different studies. Some of these studies may involve your DNA for genetic research. These research results from these studies will not be put in your medical records.

Your samples (blood, urine, and liver samples) and/or data will be stored with only an assigned dummy number and the date the sample was collected. These samples will not include your name and the "key" which links your identity and contact information to your samples and data will be kept secret and not shared with any researchers.

Your samples will be stored in two specimen banks. Your data (medical record and questionnaire information) will be stored at the statistical center for the project. If specimens and/or data are shared with researchers, it is coded with a dummy assigned number, so researchers cannot identify you.

Because this research is funded by the National Institutes of Health (NIH), a government agency, this study will be shared with a NIH database. Your name, address, or other personal identifying information will not be included on the NIH database.

Will you receive any results from the research on your samples?

You will not receive any results from research tests on you blood, urine, or liver samples. The results of the research studies will not be placed in your medical records.

What are the good things that might happen?
People also may have good things happen to them because they are in research studies. These are called "benefits." There is no immediate benefit to you for being in this study. Your blood and medical information may help researchers know who is at risk for having a liver reaction and they may be given other medicines in the future.

What alternatives are there to participation in this study?
The alternative to participating in this study is to not participate and continue with your standard medical care.

Will you get any money for being in this research?
You will not be paid any money for being in this research study.

Who should you ask if you have any questions?
For questions about the study or a research-related injury, or if you have complaints, concerns or suggestions about the research, contact Dr. *[PI]* at *[PI's Number with Area Code]* during regular business hours and at *[PI's 24-hour Number with Area Code]* after hours or on a weekend or holiday.

For questions about your rights as a research participant, contact *[the IRB name and contact number]*

If you sign your name below, it means that you agree to take part in this research study.

_____ _____
Signature of Research Subject Date

_____
Printed Name of Research Subject

_____ _____
Signature of Person Obtaining Assent Date

_____
Printed Name of Person Obtaining Assent

17.8 Parental Permission for a Minor to Participate in a Research Study – CASES

[Name of Institution]
Parental Permission for a Minor to Participate in a Research Study - CASES

IRB Study #
Consent Form Version Date:

Title of Study: Drug-Induced Liver Injury Network: A Multi-Center, Longitudinal Study of Drug- and CAM-Induced liver Injury

Principal Investigator:

Phone number:

Sponsor: National Institutes of Diabetes and Digestive and Kidney Diseases

You are being asked to give permission for your child to take part in a research study. The investigators listed above are in charge of this protocol; other professional persons may help them or act for them.

What are some general things you should know about research studies?
Research studies are designed to gain scientific knowledge that may help other people in the future. Your child may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your child's participation is voluntary. You may refuse to allow this participation, or may withdraw your consent at any time, and for any reason, without jeopardizing your family's future care at this institution or your relationship with your doctor. If your child is a patient with an illness, your child does not have to participate in research in order to receive treatment.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want your child to participate. You will be given a signed and dated copy of this consent form. You are urged to ask the investigators named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
The purpose of this study is to try to understand the reasons why some people have unwanted liver reactions to certain drugs that they take and other people do not. Your child is being asked to participate in a research study because he/she has taken a drug or complementary and alternative medication (e.g., a non-prescription, herbal preparations), and your child may have developed a liver reaction while taking the drugs in question.

What will happen if your child takes part in this study?
If you choose for your child to take part in this study, the following will occur:

- This consent form will be reviewed with you by a member of the study team at the screening/initial study visit. You and your child will have the opportunity to ask any questions you may have about the study at that time. If you are willing for your child to participate in the study, you will be asked to sign the consent forms, HIPAA authorization, and medical release of information forms in the presence of a witness. You will be able to keep a copy of all these forms. **This study does not involve any treatment for your child's symptoms.**

There will be a Screening/Initial Study Visit to the clinic. There will potentially be follow up visits at 6 months, 12 months, and 24 months. The following procedures will take place at one or more of the visits:

At the initial study visit, the following will occur:

- You will be asked for your child's age, sex and race, exposures to a variety of potential risk factors including smoking and alcohol, questions about the drug-related liver problem he/she may have experienced, questions about his/her medication history, medical history, and family history. Your child will be asked to complete some questionnaires that ask about your child's quality of life and current symptoms.

- Your child will have a physical exam, including height, weight, blood pressure, heart rate and assessment of the abdomen (liver, spleen, skin)

- Your child will also have blood drawn and a urine sample collected for standard laboratory tests and for research purposes. Blood will be drawn by a qualified person who will obtain a little more than 5 tablespoons of blood (77 ml) from a vein in your child's arm. Very rarely, we may need to contact you for a second (subsequent) blood draw. In this case, an additional 3 tablespoons (37 ml) of blood will be drawn from a vein in your child's arm. The urine sample consists of 50 ml of urine.

- Some of these blood and urine samples will be sent outside the hospital and stored in two biobanks. Your child's blood will be separated into its different types of cells, stored, and used later for many different analyses. Genetic material called DNA that is obtained from the white blood cells in your child's blood will be used to search for genes that may cause or increase the risk of having a liver injury from a drug. The results of these studies will not be put in your child's health records. Some of your child's normal blood cells will be treated and frozen so that these cells can be stored, grown, and used later by researchers. These cells along with the DNA they can provide will be used in future projects to understand why some people have reactions to drugs and others do not. You will not receive any results from tests done for research purposes from your child's blood and urine. However, standard test results and liver imaging results that may require further follow-up will be provided to you or your doctor.

- Some patients may have a liver ultrasound (picture of the liver). If your child has had a prior liver biopsy (liver sample), you will be asked to sign a medical release of information form to collect this additional information for the study.

Your child's biological samples (i.e., the blood, urine, and liver samples) will be stored with only an identification number and the date the sample was collected. Your child's name will not be on the blood tube. The "key" which links your child's identity and contact information to the blood sample will be kept separately by a member of the research team who is not doing the sample testing.

- In addition to the blood collected and the interview, a member of the research team will make efforts to obtain copies of your child's medical records around the time that he/she started taking the medication. At the time you sign consent, you will be asked to sign a release of medical information form for your child.

Patients with liver injury will have a follow-up visit at 6 months. At the 6 month visit, the following will occur:

- Your child will have a physical exam including height, weight, blood pressure, heart rate, and assessment of the abdomen.
- You will be asked questions about your child's medication and medical history (including alcohol and smoking history) since the first study visit.
- Your child will be asked questions about his or her quality of life.
- Your child will also have nearly 4 tablespoons (50 ml) of blood drawn and give a urine sample (50 ml).
- Your child may have a liver ultrasound. If your child is one of these patients, your study doctor or nurse will tell you at that time.

Some patients may also have a 12 month and 24 month follow-up visit. If your child is chosen to have these visits, the following will occur:

- A review of your child's medical record to review changes since the previous study visits. You will be asked questions about your child's medication and medical history (including alcohol and smoking history) since the last study visit.
- Your child will have a physical exam including height, weight, blood pressure, heart rate, and assessment of the abdomen.
- Your child will be asked questions about his or her quality of life.
- Your child will also have nearly 4 tablespoons (50 ml) of blood drawn and give a urine sample (50ml).
- Your child will also undergo a liver ultrasound at Months 12 and 24 and possibly be entered into an extended long-term follow-up. If your child is one of these patients your study doctor or nurse will tell you at that time.

Because this research is funded by the National Institutes of Health, data from this study will be shared with the NIH database, a centralized government funded database. However, only coded, de-identified data will be submitted to the database. We will NOT share your child's name, address, medical record number, or any other traditional identifying information with the NIH database. Depositing data in the NIH database promotes widespread data sharing and allows approved investigators to more rapidly address health problems.

How long will his/her participation last?

His/Her participation in this study may last for up to 20 years from the time that you sign this consent document. Based on the information we obtain about you or your child or what is found in your child's blood and urine samples, you or your child may be eligible to participate in future studies. You and your child will be contacted (usually by mail) each year for up to 20 years and asked to update your child's contact information.

In addition, your child may be contacted by a member of the research team for up to 20 years from the time you sign this consent document and offered participation in another study. **Signing this consent form does not mean your child is automatically enrolled in any future new studies or that your child is obligated to participate in future studies.** If you and your child are contacted in the future, the person who contacts you will explain the specific study purpose and procedures. If you and your child agree to participate in a future study, you and your child will be given a separate consent form to sign and the study will have been reviewed and approved separately.

During the conduct of this research study, routine blood work and a urinalysis will be obtained from your child. In addition, a liver imaging study (ultrasound, CT scan, MRI scan) may be obtained for diagnostic or prognostic purposes. The results of such diagnostic tests, which are part of standard medical care for your child's condition, will become a part of your child's permanent medical record per hospital policy. I also understand that any abnormalities found in these routine diagnostic studies will be provided to me and to my child's doctor.

Some information collected from your child will be for research purposes only; this information will be called your child's personal research record. For example, his/her responses to interviews, questionnaires, medication use, and family health will be collected exclusively for research purposes. In addition, blood, urine, and liver tissue samples will in part be collected for research purposes. The information and test results from these research investigations as well as the notes and summaries of the researchers, will be filed together as your child's personal research record.

Due to the exploratory nature of this research and the current lack of knowledge regarding how research results should be interpreted, I agree that access to my child's personal research records by me and my child will be suspended while this study is in progress and that this right will be reinstated at the conclusion of this research study or upon completion of data analyses.

By signing this form, you will donate your child's biological samples (i.e., the blood, urine, and liver samples) for medical research purposes. Your donation does not entitle you to compensation from any commercial use of the products that may be derived from the specimen. The research studies in which the specimens may be used have not yet been fully determined, but they may involve genetic research strictly related to finding reasons why some people may be more susceptible to having liver reactions to drugs. Your child has the right not to participate in any research study for which your consent is sought. Refusal to participate will not jeopardize your child's medical care or result in loss of benefits to which your child is entitled.

The specimens may be shared with other institutions and research studies may be conducted at several locations at the same time. Non-identifying personal information about your child will be provided to investigators from other institutions.

If in the future you or your child should decide that you no longer wish for the biological samples (i.e., the blood, urine and liver samples) to be stored, you may contact [Site PI] and/or his associates at [Institution] at [telephone number] or [Name of local IRB] at [telephone number] and request that the specimens be disposed of according to standard medical research procedures. If you or your child does not make such a request, the specimens will be stored up to 20 years. The specimens may be disposed of at any time at the discretion of the investigators.

Are there any reasons your child should not participate?

Your child should not participate in this study if:

- You do not want your child's medical records to be shared with the research team
- You do not want your child to give blood for study purposes
- You do not want your child to participate in the genetic component of this study.
- You do not want your child's information and data from the current study placed in the NIH database for sharing with approved researchers worldwide.

What are the possible risks or discomforts?

Participation in this study might involve the following risks and/or discomforts to your child:

Risk of blood drawing: The risks of having blood drawn include bleeding, pain, bruising, and/or tenderness at the site where the blood is taken, infection and feeling fait or fainting. Most of these risks are rare. Only qualified staff will be allowed to draw your child's blood. Whenever possible, the blood for research will be drawn at the same time your child has blood drawn for their clinical care.

Genetic research: Results of genetic research may affect your decisions on insurability, employability, or have a negative impact on family relationships or other problems. We plan to greatly minimize these risks by not disclosing any results of the genetic testing to you or placing test results or study information in your child's medical record. We will also code your child's genetic sample. Only the research staff at the [Institution] will be able to match your child's identifiable information with your child's coded sample. This information will be kept in a locked cabinet which will only be accessible to the research staff at [Institution]. [Institution] will not share your child's name or other identifiable information with any person or persons with which it collaborates.

A new federal law called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against your child based on their genetic information. This applies to genetic information obtained in research or in clinical care. This new federal law, however, does NOT protect your child against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Risk of breach of confidentiality: We will request your child's medical records and your contact information. It is possible that despite every effort by the research team, you and your child's confidentiality may be breached (someone finds out your child is in this study). The measures the research team is taking to protect you and your child's privacy are noted below.

Ultrasound: Diagnostic ultrasound is a safe procedure that uses low power sound waves. There are no direct risks from ultrasound. Ultrasound does have its limitations in that it doesn't travel well through air or bone; therefore, it is not an effective tool for imaging parts of your body that have gas in them or are obscured by bone.

What alternatives are there to participation in this study?

The alternative to participating in this study is to not participate and continue with your standard medical care.

What if your child is injured as a result of being in this study?
In case of injury, please contact [INSERT DOCTORS NAME AND SITE CONTACT PHONE NUMBER (24 HOUR NUMBER, IF APPLICABLE)]. Immediate necessary care is available if your child is injured as a result of taking part in this study. [However, there is no provision for free medical care or for monetary compensation for such injury.](This last statement is to be included only if the sponsor has no provision to pay healthcare needed as a result of a study-related adverse event.)

What are the possible benefits?
There will be no direct benefit to you or your child for participating in this study. However, if the research team can begin to identify factors that make people have certain drug reactions, people may be able to be tested before receiving a drug to see if they are susceptible to having an unwanted reaction. In addition, once it is discovered why some people have these reactions, it may be possible to design drugs that will be safer.

What if we learn about new risks during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your child's participation.

How will your family's privacy be protected?
No subjects will be identified in any report or publication about this study. Any study records that identify your child will be kept confidential as required by law. His/Her records may be reviewed in order to meet federal or state regulations. Reviewers may include representatives from the National Institutes of Health, the Food and Drug Administration, the [Institution's] Institutional Review Board, the Data Coordinating Center at the Duke Clinical Research Institute, [add any others as appropriate]. If his/her research record is reviewed by any of these groups, they will take will take every precaution to protect your privacy.

By signing this consent, you are authorizing such access.

Your child's privacy will be protected by not placing your child's name on the biological samples (i.e., the blood, urine, and liver samples) and by keeping your child's medical information separate from the results of testing on the blood sample. There will be a list linking your child's name and contact information with the number on the sample. This list will be kept by a separate person than the one who tests your lab samples. The person who has the list with your child's name and contact information will also have your child's medical record information. This information will be stored in a locked cabinet in the office of someone on the study team. A secure computer database will be created which is password protected and accessible only to current members of the study team.

Other protection for your child's privacy:
To help us protect your child's privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health With this Certificate, the researchers cannot be forced to disclose information that may identify your child, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify your child, except as explained below.
The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about your child or his/her involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

Publication of Data
Please note that information obtained from your child may be published in peer-reviewed medical journals. None of your child's personal-identifying information will be used in the publications.

Will you or your child be paid for participating?
You will receive [monetary amount] to cover the expenses and effort associated with participation in this study.

Will it cost you anything if your child participates?
There will be no cost to you for running any of the tests done on your child's biological samples or diagnostic tests done specifically for this study. In addition, there will be no cost to you for obtaining any of your child's medical records.

Who is sponsoring this study?
This research is funded by the U.S. National Institutes of Health. This means that the research team is being compensated by the U.S. government for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the outcome of the study.

What if you want to stop before your child's part in the study is complete?
Your child is free to leave the study at any time. If your child leaves the study before it is finished, there will be no penalty or loss of benefits to which your child may otherwise be entitled. In addition, your child's participation in this study may be terminated, with or without consent, by your physician if he/she believes it to be in your child's best interest or by the study sponsor.

What if you have questions about this study?
You and your child have the right to ask, and have answered, any questions you may have about this research. If you have further questions, or if a research-related injury occurs, you should call _____ at _____ (24 HOUR NUMBER, IF APPLICABLE).

What if you have questions about your child's rights as a subject?
This research has been reviewed and approved by _____. If you have any questions or concerns regarding your child's rights as a research subject, you may contact the _____ at _____ (24 HOUR NUMBER, IF APPLICABLE).

Subject Initials _____

Parent's Agreement:

"The purpose of this study, procedures to be followed, risks, and benefits have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to allow my child to be in this study with the understanding that I may withdraw him/her at any time. I have been told that I will be given a signed copy of this consent form."

I voluntarily agree to allow my child to participate in this study in the following way(s):

Yes	No	I agree to allow my child to contribute biological samples and associated data and allow their medical information to be used in this study and for future research related to liver injury or liver disease. Some of these studies may include genetic research.
Yes	No	In addition to studies of liver injury or liver disease, I agree my child's biological specimens and associated data collected for this study, can be used for future research for conditions including, but not limited to, heart disease, cancer, or mental illness. Some of these studies may include genetic research.

_____
Printed Name of Research Subject (Child)

_____ _____
Signature of Parent Date

_____
Printed Name of Parent

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

17.9 CASES – Addendum to Consent Form for Participating in a Research Study (HIPPA Authorization for use of Protected Health Information)

CASES - ADDENDUM TO CONSENT FORM FOR PARTICIPATING IN A RESEARCH STUDY (HIPAA Authorization for use of Protected Health Information)
[Institution]
Parent Form

IRB Study Number:
Version Date of This Form:

Title of Study: Drug-Induced Liver Injury Network: A Multi-enter, Longitudinal Study of Drug- and CAM-Induced liver Injury

Principal Investigator:

Mailing Address:

Sponsor: **National Institutes of Diabetes, Digestive and Kidney Diseases**

What is the purpose of this form?
You have been asked to give permission for your child to take part in a research study. The consent form for this study describes your child's participation, and that information still applies. This extra form is required by the federal "Health Insurance Portability and Accountability Act" (HIPAA). The purpose is to get your permission (authorization) to use health information about your child that is created by or used in connection with the research. If you are signing on behalf of someone other than yourself, this permission applies to that person's health records.

What personal health information am I allowing to be used for this research study?
The information we might use includes:

- Information about your child's medical history (other conditions, medications your child is taking)
- For cases, information about this experience he/she had with his/her liver (symptoms, results of tests and procedures, doctor's notes, laboratory evaluations); and follow up information until his/her liver returns back to normal.

- Contact information, including name, address, telephone number(s), e-mail addresses from you and close relatives; his/her social security number and date of birth.

What if I don't want my child's personal health information to be used in this research study?
You may refuse to give this permission. A decision not to sign this form will not change your child's ability to get health care outside of this research study. However, your child may not be able to participate in this research study unless you sign this permission form. You should discuss this, and any other questions, with the investigators.

Where will investigators go to find my personal health information?
We may ask to see your child's personal information in records at hospitals, clinics, or

doctor's offices where your child has received care in the past. Based on what we know at this time, the places we will seek access to your child's records include:

- If your child has been in the hospital for the problems with your child's liver, we will request records from that hospital
- Your child's local doctor if the liver problem was first identified there, or if your child continues to see that doctor to manage your child's symptoms
- Records from [Institution] Hospitals if your child is seen there.

Who will be allowed to use and disclose my child's personal health information for this research and why?

The investigators named above and their assistants will be allowed to see and to use your child's health information for this research study. We may use it to check on your child's progress during the study, or analyze it along with information from all other subjects. Sometimes research information is shared with collaborators at other institutions, or with labs running additional tests. Your child's records may also be reviewed by other employees of the University of [Institution] or representatives of the research team at collaborating institutions, or the National Institutes of Health (NIDDK) in order to check for quality, safety, or effectiveness.

If your child had a prior liver biopsy, you will be requested to sign a medical release so this information can be collected as part of the study. As part of this study, your child's biological samples (i.e., the blood, urine, and liver samples) will be sent to the NIDDK Central Repositories, a research resource supported by the National Institutes of Health. The Repository collects, stores, and distributes biological samples and associated data from people with many kinds of disorders, unaffected family members, and other healthy people. The purpose of this collection is to make samples available for use in research of the genetic and clinical factors related to your injury after the current study is completed. Sending samples to the Repository may give scientists valuable research material that can help them to develop new diagnostic tests, new treatments, and new ways to prevent disease.

In addition, his/her medical records may be reviewed in order to meet federal or state regulations. Reviewers may include representatives of the National Institutes of Health – [choose as appropriate:] the [Institution's] Institutional Review Board, the Duke Clinical Research Institute, [add others as appropriate]. If his/her research record is reviewed by any of these groups, they may also need to review the entire medical record.

This information may be further disclosed by those receiving it from us. If so, the information may no longer be covered by the federal privacy regulations.

What are the privacy protections for my child's health information used in this research study?

The federal privacy regulations (HIPAA) apply to personal health information in the records of health care providers and other groups that share such information. There are some differences in how these regulations apply to research, as opposed to regular health care. One difference is that you may not be able to look at your child's own records that relate to this research study, at least until the study is over. The HIPAA privacy protections may no longer apply, once your child's personal health information has been shared with others who may be involved in this research.

How long does this permission allow my personal health information to be used?

If you decide to allow your child to be in this research study, your permission to access and use your child's health information in this study will not expire, unless you revoke or cancel it. Otherwise, we will use your child's information as long as it is needed for the study.

Subject Initials _____

What if I change my mind after I give this permission?

You have the right to cancel this permission to use your child's personal health information for research. In this case, we will not get any more of your child's health information for use in this research. However, canceling this authorization will not reverse uses of your child's personal health information that have already happened, or uses that have already been promised and cannot reasonably be reversed. If you want to cancel this permission, you must put this in writing and deliver to [Site PI] at the mailing address listed at the top.

SUBJECT'S PARENT AUTHORIZATION

I have read the information provided above. By signing this form, I am giving permission for my child's personal health information to be used in research as described above. I will be given a copy of this authorization form after I have signed it.

Printed Name of Research Subject (Child)

_____ _____

Signature of Parent Date

_____

Printed Name of Parent

*Only if consent/authorization by someone other than immediate subject was approved by IRB. If used, also include description of Representative's relationship to subject, and their authority to act on subject's behalf (parent, legal guardian, etc.)