Drug-Induced Liver Injury Network (DILIN)

Idiosyncratic Liver Injury Associated with Drugs (ILIAD): A Retrospective Study

Protocol

Compiled by:

The DILIN Research Group

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Idiosyncratic Liver Injury Associated with Drugs (ILIAD): A Retrospective Study

Protocol

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PROTOCOL CHANGES FROM VERSION 2.4 TO VERSION 2.5

In this section, changes to Version 2.4 of the ILIAD protocol are described. This excludes corrections to 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 has been revised since the enrollment of controls has been clarified. The protocol will not be enrolling control subjects and the Table of Contents has been revised throughout, deleting any reference to controls.

Executive Summary

Section 1.0, the Executive Summary, has been revised through the removal of controls and a section on power calculations has been added. 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.

Controls

The ILIAD protocol has been significantly revised accordingly because of the decision to no longer enroll controls in the study. This includes the deletion of former Sections 5.2, 7.7, 7.8, 7.8.1, 7.8.2, 7.8.4, 11.1, and 11.3. Also former protocol sections 7.1 and 7.3 have been revised accordingly with the deletion of controls, since controls will not be enrolled in the study.

Statistical Considerations

Section 11 has been completely revised. Genetic studies are one of the main focuses in DILIN. For genetic analysis, drug-matched controls may not be necessary. 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 will be carried-out based on the proposal genetic analyses using existing population control subjects.

Study Administration

Former section 13.1 on Cooperative Agreement Mechanism is removed and the new section 13.3 describes the role of Data and Safety Monitoring Board (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 the DILIN studies. The description of DSMB activities is included in the DSMB Charter.

PROTOCOL CHANGES FROM VERSION 2.3 TO VERSION 2.4

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

Executive Summary:

Specific Aims and Objectives [Section 3]:

- Four specific drugs has been changed to seven specific drugs and one drug class (quinolone antibiotics)

Targeted Drugs [Section 3]:

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics have been added the listing of targeted drugs.
- Four has been removed before the word "drugs."

Basic Study Design [Section 4]:

- "Arrangements will be made for a blood sample at a time and place convenient for the participant." has been changed to "Arrangements will be made for drawing a blood sample at a time and place convenient for the participant"
- "The blood sample will be shipped to the Rutgers University Cell and DNA Repository (RUCDR) where DNA will be extracted and lymphocytes will be immortalized" has been changed to "The blood sample will be shipped to the Rutgers University Cell and DNA Repository (RUCDR) where DNA, plasma and lymphocytes will be extracted and lymphocytes may be immortalized"
- Five has been removed from clinical sites in paragraph one.

Study Population [Section 5]:

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics have been added to isoniazid, phenytoin, clavulanic acid/amoxicillin and valproic acid.
- Trimethoprim-sulfamethoxazole, and quinolone antibiotics have been added to **"**For INH, phenytoin, clavulanic acid/amoxicillin, severe liver injury is defined as a documented serum total bilirubin > 2.5 mg/dl".
- Severe injury for nitrofurantoin and minocycline has been added as; "For nitrofurantoin and minocycline, severe liver injury is defined as a documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy".
- "Ever undertaken an allogeneic bone marrow transplant" has been added as an exclusion criterion.

Control Subjects [Section 5.2]:

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics have been added to "For DILI due to clavulanic acid/amoxicillin, controls will be drawn from patients who are taking these antibiotics in adult and pediatric primary care clinics affiliated with the DILIN Clinical Center".
- "These antibiotics" replace "who are taking clavulanic acid/amoxicillin".
- "For each of the study drugs" replaces "for each of the four study drugs, selection of controls will only begin once a decision is reached that the minimum of 50 cases can be attained in this study.
- These two sentences have been added to this section: "It is possible that population controls may be sufficient for some or all of the genetic analyses planned, and that drug treated con-

trols will not be necessary. These decisions will be made for each target drug by the Genetics Subcommittee of the network".

- Four has been removed before "study drugs".

Informed Consent Procedures [Section 12.2]:

- In sentence 2, five has been removed before clinical sites.

#2: Background and Rationale:

- "An important and unanswered question is whether genetic susceptibility for DILI exists that is independent of the causative agent (for example, deficiencies in common pathways involved in the adaptation response)" has been added to paragraph seven.
- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics have been added to isoniazid, phenytoin, combination clavulanic acid/amoxicillin, valproic acid in paragraph seven.
- "Initial" has been removed from "The initial drugs to be targeted are isoniazid, phenytoin, combination clavulanic acid/amoxicillin, valproic acid" in paragraph seven.
- "Several considerations were taken into account in selecting these drugs" and "First, surveys of various databases suggest that these are the drugs most frequently causing severe idiosyncratic DILI over the last decade" has been replaced with "These drugs were chosen because, surveys of various databases, including the database for the prospective protocol being conducted by the DILIN, indicate that these are the drugs most frequently causing severe idiosyncratic DILI over the last decade" in paragraph eight.

#3: Specific Aims and Objectives:

- Removed "initially" from "The network will initially identify people who have developed jaundice as a result of treatment with isoniazid, phenytoin, or combination clavulanic acid/ amoxicillin" in sentence one.
- Changed "jaundice" to "liver injury" in "The network will identify people who have developed jaundice asa result of treatment with soniazid, phenytoin, or combination clavulanic acid/ amoxicillin" in sentence one.
- Added nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics to "The network will identify people who have developed jaundice as a result of treatment with isoniazid, phenytoin, or combination clavulanic acid/amoxicillin" in sentence one.

#4: Basic Study Design

- The number of sites has been removed from bullet one.
- The toll free number, an email address or an enclosed self-addressed postcard has been removed from bullet one.
- The number of sites has been removed from bullet two.
- The number of sites and the number of drugs has been removed from bullet three.

#5: Study Population/5.1 Screening Criteria:

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics have been added to the third bullet.
- Trimethoprim-sulfamethoxazole and quinolone antibiotics have been added to bullet five.
- For nitrofurantoin or minocycline, either total serum bilirubin >2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy has been added as bullet six.

#5.2: Study Controls:

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics has been added to: "This study is designed to collect and maintain a database of clinical and demographic information and to store DNA, serum, plasma and lymphocytes from cases and controls to support future studies of genetic, clinical, and demographic risk factors for DILI in patients treated with isoniazid, phenytoin, valproic acid, clavulanic acid/amoxicillin" in paragraph one.
- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics has been added to: "The disease in this study is DILI, (a) judged to have been caused by isoniazid, phenytoin, valproic acid, clavulanic acid/amoxicillin, and (b) ascertained through one of the DILIN clinical centers" in paragraph two.
- Four has been removed from: "For each of the four study drugs, the selection of controls will begin once a decision is reached that the minimum of 50 cases specified in section 11.1 below can be attained in this study" in paragraph three.
- Four has been removed from in front of "study drugs" in paragraph six, sentence three.
- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics has been added to sentence 1, paragraph ten AND "these drugs" have been added to replace calvulanic acid/amoxicillin.
- "Whether 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 has recently formed a Genetics Subcommittee which will determine whether drug treated controls are appropriate for each drug." has been added to the last paragraph.

#6.1 Recruitment Procedures

- Removed five in from of clinical sites in paragraphs two and three.
- The toll free number, an email address or an enclosed self-addressed postcard has been removed from paragraph three.

#6.4 Initial Confirmation of DILI Case

- Phenytoin: "Onset within a few days to 6 months of starting therapy" has been changed to "Onset within a few days to 2 months of starting therapy".
- Nitrofurantoin: "There is a spectrum of clinical presentations of DILI associated with this drug – from acute hepatocellular, mixed or predominantly cholestatic liver injury to chronic hepatitis with insidious onset of symptoms. The acute DILI generally occurs within 6 weeks of starting therapy, but has been reported a year or more after initiation of treatment. The chronic liver injury may mimic autoimmune hepatitis both serologically and histologically. Documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy "has been added to this section.
- Minocycline: "There is also a spectrum of clinical presentations of DILI associated with this drug – from acute hepatocellular, mixed or predominantly cholestatic liver injury to chronic hepatitis with insidious onset of symptoms. The acute DILI generally occurs within 6 weeks of starting therapy, but has been reported a year or more after initiation of treatment. The chronic liver injury may mimic autoimmune hepatitis both serologically and histologically. Documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy" has been added to this section.
- Quinolone antibiotics: "Onset of hepatocellular or mixed injury typically occurring within the first week of treatment. Documented serum total bilirubin > 2.5 mg/dl" has been added to this section.
- Trimethoprim-sulfamethoxazole: "Onset of a mixed hepatocellular/cholestatic injury between 5-14 days after starting treatment. Signs of hypersensitivity are frequently present,

including skin rash, fever and eosinophila, but need not be present for diagnosis. Documented serum total bilirubin > 2.5 mg/dl" has been added to this section.

#7.2 Time Frames

- The "date of DILI onset" definition was changed from "is defined as the first documented date after starting the implicated medication at which the serum total bilirubin > 2.5 mg/dl" to "is defined as the first documented date when the patient entrance criteria for the study have been satisfied."
- "For valproic acid cases, date of onset is the date of hospitalization or the date of the first significant biochemical liver dysfunction as defined in Section 5.1 above whichever comes first." has been removed.

#7.4 Data Collected During Interview with Cases

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics have been added to bullet two.

#7.5.1 DILI Episode

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics has been added to bullet one.

#7.7 Data Collected During Interview with Control Subjects

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics have been added to bullet two.

#7.8.1 Target Medication History

- Nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics have been added to bullet one.

APPENDIX C has been added.

APPENDIX C Title

- "Draft Letter from Site PI to Potential Subject Describing DILIN and Its Purpose" has been changed to "Proposed Personalized Letter From Referring/Collaborating Physician to Potential Patient:. DILIN's Retrospective Study (ILIAD)"

APPENDIX C Text

- The sentence, "The four drugs being considered are: isoniazid (INH), phenytoin (Dilantin), combination clavulanic acid / amoxicillin (Augmentin) and valproic acid" was changed to: "The seven drugs and one drug category being considered are: isoniazid (INH), phenytoin (Dilantin), combination clavulanic acid / amoxicillin (Augmentin), valproic acid, nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics."
- The Web address has been removed.

PROTOCOL CHANGES FROM VERSION 2.2 TO VERSION 2.3

In this section, significant changes to Version 2.2 of the ILIAD protocol are described, excluding corrections to simple typographical and grammatical errors.

Section 3:

- The specific aims have been clarified to read, "The network will initially identify people who have developed jaundice as a result of treatment with isoniazid, phenytoin, or combination clavulanic acid / amoxicillin. In the case of valproic acid, eligibility requires a clinical presentation that is severe enough to prompt hospitalization or is associated with significant biochemical liver dysfunction."

Section 5.1:

- An additional screening criterion has been added: "the subject is taking only one of these medications in the period leading up to the onset of the qualifying DILI episode."
- The screening criteria for valproic acid have been changed to the following: "For valproic acid, compatible symptomatic clinical presentation that is severe enough to prompt hospitalization, or that is associated with significant biochemical liver dysfunction defined as any of the following: INR > 1.5; serum AST or ALT > 3 × ULN or > 3 times the baseline level, if the baseline level is elevated; bilirubin > 1 × ULN or > 1 times the baseline level, if the baseline level is elevated; unexplained elevated arterial or venous NH3 levels; or, liver biopsy showing steatosis."
- An additional exclusion criterion has been added: "are diagnosed with a specific seizure syndrome associated with known genetic defects if the implicated drug is valproate or phenytoin."

Section 5.2:

- Individuals with genetic defects cannot be included as controls for valproate or phenytoin. Thus, the following sentence has been added to the 9th paragraph in this section: "As with cases, children with genetic bases for seizures cannot serve as controls.

Section 7.2:

- In the first paragraph, the screening criteria for valproic acid have been changed as described above.

Section 7.3:

The following information will also be collected during the screening process to verify eligibility:

- The participant's date of birth;
- the name and start date of the implicated medication, month and year of the onset of symptoms while taking the implicated medication; recollection of jaundice during the episode;
- Willingness to receive the informed consent and other documents, and participate in the genetics component of the study.

Section 7.4:

- Indication for use of the implicated DILI medication will only be captured from the participant's chart and medical records and has been removed from this section.
- In addition to prescription and herbal medications, the use of over-the-counter medications (e.g., aspirin and acetaminophen) is recorded during the two time frames identified in Section 7.2.
- "Toxin exposures" will not be recorded because it is unclear whether this information can be collected reliably.
- "Outcome information," e.g., duration of the episode, persistent symptoms and current health status, will be recorded.
- The "cause" of any prior liver disease will not be recorded.
- "Current health status" has been removed in favor of outcome information from the DILI event.

Section 7.5:

- The participant's height and weight around the time of onset will be recorded.
- In addition to prescription and herbal medications, the use of over-the-counter medications (e.g., aspirin and acetaminophen) is recorded during the two time frames identified in Section 7.2.
- Section 7.5.3 has been split into two sections. Now, Section 7.5.3 refers exclusively to liver tests, while Section 7.5.4 refers to other laboratory tests. The description of the timing of the observations in Section 7.5.3 remains the same.
- In Section 7.5.4, other laboratory tests will only be collected at two time points, i.e., within two weeks of the onset of the DILI event, and at that time point corresponds to most abnormal value during the event.
- In Section 7.5.4, creatinine phosphokinase, amylase and lipase have been added to the list of other laboratory tests.

Section 7.7:

- Indication for use of the implicated DILI medication will only be captured from the participant's chart and medical records.
- In addition to prescription and herbal medications, the use of over-the-counter medications (e.g., aspirin and acetaminophen) is recorded during the two time frames identified in Section 7.2.
- "Toxin exposures" will not be recorded because it is unclear whether this information can be collected reliably.
- The "cause" of any prior liver disease will not be recorded.
- Height and weight around the time of starting the implicated medication will be recorded.
- Prescription and over-the-counter medications (e.g., acetaminophen, aspirin), herbal preparations taken during the two time frames identified in Section 7.2.

Section 7.8:

- In Section 7.8.3, other laboratory tests will only be collected at two time points, i.e., within two weeks of starting the implicated medication, and at that time point corresponds to most abnormal value while receiving the drug for the relevant timeframe of interest.
- In Section 7.8.3, creatinine phosphokinase, amylase and lipase have been added to the list of other laboratory tests.

Section 8.1:

- For adults, provision has been for an additional blood draw if the DNA yield from the initial blood draw is less than 50ug DNA/ml blood. In the second paragraph, the following sentences have been added: "If the initial DNA yield from the submitted whole blood sample is less than 50ug 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) 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." No changes in the amount of blood drawn from children have been made.

  The corresponding description in Section 4 on the Basic Study Design has been updated.

Section 11.3:

- In Section 11.3, the sentence, "Because the design is stratified by clinical site, clinical center will be included as a stratification variable in all statistical analyses." Clinical site is a matching variable, and it is therefore not necessary to include it in the logistic regression model.

Appendix B – Informed Consent and HIPAA Authorization Templates:

- Sentences have been added to informed consent template for adults to indicate that a repeat blood sample may be taken if the initial DNA yield is less than 50ug DNA/ml blood. Paragraph 3 now reads as follows:

"3. At the time of this conversation, arrangements will also be made to obtain blood from you. We will attempt to give you a choice of locations so you can choose a place and time convenient for you. Blood will be drawn by a qualified person who will obtain approximately 3 tablespoons (37 ml) of blood from a vein in your arm. This blood will usually be sent by mail to the study center. 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."

- The following paragraph has been added to the informed consent template for adults. A similar paragraph has been added to the parental consent for children. The purpose of this addition is to safeguard the integrity of this research study while the study is in progress.

  "7. The personal research records for this study include all interview data, questionnaires, investigator notes and summaries, as well as results of tests done for research purposes, including genetic testing. Due to the exploratory nature of this research, and the current lack of knowledge regarding how research results should be interpreted, you agree that your right to access your personal research records 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."

- 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."

  A similar change has been made to the parental consent for children.

- In the HIPAA Authorization for use of Protected Health Information, the paragraph indicating that the blood sample will be sent to the Rutgers University Cell and DNA Repository has been expanded as follows:

  "As part of this study, your blood sample 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. This genetic information will be extracted and stored for 20 years, and the storage location will be forwarded to a Data Coordinating Center at the Duke Clinical Research Institute."

  A similar change has been made to the HIPAA Authorization template for children.

PROTOCOL CHANGES FROM VERSION 2.1 TO VERSION 2.2

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

Section 8.1:

- The volume of blood drawn for adults has been increased. We are now collecting, two10ml NaEDTA (lavender top) and two 8.5ml ACD (yellow top), for a total of 37 ml, from the adults. The amount of blood drawn for children has not changed.

  The corresponding description in Section 4 on the Basic Study Design has been updated.

Appendix B – Informed Consent Templates:

- The informed consent template for adults has been updated to reflect the new volume of blood. The text now reads, “Blood will be drawn by a qualified person who will obtain approximately 3 tablespoons (37 ml) of blood from a vein in your arm.”
- In the assent template for children, although the volume of blood has not changed, the amount of blood to be drawn has been clarified as follows, “A blood sample (up to 4 teaspoons).” Similarly, in the informed consent form for the parents of minor subjects, the amount has been clarified as follows: “Blood will be drawn by a qualified person who will obtain up to 4 teaspoons of blood from a vein in your child’s arm based on your child’s age and other blood samples taken at the time for routine care.”

1. EXECUTIVE SUMMARY

Background and Rationale [Section 2]: Drug-induced liver injury (DILI) is the single most common reason for regulatory actions concerning drugs, including failure to gain approval for marketing, removal from the market place, and restriction of prescribing indications. DILI is also a significant cause of morbidity and mortality in many patient populations. To stimulate and facilitate research into DILI, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has recently established the Drug-Induced Liver Injury Network (DILIN). One of the initial projects to be conducted by the network is to retrospectively establish a nationwide registry of patients who have suffered severe idiosyncratic liver injury associated with drugs (ILIAD), and to collect, immortalize and store serum, DNA, and lymphocytes from these patients (hereafter referred to as the "ILIAD protocol"). This ILIAD protocol will serve as a resource for subsequent mechanistic investigations of the basis for susceptibility to severe idiosyncratic DILI.

Specific Aims and Objectives [Section 3]: The primary goal of the ILIAD protocol is to create: (a) a clinical database consisting of individuals who have experienced severe DILI caused by **seven** specific drugs **and one drug class (quinolone antibiotics)** and the relevant clinical data concerning the episode of DILI; and, (b) to create a bank of biological specimens obtained from these individuals and a similar number of appropriate controls. These biological specimens will be DNA, plasma, and immortalized lymphocytes. Immortalized lymphocytes will provide unlimited amounts of genomic DNA for study as well as living immune cells for phenotyping studies. A secondary goal of the ILIAD protocol is to maintain a registry of cases in the ILIAD database so that they may be recontacted in the future. It is expected that this will facilitate additional studies exploring the mechanisms of DILI.

Targeted Drugs [Section 3]: The drugs to be targeted in the ILIAD protocol are isoniazid, phenytoin, clavulanic acid / amoxicillin and valproic acid, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics.** The target drugs were chosen because they cause severe DILI at a high rate compared with other drugs, making our target enrollment for each drug (n = 50-100) attainable. In addition, these drugs are frequently administered to reasonably healthy patients not concurrently receiving other drugs more likely to be hepatotoxic, facilitating causation assessment.

Basic Study Design [Section 4]: The DILIN clinical centers will identify and contact patients at their own and affiliated institutions who may have suffered a liver injury due to one of the targeted drugs. They will also contact gastroenterologists, hepatologists, and other health care professionals most likely to have treated DILI cases. In the latter case, an information packet will be sent by the treat clinical sites. In either case, the subject will be given a brief description of the study's purpose and procedures, and when further interest in the study is expressed, s/he will be provided with an information packet including the informed consent document, HIPAA authorization and release of medical record forms. Once these documents have been reviewed, study staff will contact the potential subject a second time. This follow-up contact will either occur by telephone or in person at the subject's convenience, and the informed consent process described in Section 12.2 below will be conducted. At the first opportunity after the participant has provided consent to participate in the study, an interview will be conducted using either a telephone or personal interview format. Arrangements will be made for **drawing** a blood sample at a time and place convenient for the participant. The blood sample will be shipped to the Rutgers University Cell and DNA Repository (RUCDR) where DNA, **plasma and lymphocytes** will be extracted and lymphocytes **may be** immortalized. DNA, plasma and lymphocytes will be frozen and stored for future studies. A request will be made to the appropriate health care professionals to release medical records so that relevant clinical data can be abstracted. Detailed clinical information concerning the DILI event will be abstracted from the charts and entered onto case report forms. Information from the personal interview and patient charts will be reviewed by the DILIN Causality Committee and it will make the final determination on whether the patient was a true DILI case.

Study Population [Section 5]: Cases are individuals who suffered drug-induced liver injury and the implicated medication is one of the following: isoniazid, phenytoin, combination clavulanic acid / amoxicillin, valproic acid, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics.** For INH, phenytoin, clavulanic acid/amoxicillin, **trimethoprim-**

sulfamethoxazole, and quinolone antibiotics, severe liver injury is defined as a documented serum total bilirubin > 2.5 mg/dl. For valproic acid, it is defined as compatible symptomatic clinical presentation that is severe enough to prompt hospitalization, or that is associated with significant biochemical liver dysfunction, defined as any of the following: INR > 1.5; serum AST or ALT > 3 × ULN or > 3 times the baseline level, if the baseline level is elevated; bilirubin > 1 × ULN or > 1 times the baseline level, if the baseline level is elevated; unexplained elevated arterial or venous NH3 levels; or, liver biopsy showing steatosis. **For nitrofurantoin and minocycline, severe liver injury is defined as a documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy.** Cases must be living, and may have undergone liver transplantation but the DILI episode must have occurred since January 1, 1994. Subjects will be excluded if they are not willing to have medical information and blood samples taken, or if they are unable to adequately give informed consent to participate in the study. Individuals less than 2 years old at the time of study enrollment are excluded due to blood volume requirements.

Power Calculations [Section 11.1]: 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. Because the cases enrolled in the retrospective study will be combined with cases from the prospective study for genetic/genomic studies, the power calculations and analyses for genetic/genomic studies are the same as ones in the prospective study protocol. 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).

Data Collection Protocol [Section 7]: Several phases of data collection are envisaged for this study. First, during the initial contact between the patient and a DILIN clinical center, the screening criteria will be confirmed, and contact information will be collected in order to maintain a communication link with the participant. Second, at the first opportunity after the subject has provided consent to participate, a DILIN staff member will collect data using a telephone or personal interview format as convenient for the subject. This includes basic demographic information, family information, and prior history of liver disease and other medical conditions. Third, a copy of the participant's medical records and charts will be retrieved from the identified health care providers. Detailed clinical information concerning the DILI episode will be abstracted from patient charts. This includes concomitant medical conditions and illnesses, laboratory and liver function tests, as well as serological and other assays. Finally, detailed information from the NIDDK Genetics Repository will be forwarded to the DCC and merged with the clinical information.

Informed Consent Procedures [Section 12.2]: All DILIN participants will provide written informed consent using procedures reviewed and approved by each clinical center's Institutional Review Board (IRB). If the patient learned of this study through a referring gastroenterologist, hepatologist or other health care professional outside the DILIN network, informed consent will be sought only after the subject contacts one of the clinical sites. Each subject will be contacted by personnel at the appropriate DILIN clinical site and provided an information packet including the informed consent document, HIPAA authorization, and release of medical record forms. These documents will be reviewed on a follow-up contact with a member of the study staff. If possible, informed consent will be undertaken with the subject in person. Otherwise, it will be conducted over the telephone, and witnessed and documented by a third party who will be on the line. In this case, the participant must sign the informed consent and other documents and forward them to the DILIN clinical site. 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 genetic component of ILIAD. There will be parental consent forms, older subject consent forms, and assent forms in order to facilitate enrollment of subjects less than 18 years of age. Minors will be re-consented

as appropriate during the study. Subjects will then be asked to return the signed informed consent forms to the DILIN clinical site.

Data Management Procedures [Section 9]: Paper Case Report Forms (CRFs) will be designed specifically for the needs of this study. The CRF will be partitioned into "booklets" according to the type of data captured. A database will be created at the DCC specifically for this study. Personnel at clinical sites will record interview data onto a data form; and, data will be abstracted from the participant's medical charts and other source documents and written onto data forms. These Case Report Forms (CRFs) will be forwarded to the DCC using a parcel delivery system. Double data-entry on the most important data fields by two different operators will be performed at the DCC to ensure a high level of confidence in the data entered. Tracking information for the blood sample will be entered on the participant's CRF. Information from the NIDDK Genetics Repository will be merged with the clinical information in the main study database. Detailed quality control procedures will be invoked to safeguard the integrity of the accumulating database.

Quality Control Procedures [Section 10]: 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. Staff will be certified only if they can perform these exercises satisfactorily. 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.

Study Administration [Section13]: The administrative and funding mechanism used to undertake this project is an NIH "Cooperative Agreement" (U01), which is an assistance mechanism. Under the cooperative agreement, the NIDDK assists, supports, and/or stimulates and is substantially involved with investigators in conducting the study by facilitating performance of the effort in a "partner" role. The Steering Committee is the main governing body of the project. It is composed of the Principal Investigators of the field 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.

Significance: The most severe types of DILI usually present as idiosyncratic reactions. This type of DILI cannot be adequately studied in animal models or in current *in vitro* systems. Severe idiosyncratic DILI is also very difficult to study in people because only rare individuals experience the toxicity and most patients can take the implicated drug without any signs of toxicity. It seems likely that susceptibility to this type of DILI largely reflects the unique genetic makeup of the individual. It logically follows that complete understanding of severe idiosyncratic DILI will require finding and studying the rare individuals who have experienced it. This is not currently possible because there does not exist: a) a clinical database of such individuals; b) a bank of biological samples prepared from these individuals; and, c) a mechanism to contact these individuals for participation in clinical studies. The ILIAD protocol is proposed to address these deficiencies for the selected drugs. The database and bank of biological samples will represent a unique resource to investigators world wide. The registry will allow these individuals to be re-contacted and thereby have an opportunity to participate in studies aimed at understanding and correlating relevant genetic factors with patient characteristics (genotype: phenotype correlations).

2. BACKGROUND AND RATIONALE

Drug-induced Liver Injury (DILI) is the major single reason for regulatory actions concerning drugs, including failure to gain approval for marketing, removal from the market place, and restriction of prescribing indications [1]. DILI is also a significant cause of illness in many patient populations [2].

Severe DILI is usually "idiosyncratic". This means that at recommended doses, the implicated drug is safe for the vast majority of treated patients, but severely toxic to a small subset of patients. In most instances, it is unclear what is different about this subset of patients that makes them susceptible to liver toxicity, but it is reasonable to assume that genetic factors are involved. It has general-

ly not been possible to test this hypothesis because genomic DNA has never been systematically obtained from patients who have experienced severe, idiosyncratic DILI.

The most severe form of DILI, acute liver failure (ALF), is rarely due to idiosyncratic injury; idiosyncratic DILI represents only 11% of all patients with ALF in a recently published series [3]. This would suggest that there are only about 200 cases of ALF due to idiosyncratic DILI per year in the US, assuming roughly 2,000 cases of ALF occur annually [4]. On the other hand, asymptomatic elevations in liver chemistries, particularly the serum alanine aminotransferase (ALT), are commonly caused by drugs. Even high elevations in serum ALT do not necessarily indicate clinically important liver injury. This is because most patients with ALT elevations associated with DILI appear to "adapt" to the event even with continued treatment with the offending drug. Such patients have spontaneous resolution of the ALT elevation and do not develop clinically important liver injury even though they continue to receive the offending drug. This point is best illustrated with the drug tacrine, which is an acetyl cholinesterase inhibitor used to treat Alzheimer's disease [5]. An unpublished study revealed that very high ALT elevations during tacrine treatment (10-20 X upper limit of normal) can reverse and normalize with continued treatment with the drug (see Appendix, Figure 1). This "adaptation" to ALT elevation has been observed with many other drugs [1]. For example, ALT elevations > 3 times the upper limits of normal (ULN) occur in roughly 15% of patients treated with isoniazid yet less than 1:100 patients develop symptomatic liver disease with continued treatment. Two percent of patients treated with troglitazone developed ALT elevations > 3 X ULN [6], but less than 1:1000 developed symptomatic liver injury in an unmonitored situation [4]. Hence, patients with ALT elevations, even high ones, will not necessarily have the most clinically important determinants of idiosyncrasy (i.e. the susceptibility factors for irreversible injury).

In contrast to serum ALT elevations, the presence of jaundice due to a drug generally indicates clinically important liver injury. For example, it has been noted that there is approximately 10% mortality among patients who seek a physician after they become jaundiced due to hepatocellular injury (i.e. hepatocellular jaundice) [2]. Accepting the estimate of 200 cases/year of ALF due to idiosyncratic drug injury, this suggests that there might be approximately 2,000 cases of jaundice/year caused by the same drugs.

Based on the above observations, it seems likely that potentially life-threatening, idiosyncratic DILI will ultimately be shown to reflect the presence of at least two distinct sets of susceptibility factors in the host. The first set of factors involves a predisposition to low level injury (causing ALT elevations). This injury will generally be transient despite continued treatment with drug, unless the patient has a second and probably unrelated predisposition, which prevents adaptation and/or accelerates the initial injury. Prospective studies of DILI, whether performed in an academic or industry setting, are unlikely to be able to distinguish patients with this second set of predisposition factors from among those with the first set alone. This is because the implicated drug will usually be stopped when ALT elevations alone are detected. The rationale for this retrospective ILIAD protocol is to identify groups of patients who clearly had this second susceptibility.

Many patients who experience jaundice due to DILI come to the attention of gastroenterologists/hepatologists, who are the best trained physicians to distinguish DILI from other forms of liver injury. It is therefore reasonable to utilize this subspecialty community to identify patients eligible for the proposed ILIAD protocol.

Another important question is whether to include in the ILIAD protocol any patients who have suffered idiosyncratic DILI causing jaundice, or whether to target only patients who have had this injury as a result of specific drugs. It is possible that common susceptibility factors are involved independent of the causative agent (for example, deficiencies in common pathways involved in the adaptation response). **An important and unanswered question is whether genetic susceptibility for DILI exists that is independent of the causative agent (for example, deficiencies in common pathways involved in the adaptation response).** Nonetheless, it seems likely that many or most factors will be drug- or drug class-specific. For example, key events believed to be important in producing DILI, such as routes of drug metabolism and disposition, are known to be chemical structure-specific [7]. In addition, the clinical presentation of DILI can differ remarkably between drugs, suggesting different underlying mechanisms [1]. Finally, there are relatively few examples where patients have developed severe idiosyncratic DILI due to multiple drugs. It is therefore logical to target specific drugs or drug classes in creating the ILIAD protocol. The drugs to be targeted

are isoniazid, phenytoin, combination clavulanic acid / amoxicillin, valproic acid, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics**

These drugs were chosen because, surveys of various databases, including the database for the prospective protocol being conducted by the DILIN, indicate that these are the drugs most frequently causing severe idiosyncratic DILI over the last decade. For example, in a recent unpublished review of the national liver transplant database (UNOS), the most frequent drugs implicated in liver failure were isoniazid, phenytoin and valproic acid (excluding acetaminophen). The higher frequency of these forms of DILI should maximize the probability of collecting of at least 50 cases per drug, the minimal number estimated to be required for meaningful genetic analysis (see Biostatistics section below). Second, DILI due to these drugs can generally be confidently diagnosed because these drugs are commonly administered to reasonably healthy patients not receiving other drugs more likely to cause DILI. In addition, diagnosis is greatly aided by the fact that the DILI caused by each selected drug tends to have a characteristic, or "signature" clinical presentation. Third, there has been research conducted into mechanisms underlying the injury produced by each of these drugs, and potential candidate susceptibility genes have already been proposed.

Creation of the ILIAD plasma, DNA and immortalized lymphocyte bank will provide a valuable resource to investigators worldwide. Studies of the gene bank will require submission of additional protocols that must be approved by the HRCN Ancillary Studies Committee, the DILIN Steering Committee, and the relevant Institutional Review Boards (IRBs). In addition to examining genomic DNA, it may become important to recontact the people who have donated these lymphocytes to obtain additional historical information (such as follow up data), to offer them participation in studies (such as those designed to determine genotype:phenotype correlations), or to initiate family studies. Before contact could occur, the proposed study protocols would also need to be approved by the HRCN Ancillary Studies Committee, the DILIN Steering Committee, and the relevant Institutional Review Boards (IRBs).

In summary, progress in understanding the molecular basis for severe idiosyncratic DILI has been hampered by the lack of a bank of tissues prepared from patients who have suffered this condition. In addition, it has not been possible to study such individuals because there has been no registry, or mechanism to contact these individuals. The ILIAD protocol is proposed to correct these deficiencies for the selected drugs

3. SPECIFIC AIMS AND OBJECTIVES

The network will identify people who have developed **liver injury** as a result of treatment with isoniazid, phenytoin, combination clavulanic acid / amoxicillin, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics**. In the case of valproic acid, eligibility requires a clinical presentation that is severe enough to prompt hospitalization or is associated with significant biochemical liver dysfunction. The specific aims are as follows:

1. Establish and maintain a clinical database of these people that contains relevant clinical data. The target enrollment will be 50-100 individuals with DILI due to each drug.
2. Establish a bank of biological specimens (serum, DNA, and immortalized lymphocytes) prepared from cases and control in the clinical database.
3. Maintain a registry including yearly updated contact information of the subjects enrolled in the clinical database so that it is possible to recontact these individuals at a later date to offer participation in studies which are not part of the current proposal.

4. BASIC STUDY DESIGN

This is a retrospective study that will identify and enroll subjects who, in the opinion of a gastroenterologist / hepatologist, have experienced a drug-induced liver injury as defined in Section 5.1 below due to one of the target medications. Cases will be reviewed by the Principal Investigator at that DILIN clinical center enrolling the subject before referral on to the DILIN Causality Committee which will make a final decision on inclusion.

In general, the study will be conducted according to the following basic study design.

- An information packet will be prepared for distribution to patients suspected of having suffered a drug-induced liver injury since January 1, 1994. The packet will contain basic information concerning the nature of drug-induced liver injury, the goals of this particular protocol, together with information for how to contact one of the participating clinical centers.
- As described more fully in Section 6 below, there are two pathways for recruiting and enrolling subjects into ILIAD. First, the DILIN clinical centers will identify patients at their own and affiliated institutions who may have suffered a drug-induced liver injury. They will contact these patients directly.
- Secondly, the clinical sites will contact gastroenterologists, hepatologists, and other health care professionals in their respective spheres of influence most likely to have treated cases of DILI. These health care professionals will forward an information packet to any patient who may have suffered severe liver injury due to one of the target drugs. Interested subjects will be invited to contact one of the clinical sites using the methods described above.
- Procedures will be also applied to identify corresponding controls.
- For either pathway, screening criteria outlined in Section 5.1 will be evaluated and a brief explanation of the study and protocol requirements will be provided. Subjects who satisfy these requirements and express an interest will be provided full consent forms, HIPAA authorization and release of medical record forms.
- Study staff will contact the subject a second time and review the consent form, HIPAA authorization and release of medical record forms with the subject. All attempts will be made to have this second contact be on-site at the DILIN clinical center. However, some subjects will be remote from the clinical center and this second contact will need to be done by phone. Informed consent will be obtained as described in Section 12.2 below. At the first opportunity after the subject has provided consent to participate, study staff will then record requisite information using a face-to-face or telephone interview format.
- Arrangements for blood drawing will be made. If at the DILIN center for this contact, blood will be drawn at that time. If unable to come to a DILIN center, participants will be directed to a location convenient for the subject. For adults, a total of 37 ml of blood will be drawn as detailed in Section 8.1 below. If the initial DNA yield is less than 50ug DNA/ml blood, two additional 10 ml NaEDTA (lavender top) and two additional 8.5ml ACD (yellow top) will be drawn. These samples will be sent directly to Rutgers without freezing for DNA extraction and immediate immortalization. Lesser amounts will be drawn from children. Blood will be shipped to the NIDDK Genetics Repository maintained by the Rutgers University Cell and DNA Repository (RUCDR). DNA will be extracted there from one tube and stored for future studies. The two other tubes will be frozen for the option of later immortalizaton of lymphocytes. Information from this process will be forwarded to the DCC and merged with the clinical data.
- Once the medical records release form has been received at the clinical center, copies of all pertinent medical records and charts will be retrieved first from the health care provider that referred the subject. These records will then be reviewed and detailed clinical information concerning the DILI event will be abstracted from the charts and entered onto Case Report Forms.
- Once the Case Report Forms are completed, the Principal Investigator of a co-investigator at the clinical center will review the forms along with the original source documents to assure completeness. A short narrative summary of the case will be composed.
- Information will then be made available to the DILIN Causality Committee. It will make the final determination whether the patient was a true DILI case. If not, it will decide whether the patient can be included as a control, or whether the patient cannot be included in this study.

5. STUDY POPULATION

5.1 Screening Criteria

To be included in the ILIAD registry, the following criteria must be satisfied:

- The treating gastroenterologist / hepatologist or health care professional must believe that the subject suffered drug-induced liver injury;
- The subject must be alive and the date of onset of the qualifying DILI episode must have occurred on or after January 1, 1994;
- The implicated medication is one of the following: isoniazid, phenytoin, combination clavulanic acid / amoxicillin, valproic acid, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, and quinolone antibiotics.**
- The subject is taking only one of these medications in the period leading up to the onset of the qualifying DILI episode;
- **For INH, phenytoin, clavulanic acid/amoxicillin, trimethoprim-sulfamethoxazole and quinolone antibiotics, total serum bilirubin > 2.5 mg/dL;**
- **For nitrofurantoin or minocycline, either total serum bilirubin >2.5 mg/dL or documented fibrosis/cirrhosis on liver biopsy.**
- For valproic acid, compatible symptomatic clinical presentation that is severe enough to prompt hospitalization, or that is associated with significant biochemical liver dysfunction, defined as any of the following:
  - INR > 1.5.
  - serum AST or ALT > 3 × ULN or > 3 times the baseline level, if the baseline level is elevated
  - bilirubin > 1 × ULN or > 1 times the baseline level, if the baseline level is elevated,
  - unexplained elevated arterial or venous NH3 levels,
  - liver biopsy showing steatosis;
- Sufficient documentation of the event for the Causality Committee to make a determination.

Subjects will be excluded according to the following criteria:

- Are not willing to have medical information and blood samples taken;
- Are unable to adequately give informed consent to participate in the study including the blood draw for the genetic component;
- Age < 2 years old at the time of study enrollment (due to blood volume requirements).
- Are diagnosed with a specific seizure syndrome associated with known genetic defects if the implicated drug is valproate or phenytoin.
- Ever undertaken an allogeneic bone marrow transplant.

6. RECRUITMENT AND ENROLLMENT

6.1 Recruitment Procedures

The primary target for publicity for the ILIAD protocol will be gastroenterologists and hepatologists within the DILIN networks as outlined in the respective grant applications. Additional subspecialists will learn of the study through a variety of sources, but primarily through word of mouth (telephone calls, national meetings), and messages from DILIN PIs and co-PIs. The head nurses at state public health clinics will be targeted to help identify INH hepatitis cases and controls.

There are two pathways for recruiting and enrolling subjects in ILIAD. First, the DILIN clinical centers will identify subjects at their own and affiliated institutions who may have suffered a drug-induced liver injury. They will contact these patients directly, and give a brief description of the study's purpose and procedures. If further interest in the study is expressed, s/he will be provided with an information packet including the informed consent document, HIPAA authorization, and release of medical record forms.

Secondly, the clinical sites will contact gastroenterologists, hepatologists, and other health care professionals outside their immediate health care systems but who are most likely to have treated cases of DILI. These health care professionals will be asked to identify patients who they or a colleague believed may have suffered severe DILI due to the target drugs. These health care professionals will be sent a number of information packets, and for each identified subject, s/he will forward an information packet to the patient. The potential subject can either ignore the letter or, if s/he would like to find out more information about the protocol, the letter instructs him/her to contact the listed DILIN site. When any subject contacts a DILIN clinical site, the subject will be given a number for tracking purposes. A member of the study staff at the DILIN center will then initiate the first contact with the subject using the means identified by the subject.

6.2 Enrollment

During the first contact (in-person in the clinic or more usually by telephone), the screening criteria outlined in Section 5.1 above will be reviewed. A brief explanation of the study and review of the protocol requirements will be provided. Subjects who satisfy these requirements and express an interest will be provided full consent forms, HIPAA authorization, and release of medical record forms. Arrangements will be made for a follow-up contact a short time later after the potential subject has received these documents. (If this initial contact is face-to-face, the review of the documents, the consenting process and blood drawing may all occur at this initial visit at the convenience of the participant.)

During the follow-up contact, informed consent forms, HIPAA authorization, and release of medical record forms will be reviewed with the potential subject. All attempts will be made to have this follow-up contact occur in person, but it may be necessary for it to occur by telephone. The informed consent and other documents will be reviewed with the subject at this time and informed consent will be obtained as described in Section 12.2 below. At this point, the participant will be considered "enrolled" in the study.

6.3 Screening Failures

Potential subjects will be considered screen failures if any of the following occur:

- The potential subject was contacted directly by a DILIN clinical site, but s/he declined participation in the study.
- Physician has sent letter to subject but no contact was then made between the subject and any DILIN site. Because the identity of the patients receiving the letters is not known to the DILIN investigators, they never possess protected health information (PHI).
- Subject contacted DILIN site but declined participation during initial telephone contact.
- Subject agreed to receive consent documents, but declined participation after review of consent documents during the second contact.

For screening failures, the only information that will be retained will be study number, referring health care professional, and reason for failure (no response, declined participation, ineligible). In all cases, any PHI collected by DILIN investigators and staff will be destroyed.

6.4 Initial Confirmation of DILI Case

Once the subject has provided consent to participate, detailed data will be collected concerning the patient and DILI episode as described in Section 7 below. In particular, these data will be reviewed to insure that the liver injury is consistent with the characteristic injuries for each drug as defined by the published literature. A brief description of these characteristics follows:

- Isoniazid: Taking INH as a sole agent in prophylaxis for latent TB (usually a positive PPD skin test). The onset between one week and 12 months on therapy, and available data are consistent with a predominantly hepatocellular injury. Typically, no rash or eosinophilia is present.
- Phenytoin: Receiving phenytoin as sole antiseizure medication. Onset within a few days to 2 months of starting therapy. The patient should have fever and skin rash. Lymphadenopathy and splenomegaly are typically present. The available data are consistent with a predominantly hepatocellular injury.
- Clavulanic acid / amoxicillin: Onset of jaundice between 1-8 weeks of starting therapy, and can occur up to 35 days after stopping therapy. Increase in serum alkaline phosphatase above normal with serum ALT less than 10 X ULN; however, patients with ALT > 10 X ULN will be considered.
- Valproic acid: Receiving valproic acid as antiseizure medication, but may also be receiving other antiseizure medications. Onset within 1-6 months of starting therapy with characteristic prodrome of severe nausea, vomiting, anorexia, drowsiness, and/or changes in mental status leading to hospitalization. Serum ALT is elevated, but generally less than 10 times the upper limit of normal. Evidence of liver dysfunction (INR>1.5 or bilirubin > 2.5 mg.dl or AST, ALT > 3 X ULN, and/or characteristic changes on liver biopsy.)
- Nitrofurantoin: There is a spectrum of clinical presentations of DILI associated with this drug – from acute hepatocellular, mixed or predominantly cholestatic liver injury to chronic hepatitis with insidious onset of symptoms. The acute DILI generally occurs within 6 weeks of starting therapy, but has been reported a year or more after initiation of treatment. The chronic liver injury may mimic autoimmune hepatitis both serologically and histologically. Documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy.
- Minocycline: There is also a spectrum of clinical presentations of DILI associated with this drug – from acute hepatocellular, mixed or predominantly cholestatic liver injury to chronic hepatitis with insidious onset of symptoms. The acute DILI generally occurs within 6 weeks of starting therapy, but has been reported a year or more after initiation of treatment. The chronic liver injury may mimic autoimmune hepatitis both serologically and histologically. Documented serum total bilirubin > 2.5 mg/dl or documented fibrosis/cirrhosis on liver biopsy.
- Quinolone antibiotics: Onset of hepatocellular or mixed injury typically occurring within the first week of treatment. Documented serum total bilirubin > 2.5 mg/dl.
- Trimethoprim-sulfamethoxazole: Onset of a mixed hepatocellular/cholestatic injury between 5-14 days after starting treatment. Signs of hypersensitivity are frequently present, including skin rash, fever, and eosinophilia, but need not be present for diagnosis. Documented serum total bilirubin > 2.5 mg/dl.

7. DATA COLLECTION

7.1 Overview of Data Collection Process for Cases

Several phases of data collection are envisaged for this study.

1. As described in Section 6.2 above, the screening criteria will be reviewed during the initial contact with the subject, and contact information will be collected in order to maintain a communication link with the participant.
2. At the first opportunity after the participant has provided consent to participate in the study, a DILIN staff member will record requisite data from the subject using a telephone or personal interview format.
3. Arrangements will be made for a blood sample to be drawn from the participant and forwarded to the Rutgers University Cell and DNA Repository. Relevant information from the Repository will be recorded and ultimately merged with the subject's clinical data at the DCC.
4. Once copies of the participant's medical records and charts have been retrieved from the identified health care providers, the third phase of data collection will be conducted. Relevant data will be abstracted from these source documents and written onto appropriate DILIN case report forms.
5. Once the Case Report Form for any subject is completed, the principal investigator or co-investigator of the clinical center will review the forms along with the original source documents to assure completeness. A short narrative summary of the case will then be composed.
6. Finally, relevant data from the case report form and the clinical narrative will be forwarded to the DILIN Causality Committee. A final determination of whether the participant fulfills the criteria for a DILI case or control as appropriate will be made. If not, a decision must be made on whether and how to include the subject in the ILIAD database. This decision will be communicated to the DCC and entered in the study database.

7.2 Time Frames

For the purposes of data collection, the following time frames are identified. First, "drug start date" is the date when the subject started taking the implicated medication prior to the DILI event. The "date of DILI onset" is defined as the first documented date **when the patient entrance criteria for the study have been satisfied**.

For some data sections, as described below, information from a DILI case will be collected for two distinct time frames. The first is the one-month period prior to drug start date with the implicated medicine. The second is as follows. For DILI occurring within 3 months of starting drug, this is the period from the drug start date to the date of DILI onset. For DILI occurring more than 3 months after starting drug, this is the period from 3 months prior to the onset of the DILI event until the date of DILI onset.

Moreover, because controls are individually matched to a specific case and the duration of therapy for a control must be at least as long as that for his/her case, the time frames for any control are exactly the same as those for the case to which s/he is matched. For example, consider a DILI case from valproic acid occurring exactly 5 months after starting therapy. Then, the time frames for the case and matched control are the one month period before starting therapy, and from Month 2 to Month 5 following the drug start date. In this way, the exposure time for the control is the same as that for the corresponding case.

7.3 Contact Information and Screening Criteria for Cases

The following information will be collected during the initial contact with the participant. For controls, this contact may be made via telephone or in person depending on the location and referral source. In either case, this initial contact is intended to be brief, and because formal informed consent has not been obtained, only essential information will be collected.

- The subject's name, address, telephone number, and other contact information so that the information packet can be forwarded and communication can be maintained with the participant.

- The name of the gastroenterologist, hepatologist or other health care professional that referred the subject to the study will be recorded for recruitment tracking purposes.
- The participant's date of birth.
- The name and start date of the implicated medication, month and year of the onset of symptoms while taking the implicated medication; recollection of jaundice during the episode.
- Willingness to receive the informed consent and other documents, and participate in the genetics component of the study.

7.4 Data Collected during Interview with Cases

At the first opportunity after the subject has consented to participate in the study, an interview will be conducted using either a telephone or personal interview format. During this time, the following information will be collected:

- Demographic information, e.g., gender, self-reported race/ethnicity, country of birth and highest educational level attained, plus current height and weight;
- Implicated DILI medication, i.e., isoniazid, phenytoin, clavulanic acid / amoxicillin, valproic acid, **nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics**; any previous administration of the medication;
- Any rechallenge with target medication;
- Prescription and over-the-counter medications (e.g., acetaminophen, aspirin), herbal preparations, or during the two time frames identified in Section 7.2;
- Average ethanol consumption and smoking history during the two time frames identified in Section 7.2;
- Names and addresses of responsible physicians and other health care professionals during the event;
- Whether a liver biopsy was performed, together with date and location as appropriate;
- Whether the subject was hospitalized, together with the name and location of the hospital/clinic as appropriate;
- Whether a liver transplantation was performed, together with date and location as appropriate;
- Outcome information, e.g., duration of the episode, persistent symptoms and current health status.
- Any prior history of having suffered an adverse reaction to any drug severe enough to require a visit to a health care professional;
- Any prior history of jaundice;
- Any prior of history liver disease;
- Vital status and demographics of all first-degree relatives including biological parents, all siblings, and biological children, together with a history of liver diseases and a history of liver reactions to drugs as appropriate.
- For twins and multiple births, further information concerning zygosity as well as a history of liver disease for these individuals.

7.5 Data Abstracted from Patient Records and Charts for Cases

Once the participant's medical records and charts have been retrieved, the following data will be recorded. There is some redundancy between the data collected directly from the subject and those abstracted from these source documents. Medical documentation is expected to be more accurate and complete than the participant's memory. Thus, in the case of discrepancy between the two sources, data in the participant's records and charts will generally be considered as having higher credibility.

7.5.1 DILI Episode

- Implicated DILI Medication, i.e., INH, phenytoin, clavulanic acid / amoxicillin, valproic acid,
- **Implicated DILI Mediations, i.e., INH, phenytoin, clavulanic acid/amoxicillin, valproic acid, nitrofurantoin, trimethoprim-sulfamethoxazole, minocycline, or quinolone antibiotics** (from which eligibility will be confirmed), corresponding dosage, indication for use;
- Date of DILI onset as defined in Section 5.1 (from which age at onset will be derived);
- Drug start date and stop date (from which duration of therapy will be derived);
- Height and weight around the time of onset;
- Symptoms at onset of the DILI event, e.g., jaundice, abdominal pain, nausea, vomiting, anorexia, drowsiness, changes in mental status, dark urine, rash, fever, encephalopathy, ascites, itching;
- Whether a liver biopsy was performed, together with date and location as appropriate;
- Whether the subject was hospitalized, together with the name and location of the hospital/clinic as appropriate;
- Whether a liver transplantation was performed, together with date and location as appropriate;
- Whether the patient was rechallenged with the implicated drug, together with the start date and stop date of the rechallenge as appropriate;
- Prescription and over-the-counter medications (e.g., acetaminophen, aspirin), herbal preparations taken during the two time frames identified in Section 7.2.

7.5.2 Concomitant Medical Conditions and Illnesses

All medical conditions and illnesses occurring during the two time frames identified in Section 7.2 including, for example:

- Autoimmune/collagen vascular diseases
- Chronic liver disease
- HCV
- HBV
- Nonalcoholic fatty liver disease
- Alcohol-related liver disease
- Wilson's disease
- Hemochromatosis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis
- Abnormal liver tests
- Diabetes/endocrine disorder
- Infectious diseases, including TB, HIV
- Psychiatric disease
- Neurological disease, including seizure disorder
- Other comorbid diseases, including heart disease, hypertension, renal disease, pulmonary disease, gastrointestinal disease, Gilbert's Syndrome, malignancy, organ transplantation
- Family history of liver disease.

7.5.3 Liver Tests

The results from liver chemistries will be recorded at a minimum of three distinct time points relative to the DILI event. The first is the proximal to the event, i.e., the most recent time point prior to the onset of the liver injury. The second corresponds to peak values during the event, recognizing that peak values for the different tests may occur at different time points. The third is the value following resolution of the DILI event. The corresponding times will be recorded. Results will be recorded in more detail if they are available in the participant's records and charts. The following data will be collected:

- Prothrombin time (sec); INR
- Serum albumin (g /dL)
- Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (Alk Phos) (IU/L) levels
- Serum total bilirubin (from which eligibility will be confirmed) (mg/dL)

7.5.4 Other Laboratory Tests

The results from laboratory assays will be recorded at a minimum of two distinct time points relative to the DILI episode. The first is within two weeks of the onset of the DILI event. The second corresponds to most abnormal value during the event, recognizing that abnormal values for the different tests may occur at different time points. The corresponding times will be recorded. The following data will be collected:

- Complete blood count with differential including percent and total eosinophil count
- Platelet count (# /uL)
- Prothrombin time (sec); INR
- Serum total protein (g/dL)
- Serum lactic dehydrogenase (LDH) (IU/L) level
- Serum creatinine (mg/dL)
- BUN (mg/dL)
- Creatinine phosphokinase
- Amylase and lipase

In addition, the results from any liver imaging studies (e.g., CT, MRI, ultrasound) will be recorded.

7.5.5 Serological and Other Assays

The results from serological and other assays performed during the evaluation of the DILI episode will be recorded including for example:

- Anti HAV IgM
- HBsAg
- Anti HB core Ab
- Anti HB core Ab IgM
- Anti HBs
- HCV RNA
- Anti-HIV
- Anti nuclear antibodies (ANA)
- Anti smooth muscle antibodies (ASMA)
- Serum ceruloplasmin (mg/dL)
- Anti mitochondrial antibodies (AMA)
- Serum alpha-1-antitrypsin (mg/dL)

- Serum iron (mcg/dL)
- Serum total iron binding capacity (mcg/dL)
- Serum ferritin (ng/mL)

7.6 Causality Adjudication

On the basis of the information collected above and the clinical narrative provided for each potential DILI case, a determination concerning causation will be made by the DILIN Causality Committee. Source documents will also be made available to the committee as deemed necessary.

8. BLOOD DRAW AND REPOSITORY ACTIVITIES

8.1 Collection and Submission of Blood Samples

After informed consent has been obtained from each patient and/or control, staff at each clinical center will draw blood for the purposes of DNA storage, PBMC cryopreservation, and plasma storage in the central repository. Cryopreservation of PBMC's (buffy coat) from two ACD Vacutainer blood tubes, DNA extraction, and plasma separation and aliquoting of plasma from one NaEDTA Vacutainer blood tube will be performed by the Rutgers University Cell and DNA Repository (RUCDR) in collaboration with the clinical centers.

Sample collection kits for this purpose will be prepared, provided and shipped by the RUCDR to each clinical site. These will include instructions, a collection form, Vacutainer blood collection tubes, packaging, and a pre-addressed Federal Express label. Kits will be prepared and shipped to the participating sites by the RUCDR as needed. For adults, two 10ml NaEDTA (lavender top) and two 8.5ml ACD (yellow top) 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) 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.

Lesser amounts will be drawn for children. For children under 30 kg, two 3 ml yellow-top ACD tubes will be drawn; for children 30-45 kg, one 3.0 ml NaEDTA for DNA extraction and one 8.5 ml ACD tube for a buffy coat will be drawn; for children > 45 kg, one 10 NaEDTA tube and one 8.5 ml ACD tube will be drawn. These quantities are less than 0.5ml/kg for all children in these age ranges at or above the 3rd percentile of weight. No additional blood draws will be made from children.

The tubes will be pre-labeled by the participating center with the DILIN Study ID number, an Alternate ID number, and age of subject, gender, and collection date. The DILIN ID number will serve as the Study ID number, and will also serve as a cross-linking field between the RUCDR biological material and the clinical and genetic data. The Alternate ID number is a secondary ID number that will serve as a secondary cross-reference between the collection site and the RUCDR for resolving potential labeling discrepancies during collection and submission of blood samples to the RUCDR. The blood collection tubes or data collection sheets will not include personal identifiers of the subject.

Collected specimens will be shipped to the RUCDR by Federal Express priority overnight. Shipping is at room temperature in the supplied Styrofoam insulated blood mailers approved for blood shipment. Prior notification of incoming samples should be provided to the RUCDR on the day of shipment, preferably by email. Prior notification will include the DILIN ID number of each sample being shipped and the Federal Express tracking number. Weekend shipments will be accepted on Saturdays.

All samples will be logged into secure computer databases with all coded identifiers received from the DILIN clinical site. A unique in-house RU cell line ID number will be assigned as samples are logged into the RUCDR database. This RU ID number for each cell line will be used to track the sample throughout processing, transformation and DNA extraction. The DILIN ID number and RU cell line ID number will be cross-referenced in the computer database. The DILIN clinical site will be notified by email or fax on the day that the samples arrive at the RUCDR.

8.2 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.

8.3 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 ILIAD Manual of Procedures.

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

Cryopreservation of PBMC's (peripheral blood mononuclear cells)**:** PCMB's will be isolated from ACD Vacutainer tubes as described in the ILIAD 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 ILIAD 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.

8.5 Distribution of DNA, Plasma and Cryopreserved Transformed Lymphocyte Cell Lines

Distribution of Plasma Samples: Plasma aliquots will be shipped on dry ice to the Genetics 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. The RUCDR will require an NIDDK approved Material Transfer Agreement (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.

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.

9. DATA MANAGEMENT PROCEDURES

9.1 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. Clintrial will be used for 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 will be used to provide supplementary functions 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.

9.2 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 recorded on paper case report forms (CRFs), and forwarded via parcel delivery service to the DCC for data entry. Additionally, a blood sample will be drawn from study participants and sent to the NIDDK Genetics Repository. Tracking information for this sample will be recorded on the participant's CRF. Information at the NIDDK Genetics Repository will be recorded, forwarded to the DCC and merged with the clinical information in the study database.

9.3 Data Management Activities

In general, the following data management procedures will be applied.

1. Paper Case Report Forms (CRFs) will be designed specifically for the needs of this study. The CRF will be partitioned into "booklets" according to the type of data captured (e.g., Screening, telephone interview, clinical data, etc.). Identification information will identify key fields, e.g., the participant's ID number, initials, and date of birth as well the type and date of the evaluation.
2. The CRF will be printed on two-part NCR paper. At regular intervals, the different parts of the CRF will be separated. One part will remain at the clinical sites while the other will be forwarded to the DCC using a parcel-delivery system.
3. Personnel at clinical sites will record the data mandated by the protocol on the CRFs. They will be abstracted from the participant's medical charts and other source documents. All CRFs will be completed according to the current Good Clinical Practices (GCP) guidelines [8,9]. Training on completing the CRFs will be included in the training session described in Section 10.2 below.
4. A blood sample will be drawn from study participants and sent to the Rutgers University Cell and DNA Repository (RUCDR). Tracking information for this sample will be entered on the par-

ticipant's CRF. Information from the RUCDR concerning the biological samples will be forwarded to the DCC and merged with the clinical database.

5. A database will be created on the DCRI computer network specifically for this study. As described above, the database will be managed with Oracle using Clintrial.
6. For every record type, the data dictionary will identify key fields (e.g., the participant's ID, and the type and date of evaluation); the field type (e.g., numeric, character, checklist, or date) and ranges for impossible and improbable values.
7. All CRFs will be entered into the study database. Double data-entry, by two different operators [10-13] will be performed to ensure a high level of confidence in the data entered.

A series of computerized validation checks will be performed at the DCC. "Queries" will be generated, and data clarification forms (DCFs) for problems and exceptions uncovered will be forwarded to the clinical sites for investigation and resolution. Corrections will be made on the DCF using current GCP standards and forwarded to the DCC. If corrections are needed to CRF form prior to the initial submission to the DCC a single line will be drawn through the old value so that the original entry is still visible. The correct value will be written close to the field, and the correction initiated and dated by the DILIN staff member making the change.

9.4 Data Quality Control Procedures

Four levels of database quality control will be performed. The first level is the double data-entry process as described above. The second level consists of programmatic consistency checks and/or range checks. The second level of database quality is a record or panel level of control. Programs will be written to identify suspected duplicate and blank or missing records and records not double entered within and across database tables. An independent auditing group will perform the fourth level of database quality control. These internal data quality and process compliance audits are routinely conducted on internal ongoing studies to document the frequency of random errors and identify systematic deviations so that they can be corrected. An audit will be performed when 100% of data in house or at the end of a study. Other periodic quality control checks will document the frequency of random entry errors and identify systematic and process errors.

In 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.

9.5 Data Management Reports

A variety of progress reports will be prepared during the course of a trial and include [14]:

- Data Status Reports: Lag in entering CRFs into the database, 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.
- Baseline Characteristics: Summary of the demographic and other characteristics of study participants.

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

10. QUALITY CONTROL ACTIVITIES

10.1 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.

10.2 Training Sessions and Certification Procedures

Training is an important method for ensuring that all study procedures are performed consistently, accurately and reliably [15]. Site training will be specifically provided on the MoP, data collection activities as described in Section 7 above, and for data management activities as described in Section 9 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.

10.3 Site Visits

Site visits will be conducted during this study. They ensure a high level of consistency across the clinical sites [16], 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: Case Report forms (CRFs) are sensitive and specific, e.g., all evaluations recorded on the CRFs are substantiated by information in source documents, and conversely all information in source documents is recorded on the CRFs; ensure that CRFs are completed according to Good Clinical Practice (GCP) guidelines.
4. Data Accuracy: Data values written on the CRFs 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.

11. STATISTICAL CONSIDERATIONS

11.1 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 caused by one of the identified drugs, and to create a bank of biological specimens obtained from these individuals so that genomic DNA as well as living immune cells for phenotyping studies is available for genetic studies. (Plasma will also be stored since it is automatically obtained during isolation of the lymphocytes and because it may be useful in future studies). It is also expected that ILIAD patients will be recontacted for future studies exploring a variety of biomedical mechanisms of drug-induced liver injury.

ILIAD is designed as a multi-center, retrospective, registry study. DILI cases will be identified and enrolled in the study. Detailed exposure information will be recorded using a telephone interview with the participants as well as from patient charts and medical records.

Although we would like to include thousands of DILI cases in the registry, this study is limited by the network's capacity to find and enroll DILI cases over the timeframe of this study. After careful consideration, it is considered feasible to enroll 50-100 DILI cases for each of implicated drugs. 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. Because the cases enrolled in the retrospective study will be combined with cases from the prospective study for genetic/genomic studies, the power calculations and analyses for genetic/genomic studies are the same as ones in the prospective study protocol. The specific detail is laid out below.

For Genetic studies, case-control analyses will utilize existing collections of population control subjects with available genomic data in case-control analyses already available. 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 (Daly AK et al, 2009; Daly AK et al, 2010 (under review)) 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 $p < 10\text{-}8$ (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 ($p \sim 10\text{-}32$) and amoxicillin/clavulanate-DILI ($p \sim 10\text{-}14$).

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 predis-posing 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 (Menashe I 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. $p < 10\text{-}8$ 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 15.1. 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).

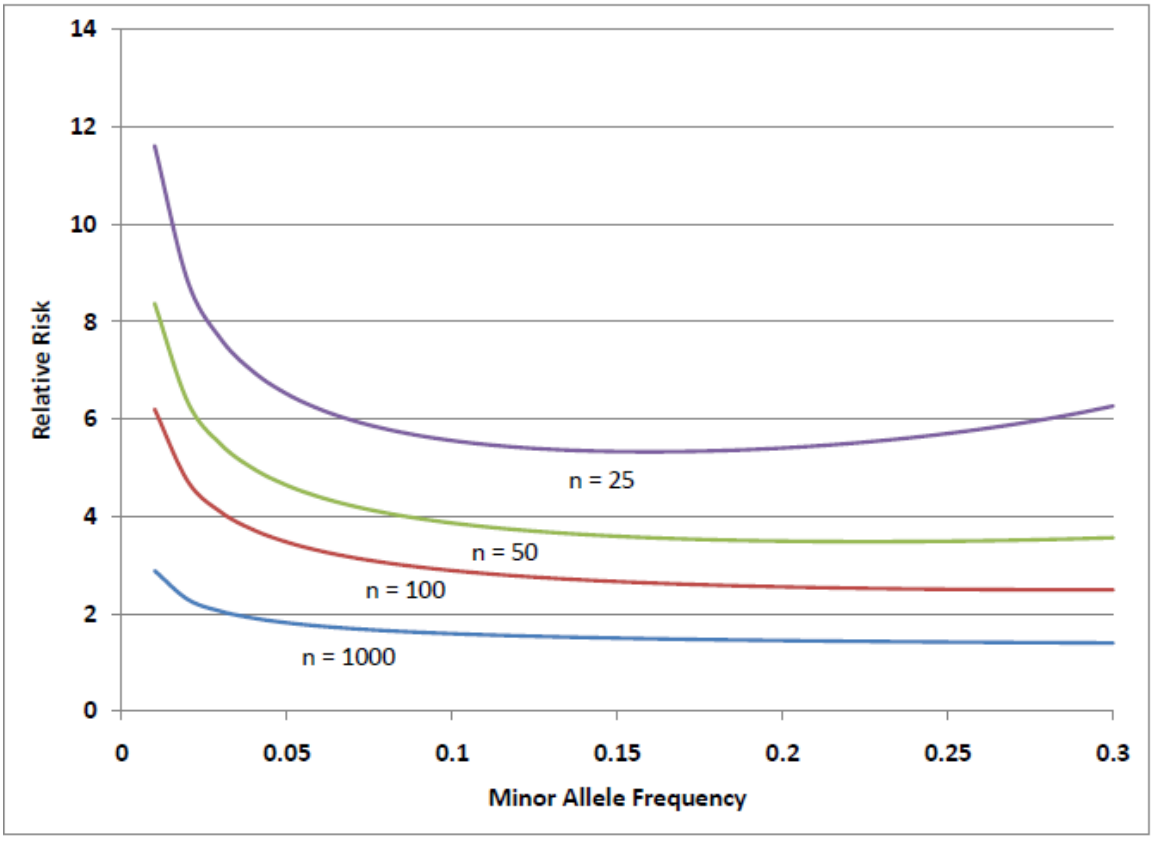

Figure 15.1. Range of detectable genetic risk markers for DILI in a genome-wide association study (GWAS). Assuming 5,000 available controls, a population prevalence of DILI of 1:10,000, co-dominant 1-degree-of-freedom genetic model, and an uncorrected p-value threshold of 10^{-8} we estimate the minimum 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).

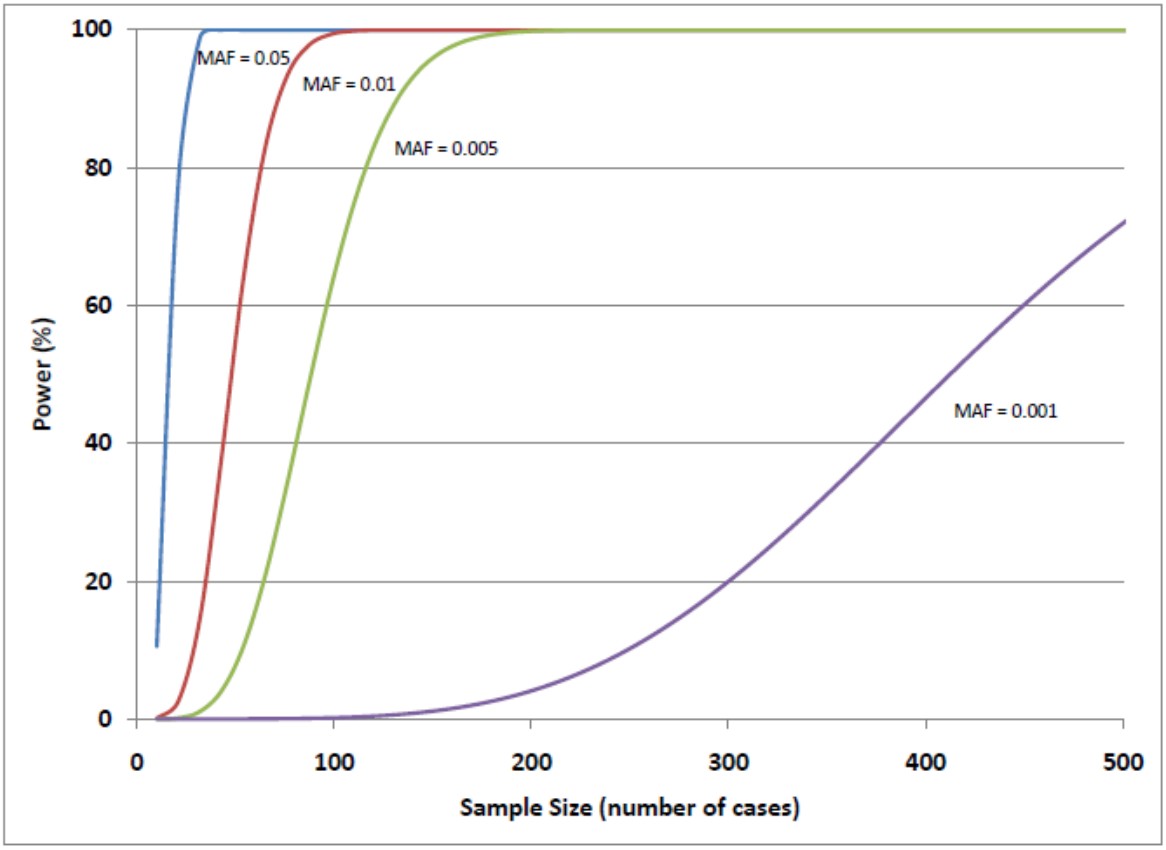

Figure 15.2. Relationship between power and sample size for low-frequency variants.

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 15.2 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 15.1).

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 (Li and Leal, European Journal of Human Genetics, 83:311-21, 2008)

12. PATIENT RIGHTS AND CONFIDENTIALITY

12.1 Confidentiality and HIPAA Considerations

Confidentiality will be protected throughout each study procedure. Initial contact with the subject will only be made by the treating physician, and no contact or identifying information will be known to the DILIN network until the subject initiates interest in the protocol by contacting a DILIN clinical site or the Data Coordinating Center using one of the methods described. Only in this way does the subject signify his/her desire to be contacted and to learn more about the protocol

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, all 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 ILIAD 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.

12.2 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). As described above, the informed consent process will only be undertaken after the initial contact between the potential subject and one of the clinical sites as described in Section 6.1 above. At this time, 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. The subject has the option of declining further participation in the study at that point. If the initial meeting is in person (such as in a clinic), the consenting process will be offered in the clinic at that time. Otherwise, study staff will contact the potential subject a second time to perform the informed consent process. If possible, informed consent will be undertaken with the subject in person. Otherwise, it will be conducted over the telephone, and witnessed and documented by a third party who will be on the line. In this case, the participant must sign the informed consent and other documents and forward them to the DILIN clinical site. Medical records will be retrieved

from the appropriate health care professionals and the blood sample will be drawn only after 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 ILIAD. 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 genetics 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 Appendix B but will be modified according to the specific needs of each participating clinical site.

12.3 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. Because the gastroenterologist, hepatologist or other health care provider originally involved in the care of the patient is only utilized to publicize the study (i.e. send the form letter to potential DILI cases), approval by this physician's IRB is not necessary. 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.

13. DILIN STUDY ADMINISTRATION

13.1 Cooperative Agreement Mechanism

The administrative and funding mechanism used to undertake this project is a "Cooperative Agreement" (U01), which is an assistance mechanism. Under the cooperative agreement, the NIDDK assists, supports, and/or stimulates and is substantially involved with investigators in conducting the study by facilitating performance of the effort in a "partner" role. The NIDDK Project Scientist serves on the Steering Committee, and he or another NIDDK scientist may serve on other project committees, when appropriate. At the same time, however, NIDDK does not assume a dominant role, direction, or prime responsibility for this research program.

As described below, governance of the project is conducted through a Steering Committee. Principal Investigators have lead responsibilities in all aspects of their trials and the project, including any modification of trial designs, conduct of the trials, quality control, data analysis and interpretation, preparation of publications, and collaboration with other investigators, unless otherwise provided for by the Steering Committee.

Principal Investigators retain custody of and have primary rights to their center-specific and collaborative data, subject to Government rights of access consistent with current HHS, PHS, and NIH policies. The protocols and governance policies call for the continual submission of data centrally to the Data Coordinating Center for the collaborative database, which at a minimum will contain the key variables selected by the Steering Committee for standardization across all field centers; the submittal of copies of the collaborative datasets to each Principal Investigator upon completion of the project; procedures for data analysis, reporting and publication; and procedures to protect and ensure the privacy of medical and genetic data and records of individuals. The NIDDK Project Scientist, on behalf of the NIDDK, will have the same access, privileges, and responsibilities regarding the collaborative data as the other members of the Steering Committee.

Principal Investigators are also encouraged to publish and to publicly release and disseminate results, data, and other products of the project, concordant with the project protocols and governance, and the approved plan for making data and materials available to the scientific community and to the NIDDK. However, during or within three years beyond the end date of the project period of NIDDK support, unpublished data, unpublished results, data sets not previously released, or other study materials or products are to be made available to any third party only with the approval of the Steering Committee.

Upon completion of the project, Principal Investigators are expected to put their intervention materials and procedure manuals into the public domain and/or make them available to other investigators, according to the approved plan for making data and materials available to the scientific community and the NIDDK, for the conduct of research at no charge other than the costs of reproduction and distribution.

The NIDDK reserves the right to terminate or curtail the project (or an individual award) in the event of (a) failure to develop or implement mutually agreeable collaborative measurement, participant eligibility, and data management sections of the protocols, (b) substantial shortfall in participant recruitment, follow up, data reporting, quality control, or other major breech of protocol, (c) substantive changes in the agreed-upon protocols with which NIDDK cannot concur, (d) reaching a major project outcome substantially before schedule with persuasive statistical significance, or (e) human subject ethical issues that may dictate a premature termination.

Any disagreement that may arise in scientific/programmatic matters (within the scope of the award) between award recipients and the NIDDK may be brought to arbitration. An arbitration panel will be composed of three members -- one selected by the Steering Committee (with the NIDDK member not voting) or by the individual Principal Investigator in the event of an individual disagreement, a second member selected by NIDDK, and the third member selected by the two prior members. This special arbitration procedure in no way affects the Principal Investigator's right to appeal an adverse action that is otherwise appealable in accordance with the PHS regulations at 42 CRF part 50, Subpart D and HHS regulation at 45 CRF part 16 or the rights of NIDDK under applicable statutes, regulations and terms of the award.

13.2 Steering Committee

The Steering Committee is the main governing body of the project. It is composed of the Principal Investigators of the field centers, the Principal Investigator of the Data Coordinating Center, and the NIDDK Project Scientist. The field 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; oversees 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.

13.3 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.

13.4 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) was established in December 2009 to monitor the progress of the DILIN registry protocols and approve the initiation of additional clinical studies. The DSMB is a multidisciplinary group which serves in an advising capacity to DILIN. Members are appointed by the NIDDK and shall have no financial, scientific, or other conflict of interest with the study. Membership includes experts in the fields of Hepatology, toxicology, electronic data capture, and biostatistics.

The DSMB meets a minimum of twice a year or at the call of the DSMB Chairperson, with advance approval of the NIDDK Program Official. The DSMB will review the research protocols; model informed consent documents; monitor recruitment, retention, evaluate data completeness and data quality; and ensure safety of participants is adequately addressed. It reports directly to the NIDDK Program Official; and makes recommendations on all study activities including terminating the study for safety or operational reasons.

14. REFERENCES

1. Kaplowitz N. Drug-induced liver disorders: implications for drug development and regulation. *Drug Safety* 2001; 24(7):483-90.
2. Zimmerman H. *Hepatotoxicity*. Philadelphia: Lippincott, Williams and Williams, 1999: 428.
3. Schiodt FV, Atillasoy E, Shakil AO, Schiff ER, Caldwell C, Kowdley KV, Stibling R, Crippin JS, Flamm S, Somberg KA, Rosen H, McCashland TM, Hay JE, Lee WM, and the Acute Liver Failure Study Group. Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transplantation and Surgery* 1999; 5(1):29-34.
4. Bissell DM, Gores GJ, Laskin DL, Hoofnagle JH. Drug-Induced liver injury: Mechanisms and test systems. *Hepatology* 2001; 33:1009-13.
5. Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI, Lewis KW. Hepatotoxic effects of tacrine administration in patients with Alzheimer's Disease. *JAMA* 1994; 271:992-998.
6. Watkins PB, Whitcomb R. Hepatic dysfunction associated with Troglitazone. *New England Journal of Medicine* (letter to editor) 1998; 338(13):916-7.
7. Watkins PB. Mechanisms of drug induced liver disease. In: Schiff E.R., Sorrell M.F., Maddrey W.C., eds. *Schiff's Diseases of the Liver*, 8th ed. Philadelphia: Lippincott-Raven, 1999:1065-1082.
8. Allen ME. Good Clinical Practice: current state of the art for clinical trial documentation. *Med Leg J* 1998; 66:61-4.
9. Bohaychuk W, Ball G. *Conducting GCP-Compliant Clinical Research*. New York: Wiley, 1999.
10. Reynolds-Haertle RA, McBride R. Single vs. double data entry in CAST. *Controlled Clin Trials* 1992; 13:487-494.
11. Gibson D, Harvey AJ, Everett V, Parmar MKB. Is double data entry necessary? The CHART trials. *Controlled Clin Trials* 1994; 15:482-488.
12. Mullooly JP. The effects of data entry error: an analysis of partial verification. *Comp Biomed Res* 1990; 23:259-267.
13. Day S, Fayers P, Harvey D. Double data entry: What value, what price? *Controlled Clin Trials* 1998; 19:15-24.
14. McBride R, Singer SW. Interim reports, participant closeout, and study archives. *Controlled Clin Trials* 1995; 16:137S-167S.
15. Neaton JD, Duchene AG, Svendsen KH, Wentworth D. An examination of the efficiency of some quality assurance methods commonly employed in clinical trials. *Stat Med* 1990;9:115-124.
16. Weiss RB, Vogelzang NJ, Peterson BA, Panasci LC, Carpenter JC, Gavigan M, Sartell K, Frei E, McIntyre OR. A successful system of scientific data audits for clinical trials. A report from the Cancer and Leukemia Group B. *JAMA* 1993; 270:459-464.

APPENDIX A: SERUM ALT VALUES IN 5 MEN TREATED WITH TACRINE

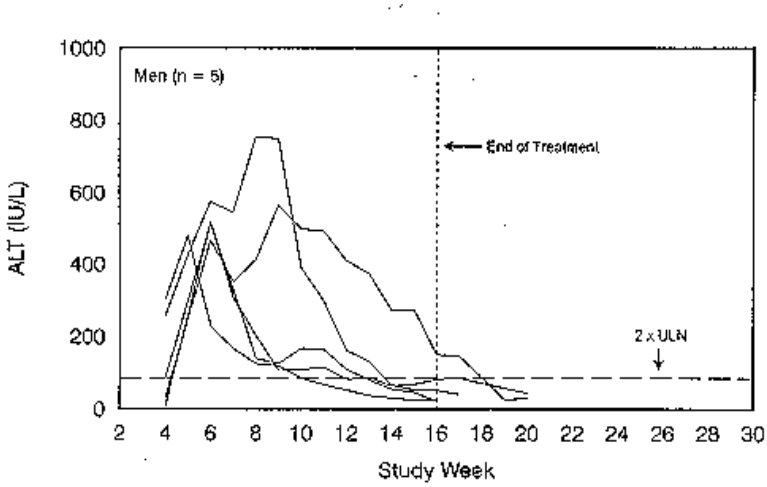

Figure 1: Serum ALT values in 5 men treated with tacrine. In this unpublished study, which was designed and monitored by Drs. Watkins, Zimmerman and Maddrey, patients were carefully followed and drug treatment was continued as long as the patients were asymptomatic and their serum ALT did not exceed 20 X ULN. Shown are the serial ALT values obtained in 5 men who had normalization of serum ALT despite continuing drug to 16 weeks. (Approximately an equal number of patients were withdrawn from therapy when their serum ALT rose greater than 20 X ULN).

APPENDIX B: INFORMED CONSENT TEMPLATES FOR CASES AND CONTROLS

INFORMED CONSENT TEMPLATES FOR RETROSPECTIVE CASES

[Name of Institution]

Consent to Participate in a Research Study

Adult consent

IRB Study #

Consent Form Version Date:

Title of Study: DILIN Protocol #1 - Idiosyncratic Liver Injury Associated with Drugs (ILIAD)

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 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 to certain drugs that they take and other people do not.

Your doctor has contacted you because you are one of the patients in his/her practice who took one of the drugs in question.

Subject Initials _____

How many other people will be participating in this study?

Approximately 400-800 subjects may be enrolled in this study.

What will happen if you take part in this study?

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

1. This consent form will be reviewed with you by a member of the study team in person or by phone. You will have the opportunity to ask any questions you may have about the study at that time. If you are willing 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. One set should be returned to the study center at the [Institution]. **This study does not involve any treatment for any symptoms.**

2. Once you have signed the consent form, you will be asked for your contact information, date of birth, sex, and race. In addition, you will be asked a number of questions about your family history, medical history (including history of jaundice and liver disease), prescription and over-the-counter medications, average alcohol consumption and smoking history, and names/addresses of health care professionals you have seen. In addition, you will be asked about exposures to a variety of potential risk factors as well as questions about the drug-related liver problem you may have experienced..

3. At the time of this conversation, arrangements will also be made to obtain blood from you. We will attempt to give you a choice of locations so you can choose a place and time convenient for you. Blood will be drawn by a qualified person who will obtain approximately 3 tablespoons (37 ml) of blood 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.

4. Your 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 blood will be used to search for genes that may have increased your risk of having a liver injury from a drug. The results of these studies will not be put in your health records. Some of your 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.

   Your blood samples will be stored with only an identification number and the date the sample was collected. Your name will not be on the blood tube. **You will not receive any results from the testing of the sample**. The “key” which links your identity and contact information to your blood sample will be kept separately by a member of the research team who is not doing the sample testing.

5. In addition to the blood collected and the telephone interview, a member of the research team will make efforts to obtain copies of your medical records around the time that you started taking the medication. At the time you sign consent, you will be asked to sign a release of medical information form.

Subject Initials _____

6. Your participation in this study may last for up to 20 years from the time that you sign this consent document. You will be contacted (usually by mail) each year for up to 20 years and asked to update your contact information.

7. Based on the information we obtain about you, the drug reaction you had or what is found in your blood, DNA, urine, or 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. If you agree to participate in a future study, you will be given a separate consent form and the study will have been reviewed and approved separately.

8. The personal research records for this study include all interview data, questionnaires, investigator notes and summaries, as well as results of tests done for research purposes, including genetic testing. Due to the exploratory nature of this research, and the current lack of knowledge regarding how research results should be interpreted, you agree that your right to access your personal research records 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.

9. By signing this form, you are agreeing to donate your blood specimens and data 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 and/or data
10. may be used have not yet been fully determined. They may involve genetic research about why some people with liver injury recover more quickly than others, Future studies may also look at heart disease, cancer, mental health research, etcetera.

11. You also agree to provide your social security number to [Site PI] and his associates for the purpose of compensation for study participation.

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 you will be provided to investigators from other institutions.

If in the future should you decide that you no longer wish for the specimens 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 do not make such a request, the specimens will be stored up to 20 years. They may be disposed of at any time at the discretion of the investigators.

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.

Subject Initials _____

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 data sharing 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 will take every precaution to protect your privacy.

Will I receive any results from the research on my 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.

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.

Subject Initials _____

What are the possible risks of participating in the study?

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

Physical Risks:

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 faint or fainting. Most of these risks are rare. Only qualified staff will be allowed to draw blood.

Non Physical Risks:

The greatest risk to you is the breach of your privacy or the confidentiality of your information, including the storage of your data on the government database.

As with any research study, there may be additional risks that are unknown or unexpected.

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.

Privacy and Confidentiality

Your privacy, and the confidentiality of your information, is very important to us and we will use many safety measures to protect your privacy. However, in spite of all 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 predict future health problems for you and your blood relatives, this information might be of interest to health providers, life insurance companies, and others.

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 genetic information. This applies to genetic information obtained in research or 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.

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 biological 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 a 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.

Subject Initials _____

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. 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.

If the results of the study are published, you *will not* be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. In some cases, your information in this research study could be reviewed by representatives of the research sponsors, or government agencies for purposes such as quality control or safety.

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, 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 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 biological specimens and/or information gathered 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.

Will you be paid for participating?

You will receive [monetary amount] to cover the expenses and effort associated with obtaining a sample of your blood. The check will be mailed to you after your blood has been drawn.

Will it cost you anything to participate?

There will be no cost to you for running any of the tests done on your blood samples. In addition, there will be no cost to you for obtaining any of your 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 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.

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, and the 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 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):

(Circle one)

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.

Yes No In addition to studies of liver injury or liver disease, I agree that the biological specimens and associated data collected on 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

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: DILIN Protocol #1 - Idiosyncratic Liver Injury Associated with Drugs (ILIAD)

Principal Investigator:

Mailing Address:

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

What is the purpose of this form?

You have been 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 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 conditions and medications you were taking, family history, alcohol consumption and smoking history, and information about exposures to a variety of potential risk factors.Information about experience you had with your liver (symptoms, results of tests and procedures, doctor's notes, and laboratory evaluations.
- Contact information, including name, address, , telephone number(s), e-mail address(es) from you and close relatives.

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 pastBased 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 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] 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 labs running additional tests. Personal health information from all the individuals who participate in this study will be kept confidential 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 (HIPPA). The law prohibits the unauthorized or unapproved use of 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 research; we will continue to use it as long as it 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 mail it 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'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

_____	_____	_____

of Representative's relationship to subject, and their authority to act on subject's behalf (parent, legal guardian, etc.)

[Name of Institution]

Minor Assent for Participation in a Research Study

IRB Study #

Assent Form Version Date:

Title of Study: DILIN Protocol #1 - Idiosyncratic Liver Injury Associated with Drugs (ILIAD)

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 others do not.

How many other people will be participating in this study?

Approximately 400-800 subjects may be enrolled in this study

Subject Initials _____

What will happen during this study?

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

During this study you will be asked for two things: a blood sample (up to 4 teaspoons) and 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 medical records from the hopsital.

If you decide to participate in this study, you will have the opportunity to ask any questions you may have. Once you have signed this assent form, you or your parents will be asked for your contact information, date of birth, sex, and race. In addition, you will be asked a number of questions about your family history, medical history (including history of jaundice and liver disease), prescription and over-the-counter medications, average alcohol consumption and smoking history, and names/addresses of health care professionals you have seen. In addition, you will be asked about exposures to a variety of potential risk factors as well as questions about the drug-related liver problem you may have experienced.

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 either 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.

- 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 your samples and / or data?

Your blood will be separated into its different types of cells, stored, and used later 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.

Subject Initials _____

Your samples (blood, urine, and liver samples) and / or data will be stored with 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 this 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 your blood, DNA, urine, or liver samples. The results of this 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 the 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	

[Name of Institution]

Parental Permission for a Minor Subject to Participate in a Research Study

IRB Study #

Consent Form Version Date:

Title of Study: DILIN Protocol #1 - Idiosyncratic Liver Injury Associated with Drugs (ILIAD)

Principal Investigator:

Phone number:

Sponsor: National Institutes of Diabetes and Digestive and Kidney Diseases

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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's doctor has contacted you because your child is one of the patients in his/her practice who took one of the drugs in question.

How many other people will be participating in this study?

Approximately 400-800 subjects may be enrolled in this study

Subject Initials _____

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:

1. This consent form will be reviewed with you by phone by a member of the study team. 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 also have the option at the end of this consent form to give permission for someone from the study team to contact you or your child for up to 20 years from this time to offer your child participation in future studies. You will be able to keep a copy of all these forms. **This study does not involve any treatment for your child's symptoms.**

2. Once you have signed the consent form for your child to participate, you will be asked for your child's contact information, date of birth, sex and race. In addition, you will be asked a number of questions about your child's family history, medical history (including history of jaundice and liver disease), prescription and over-the-counter medications, average alcohol consumption and smoking history, and names/addresses of health care professionals you have seen. In addition, you will be asked about your child's exposures to a variety of potential risk factors as well as questions about the drug-related liver problem your child may have experienced.

3. At the time of this telephone conversation, arrangements will also be made to obtain blood from your child. We will attempt to give you a choice of locations so you can choose a place and time convenient for you and your child. Blood will be drawn by a qualified person who will obtain up to 4 teaspoons of blood from a vein in your child's arm based on your child's age and other blood samples taken at the time for routine care. This blood will usually be sent by mail to the study center.

4. 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.

   Your child's blood 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. **You will not receive any results from the testing of the sample**. 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.

5. In addition to the blood collected and the telephone 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.

Subject Initials _____

6. 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 this 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 datasharing and allows approved investigators to more rapidly address health problems.

7. 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 have obtained about your child and the drug reaction (if any) your child had, or based on what is found in your child's blood sample, you and your child may be eligible to participate in future studies. If you have given permission for us to re-contact your child at a later date (this is at the end of this consent form), you will be contacted (usually by mail) each year for up to 20 years and asked to update your 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 a 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 making the contact then will explain the specific study purpose and procedures. If you and your child agree to participate in a future study, you will be given a separate consent form to sign and the study will have been reviewed and approved separately. If your child turns 18 during the study period, your child will be asked to sign their own consent form to continue participation in the study.

8. The personal research records for this study include all interview data, questionnaires, investigator notes and summaries, as well as results of tests done for research purposes, including genetic testing. Due to the exploratory nature of this research, and the current lack of knowledge regarding how research results should be interpreted, you agree that your child's right to access his/her personal research records 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.

9. By signing this form, you will donate your child's blood specimens 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 <u>strictly related to finding reasons why some people may be more susceptible to having liver reactions to drugs</u>. 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.

10. You also agree to provide your child's social security number to [Site PI] and his associates for the purpose of compensation for study participation.

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.

Subject Initials _____

If in the future you or your child should decide that you no longer wish for the specimens 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 do not make such a request, the specimens will be stored up to 20 years. They may be disposed of at any time at the discretion of the investigators.

What will happen to your child's biological samples and/or data?

Your child's 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 child's 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 child's 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 child's 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 child's name will not be kept with these biological samples or data. The "key" which links your child's identity and contact information to your child's 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 child's coded specimen or information will be given to researchers. Researchers will not obtain your name or other traditional ways to identify your child.

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 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.

Any research records that identify your child 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 will take every precaution to protect your privacy.

Will I receive any results from the research on my child's biological samples?

You will not receive any results from research tests on your child's 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 child's medical record.

Subject Initials _____

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 child's doctor. The results of such clinical 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.

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 for 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 faint or fainting. Part of these risks are rare. Only qualified staff will be allowed to draw your child's blood.

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.

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.

Subject Initials _____

What about your child's Privacy and Confidentiality?

Your child's privacy will be protected by not placing your child's name on the blood 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.

Your child's privacy, and the confidentiality of your child's information, is very important to us and we will use many safety measures to protect your child's privacy. However, in spite of all the safety measures that we will use, we cannot guarantee that your child's 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 child's name, address, telephone number, or medical record number, in the future, people may develop ways that would allow someone to link your child's genetic or medical information in the databases back to your child. Since some genetic variations can help predict future health problems for you and your blood relatives, this information might be of interest to health providers, life insurance companies, and others.

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 genetic information. This applies to genetic information obtained in research or 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.

In addition to the federal law, GINA, we plan to greatly minimize risks to your child 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 biological 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 a 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.

Subject Initials _____

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. 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.

If the results of the study are published, your child *will not* be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. In some cases, your child's information in this research study could be reviewed by representatives of the research sponsors, or government agencies for purposes such as quality control or safety.

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 allow your child's participation.

Will you or your child be paid for participating?

You will receive [monetary amount] to cover the expenses and effort associated with obtaining a sample of blood. The check will be mailed to you after your child's blood is drawn.

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 blood samples. 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.

Subject Initials _____

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

You can withdraw your child from this study at any time, without penalty. The investigators also have the right to stop your child's participation at any time.

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 ***(PI's Name)*** at ***(PI's Number with Area Code)*** (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 (Institutional Review Board Name) _____. If you have any questions or concerns regarding your child's rights as a research subject, you may contact *[the IRB name and contact number]*

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Parent's Agreement:

I have read the information provided above. I voluntarily agree to allow my child to participate in this study in the following way(s):

(Circle one)

Yes No I agree to allow my child to contribute a blood sample 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 blood sample 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

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: DILIN Protocol #1 - Idiosyncratic Liver Injury Associated with Drugs (ILIAD)

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 from my child's medical records am I allowing to be used for this research study?

The information we might use includes:

- Information about your child's medical history (including history of jaundice and liver disease and any other conditions), average alcohol consumption and smoking history, risk factors, and medications (both prescription and over-the-counter) that your child is taking
- Information about experience he/she had with his/her liver symptoms, results of tests and procedures, doctor notes, and lab evaluations.
- Contact information, including name, address, telephone number(s), e-mail address(es) 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.

Subject Initials _____

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.
- Results of testing, procedures, and lab evaluations.

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 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 child's 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 child's information in a way that nobody can tell it is your child's information.

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

A federal law exists that protects the privacy of your child's personal health information (Health Insurance Portability and Accountability Act (HIPPA). The law prohibits the unauthorized or unapproved use of personal health information (PHI). Therefore, we are asking your permission (authorization) to use your child's 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 child's 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 child's 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 mail to [Site PI] at the mailing address listed at the top. You should clearly state that you want to cancel this permission to use your child's personal health information in this particular research study.

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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.)