NASH CRN

Nonalcoholic Steatohepatitis

Clinical Research Network

The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial

Standard Operating Procedures

Part I: Clinical Center Operations

01 February 2012

FLINT SOP Part I: Clinical Center Operations

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1. Design overview

1.1. Design synopsis

Title	The Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial
Sponsor	NIDDK
Type of study	Phase IIb randomized placebo controlled clinical trial
Objective	To evaluate whether 72 weeks of treatment with obeticholic acid compared to treatment with placebo improves nonalcoholic fatty liver disease (NAFLD) as determined by hepatic histology
Study design	Multicenter, double-masked, placebo-controlled study with 2 parallel groups
Treatment groups	Group 1: Obeticholic acid (25 mg q.d.) Group 2: Placebo (q.d.)
Study duration	Up to 16 weeks screening prior to randomization, 72-week treatment period 24-week post treatment washout period
Sample size	280 (140 per group)
Number of clinics	8
Inclusion criteria	- Histological evidence of definite or borderline NASH based on standardized scoring of a liver biopsy obtained no more than 90 days prior to randomization, and a NAFLD activity score (NAS) of 4 or greater with a score of at least 1 on each component of the NAS (steatosis scored 0-3, lobular inflammation scored 0-3, ballooning scored 0-2) - Age 18 years or older at initial screening interview

Exclusion criteria

- Significant alcohol consumption or inability to reliably quantify alcohol intake
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, vamproic acid, other known hepatotoxins) for more than 2 weeks in the past year prior to randomization
- Prior or planned bariatric surgery (e.g., gastroplasty, roux-en-Y gastric bypass)
- Uncontrolled diabetes (HbA1c 9.5% or higher within 60 days prior to enrollment)
- Presence of cirrhosis on liver biopsy
- A platelet count below 100,000/mm^3
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Serum creatinine of 2.0 mg/dL or greater
- Inability to safely obtain a liver biopsy
- History of biliary diversion
- Known positivity for Human Immunodeficiency Virus infection
- Active, serious medical disease with likely life expectancy less than 5 years
- Active substance abuse including inhaled or injected drugs, in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in an IND trial in the 30 days prior to randomization
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Failure to give informed consent
- Clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities:
  - Serum albumin less than 3.2 g/dL
  - INR greater than 1.3
  - Direct bilirubin greater than 1.3 mg/dL
  - History of esophageal varices, ascites or hepatic encephalopathy
- Evidence of other forms of chronic liver disease

Outcome measures

Primary:

- Centrally scored histological improvement in NAFLD from baseline to the end of 72 weeks of treatment, where improvement is defined as:
  (1) No worsening in fibrosis and
  (2) Decrease in NAFLD activity score (NAS) of at least 2 points

1.1. Design synopsis

65 weeks from 1st patient randomized

- Change in serum ALT from baseline to 24 weeks
- Adverse events and other safety measures through interim analysis

Secondary:

- NASH diagnosis (from definite or indeterminate NASH or not-NASH)
- Fibrosis score
- Hepatocellular ballooning score
- Each component score in the NAS
- Change in serum aminotransferase and gamma-glutamyl transpeptidase (GGT) levels
- Change in MRI-determined hepatic fat
- Change in fasting markers of insulin resistance (HOMA, adipo-IR index)
- Change in post-glucose parameters of insulin responsiveness (2-hour glucose and fatty acids)
- Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference)
- Change in bile acid levels
- Change in cytokeratin 18 (CK-18) fragment assay
- Change in fibroblast growth factor (FGF-19) levels
- Changes in markers of hepatic apoptosis, inflammation, and fibrosis
- Change in health related quality of life (HR-QoL) scores

Randomization	Centrally administered randomization stratified by clinical center and diabetes status and blocked by calendar time
Statistical analysis	All analyses will be on an "intention-to-treat" basis
Safety monitoring	NIDDK appointed DSMB will monitor the data for safety and efficacy for outcomes such as hepatotoxicity, pregnancy, and any other outcomes or events identified as safety-related
Interim (Vanguard) analysis	An interim analysis (marking the end of the vanguard phase), will be performed 65 weeks after the first patient is randomized. The analysis will focus on interim efficacy outcomes and safety using data from 25-40% of patients who completed at least 24 weeks of followup. The criterion for efficacy to continue is serum ALT; criterion for continuation are safety data to ascertain emergent safety issues.

1.2. Data collection schedule

Assessment/Procedure	Screening visits			Follow-up visits Weeks from randomization								
	S		RZ	2	4	12	24	36	48	60	72	96
Consent and reaffirmation	X	.	X	.	.	.	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.	.	.	.	.	.
Follow-up medical history	.	.	.	X	X	X	X	X	X	X	X	X
AUDIT, Skinner alcohol questionnaires	A	S	.	.	.	.	.	.	.	.	.	.
Review of concomitant drugs	X	.	X	X	X	X	X	X	X	X	X	X
Review for adverse effects	.	.	.	X	X	X	X	X	X	X	X	X
Drug dispensing	.	.	X	.	.	X	X	X	X	X	.	.
Review of study drug adherence	.	.	.	X	X	X	X	X	X	X	X	.
Physical exam	D	.	.	.	.	F	D	F	D	F	D	D
MRI for hepatic fat	.	X	.	.	.	.	.	.	.	.	X	.
Liver biopsy	X	.	.	.	.	.	.	.	.	.	X	.
HR-QOL (SF-36v2)	.	X	.	.	.	.	X	.	X	.	X	X
Fasting lipid profile	X	.	.	.	.	X	X	X	X	X	X	X
Complete blood count	X	.	.	.	.	.	X	.	X	.	X	X
Metabolic panel	X	.	.	.	.	.	X	.	X	.	X	X
Hepatic panel and GGT	X	.	.	.	.	X	X	X	X	X	X	X
Fasting glucose, insulin,	X	.	.	.	.	X	X	X	X	X	X	X
2-hour OGTT (glucose, insulin,	.	X	.	.	.	.	.	.	.	.	X	.
PT and INR	X	.	.	.	.	.	.	.	.	.	X	.
HbA1c	X	.	.	.	.	.	X	.	X	.	X	X
Pregnancy test (females)	X	.	X	.	.	X	X	X	X	X	X	.
Serum, plasma for banking (Free fatty acids, bile acids, FGF-19, CK-18)	.	X	.	.	.	X	X	X	X	X	X	X
Etiologic tests	X	.	.	.	.	.	.	.	.	.	.	.
Closeout form	.	.	.	.	.	.	.	.	.	.	.	X

Detailed (D) physical exam includes measurement of height, weight, waist, and hips; vital signs (temperature, heart rate, respiratory rate, and blood pressure); examination for scleral icterus and pedal edema and auscultation of the heart and lungs; general physical findings (hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting, or fetor).

Focused (F) physical exam includes measurement of height and weight, and vital signs (temperature, heart rate, respiratory rate, and blood pressure)

Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein.

Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.

Lipid profile: total cholesterol, triglyceride, LDL, HDL.

CBC: hemoglobin, white blood cell count, platelet count, mean corpuscular volume (MCV), hematocrit

Oral Glucose Tolerance Test (OGTT): Fasting serum glucose, insulin, 75 gram oral glucose load and then repeat serum glucose and insulin after 2 hours. Will only be done in non-diabetic patients.

Etiologic tests: Hepatitis B surface antigen (HbsAg), hepatitis C antibody (anti-HCV), ceruloplasmin (if less than 40 years old), α-1 antitrypsin level (A1AT), and autoantibody studies (ANA, ASMA, AMA), iron, ferritin, and transferrin saturation.

Fasting visits:12, 24, 36, 48, 60, 72 and 96

Safety visits: weeks 2 and 4 may be done by telephone

1.3. Blood draw schedule

Assessment/Procedure	Screening visits S		Follow-up visits Weeks from randomization 12	24	36	48	60	72	96	T
Fasting lipid profile	5	.	5	5	5	5	5	5	5	40
Complete blood count	5	.	.	5	.	5	.	5	5	25
Metabolic panel	5	.	.	5	.	5	.	5	5	25
Hepatic panel and GGT	5	.	5	5	5	5	5	5	5	40
Fasting glucose, insulin,	5	.	5	5	5	5	5	5	5	40
2-hour OGTT (glucose, insulin,	.	20	.	.	.	.	.	20	.	40
PT and INR	5	.	.	.	.	.	.	5	.	10
HbA1c	5	.	.	5	.	5	.	5	5	25
Serum banking*	.	20	10	10	10	10	10	20	10	100
Plasma banking*	.	10	10	10	10	10	10	10	10	80
Other screening (etiologic testing if needed)	20	.	.	.	.	.	.	.	.	20
DNA banking	.	20	.	.	.	.	.	.	.	20
Total	**55**	**70**	**35**	**50**	**35**	**50**	**35**	**85**	**50**	**465**

Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein.
CBC: hemoglobin, white blood cell count, platelet count, mean corpuscular volume (MCV), hematocrit.
Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphate.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
Oral Glucose Tolerance Test (OGTT): Fasting serum glucose, insulin, 75 gram oral glucose load and then repeat serum glucose, insulin, after 2 hours. Will only be done in non-diabetic patients.
Other screening including etiologic tests: Hepatitis B surface antigen (HbsAg), hepatitis C antibody (anti-HCV), ceruloplasmin (if < 40 years old), α-1 antitrypsin level (A1AT), and autoantibody studies (ANA, ASMA, AMA), iron, ferritin, and transferring saturation.
Fasting visits: S, 12, 24, 36, 48, 60, 72, and 96.

*Measurements of bile acids, free fatty acids, FGF-19 and CK-18 will be derived from banked serum and/or plasma samples.

1.4. Treatment groups

Patients who have signed an informed consent statement and who meet the eligibility criteria will be randomly assigned to one of two groups for 72 weeks of treatment:

Group 1: Obeticholic acid (25 mg capsule; daily)
Group 2: Placebo (identical capsule as active drug; daily)

The randomization plan will be prepared and administered centrally by the Data Coordinating Center (DCC) but will not require real time interaction with a DCC staff member. Requests for randomizations will be made by the clinics using a web-based application. An assignment will be issued only if the database shows that the patient is eligible, has signed the consent statement, and has had all required baseline data keyed to the database.

2. Eligibility and enrollment

2.1. Inclusion and exclusion criteria

Inclusion criteria

Patients must satisfy all of the following criteria to be eligible for enrollment:

- 18 years of age or older as of the initial screening interview and provision of consent
- Histologic evidence of definite or probable NASH based upon a liver biopsy obtained no more than 90 days prior to randomization and a NAFLD activity score (NAS) of 4 or greater with at least 1 in each component of the NAS score (steatosis scored 0-3, ballooning degeneration scored 0-2, and lobular inflammation scored 0-3)

Exclusion criteria

Patients who satisfy any of the following exclusion criteria will be ineligible for enrollment:

- Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening (significant alcohol consumption is defined as more than 20 g/day in females and more than 30 g/day in males, on average)
- Inability to reliably quantify alcohol consumption based upon local study physician judgment
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other known hepatotoxins) for more than 2 weeks in the year prior to randomization
- Prior or planned (during the study period) bariatric surgery (e.g., gastroplasty, roux-en-Y gastric bypass)
- Uncontrolled diabetes defined as HbA1c 9.5% or higher within 60 days prior to enrollment
- Presence of cirrhosis on liver biopsy
- A platelet count below 100,000 /mm^3
- Clinical evidence of hepatic decompensation as defined by the presence of any of the following abnormalities:
  - Serum albumin less than 3.2 g/dL
  - INR greater than 1.3
  - Direct bilirubin greater than 1.3 mg/dL
  - History of esophageal varices, ascites or hepatic encephalopathy
- Evidence of other forms of chronic liver disease:
  - Hepatitis B as defined by presence of hepatitis B surface antigen (HBsAg)
  - Hepatitis C as defined by presence of hepatitis C virus (HCV) RNA or positive hepatitis C antibody (anti-HCV)
  - Evidence of ongoing autoimmune liver disease as defined by compatible liver histology

  - Primary biliary cirrhosis as defined by the presence of at least 2 of these criteria
    (i) Biochemical evidence of cholestasis based mainly on alkaline phosphatase elevation
    (ii) Presence of anti-mitochondrial antibody (AMA)
    (iii) Histologic evidence of nonsuppurative destructive cholangitis and destruction of interlobular bile ducts
  - Primary sclerosing cholangitis
  - Wilson's disease as defined by ceruloplasmin below the limits of normal and compatible liver histology
  - Alpha-1-antitrypsin(A1AT) deficiency as defined by diagnostic features in liver histology (confirmed by alpha-1 antitrypsin level less than normal; exclusion at the discretion of the study physician)
  - History of hemochromatosis or iron overload as defined by presence of 3+ or 4+ stainable iron on liver biopsy
  - Drug-induced liver disease as defined on the basis of typical exposure and history
  - Known bile duct obstruction
  - Suspected or proven liver cancer
  - Any other type of liver disease other than NASH
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Serum creatinine of 2.0 mg/dL or greater
- Inability to safely obtain a liver biopsy
- History of biliary diversion
- Known positivity for Human Immunodeficiency Virus (HIV) infection
- Active, serious medical disease with likely life expectancy less than 5 years
- Active substance abuse including inhaled or injection drugs in the year prior to screening
- Pregnancy, planned pregnancy, potential for pregnancy and unwillingness to use effective birth control during the trial, breast feeding
- Participation in an IND trial in the 30 days before randomization
- Any other condition which, in the opinion of the investigator, would impede compliance or hinder completion of the study
- Failure to give informed consent

2.2. Run-in period

Patients must not have used any medications historically associated with NAFLD for more than 2 weeks in the year prior to randomization. Such medications include amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other known hepatotoxins.

Patients will be allowed to use thiazolidinediones (e.g., pioglitazone or rosiglitazone), prescription anti-diabetic, antihypertensive or hyperlipidemic agents, vitamins (e.g., Vitamin E), and supplements (e.g., fish oils, ginko biloba, etc). Patients will be interviewed in a detailed fashion at screening and at clinic visits after randomization to document such use.

2.3. Guidelines for repeat determinations of eligibility

While certain inclusion and exclusion criteria are more objective and are unlikely to change, others are more subjective and may change over time. Thus, participants who are deemed ineligible at the time of initial screening may be re-screened at a later time as follows:

- An ineligible liver biopsy – the participant may be re-screened after 12 months at the discretion of the investigator
- Age < 18 years – the participant may be re-screened after his or her 18th birthday
- Ineligibility determined on measurements of albumin, INR, direct bilirubin, ALT, HbA1c, platelet count, and creatinine – the participant may be re-screened at the discretion of the investigator
- Pregnancy – the participant may be re-screened 3 months following end of pregnancy and breast feeding at the discretion of the investigator
- Unwilling to participate – the participant may be re-screened after 3 months at the discretion of the investigator

2.4. Co-enrollment in NAFLD Adult Database 2

- When a NAFLD Adult Database 2 patient enrolls in FLINT, the visit schedule and requirements of the trial take precedence over the requirements for the NAFLD Adult Database 2 – Database requirements are suspended for the duration of the participant's time in the treatment trial.

- NAFLD Adult Database 2 patients interested in enrolling in the FLINT trial must comply with the FLINT eligibility time window for a liver biopsy.

- A NAFLD Adult Database 2 Closeout form (CO) must be completed for patients randomized into FLINT. Once the CO form is completed and keyed, the patient is exempt from Adult Database 2 visits.

- Data requirements are not suspended while a FLINT patient participates in a NASH CRN ancillary study or pilot or feasibility study.

- All FLINT data collection forms and procedures must be completed anew.

2.5. Randomization and eligibility checking

Randomization steps

- Complete collection of baseline data and key baseline data forms.
- Run electronic check on eligibility (i.e., run the Randomization Task, but opt out of randomization and resolve any ineligibility conditions).
- Run the Randomization Task and confirm that you want to randomize the patient "now." This task will officially randomize the patient in FLINT and the randomization bottle numbers and materials needed in follow-up will be generated (i.e., labels, visit time window)

Randomization

- The date the clinical center runs the Randomization Task and confirms that the patient is to be randomized "now" and the treatment group is assigned is the date of randomization.
- The "time zero" for reckoning the time windows specified on the patient's FLINT visit time window guide is the date of randomization.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

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Patients enrolled in NAFLD Adult Database 2 who want to enroll in FLINT

- Transferring Adult Database 2 patients into FLINT necessitates that the screening procedures are conducted within the designated eligibility time window (e.g., liver biopsy obtained within 90 days prior to randomization in FLINT). Because the eligibility time windows in the NAFLD Adult Database 2 study and FLINT are different, Adult Database 2 procedures may not be compatible for FLINT screening purposes.
- Adult Database 2 patients **without** liver biopsy or a liver biopsy obtained ≥1 year prior to FLINT registration are good candidates for screening in FLINT.
- Physician discretion is recommended for Adult Database 2 patients **with** a recent liver biopsy as to whether the patient should register for FLINT; in this scenario, it may be reasonable to wait until the patient has completed their Adult Database 2 annual t048 visit.
- Recent liver biopsies obtained in the Adult Database 2 study and within the FLINT eligibility window (within 90 days prior to randomization) may be used for FLINT screening to determine eligibility.
- Have the patient sign the FLINT consent form.
- Complete and key the FLINT RG form but do NOT issue a new patient ID number and code.
- Blood for serum and plasma repository must be collected even if already banked for NAFLD Adult Database 2.
- Regarding blood for genetics repository:
    - If not already collected, have patient sign the FLINT genetic consent, collect a sample, and complete the FLINT CG form.
    - If blood for genetics testing was already collected, do not send another sample unless the yield was unsatisfactory.
        - If the yield on the sample drawn when the patient screened for the Adult Database 2 was satisfactory, leave the Adult Database 2 CG form in the data system and complete the FLINT CG answering 'yes' to question about prior blood draw for the Adult Database 2; the patient does not need to sign the FLINT genetic consent nor does the FLINT CG form need to be completed.
        - If the yield on the sample drawn when the patient screened for the Adult Database 2 was unsatisfactory, have the patient sign the FLINT genetic consent form and complete the FLINT CG forms; the Adult Database 2 CG form should remain in the data system.
- Lab results reported on the Adult Database 2 LR and LS forms may be used on the FLINT LR and LS forms if they were obtained within the time windows specified on the forms.
- All interviews and patient questionnaires (drinking history, AUDIT, baseline history, liver symptoms, quality of life) must be completed anew for FLINT.
- The physical exam (PE) form must be completed anew for FLINT.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

- If the biopsy used for FLINT is the same one that was used for the NAFLD Adult Database 2 (keep in mind that the biopsy for FLINT must meet date requirements not imposed in the NAFLD Adult Database 2), the Clinical Coordinator should transcribe the histology data from the Histology Worksheet (HW) onto the FLINT HF form. The FLINT SD form must be completed; transcribe information from the Adult Database 2 SD form. For items relating to slide sequence numbers, transcribe the slide numbers for the slides that were previously sent for the Adult Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the FLINT LT form must be completed; transcribe information from the Adult Database 2 LT form. Where the FLINT LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the Adult Database 2 form and write in the margin "see Adult Database 2 LT form". There will be more than one form in the data system pointing to the same numbered slides and liver tissue vials (Adult Database 2 SD/LT and FLINT SD/LT forms), but this is okay since the patient enrolled in the Adult Database 2.
- If the patient is eventually randomized in FLINT, have the patient complete FLINT follow-up visits and forms; you do not need to complete the MV form for the missed Adult Database 2 visits, but you do need to complete the Adult Database 2 Closeout (CO) form to suspend the patient's participation in the NAFLD Adult Database 2. The CO form can be completed prior to or after randomization in FLINT, but our advice is to complete it upon randomization in FLINT. The patient remains enrolled in NAFLD Adult Database 2 while participating in FLINT, but the patient is not subject to completion of NAFLD Adult Database 2 visits.
- Retain all NAFLD Adult Database 2 forms completed for the patient in the patient's NASH CRN file.
- Retain the patient's Adult Database 2 visit windows schedule since it will be needed once FLINT is completed.

Patients registered in NAFLD Adult Database 2 but never enrolled, now wants to register in FLINT

- The patient should be closed out of the NAFLD Adult Database 2 by completing and keying the Adult Database 2 EN form to document the reason(s) why the patient did not enroll in the Adult Database 2. Answer as many of the questions in sections B, C, D, E, and F of the form EN as you can, coding an item as 'm' if you do not know the answer; if the patient is eligible for the Adult Database 2 but is opting to go directly into FLINT, answer 'no' to item 22 (no longer consents) and check 'Other reason' in item 23c and write in 'opted to go directly into FLINT.'
- Have the patient sign the FLINT consent form.
- Complete and key the FLINT RG form but do NOT issue a new patient ID number and code.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

- Blood for serum and plasma repository must be collected even if already banked for NAFLD Adult Database 2.
- Blood for genetics repository:
  - If not already collected, have the patient sign the FLINT genetic consent and collect a sample and complete the FLINT CG form.
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory.
    - If the yield on the sample drawn when the patient screened for the Adult Database 2 was satisfactory, key the Adult Database 2 CG form (if not already keyed) and complete the FLINT CG form answering 'yes' to the question about prior blood draw for the Adult Database 2; the patient does not need to sign the FLINT genetic consent.
    - If the yield on the sample drawn when the patient screened for the Adult Database 2 was unsatisfactory, then have the patient sign the FLINT genetic consent form, draw the replacement sample, and complete the FLINT CG form (the Adult Database 2 and CG form can remain in the data system).
- Interviews and questionnaires must be completed on the FLINT forms:
  - If available, data from the Adult Database 2 AD and LD forms may be transcribed to the corresponding FLINT forms, but the patient should be queried regarding any changes since the previous interviews; the date in item 4 on each FLINT form should be the date you review the information with the patient.
  - The FLINT BG form should be completed anew– it is different from the Adult Database 2 BG form, and the FLINT BG form data will help establish that the biopsy is a medication free biopsy (medication use is not an issue with Adult Database 2 biopsies).
- The physical exam (PE) form must be completed anew.
- If the same biopsy is used for FLINT that was used for the Adult Database 2 (keep in mind that the biopsy for FLINT must meet date requirements not imposed in the NAFLD Adult Database 2), the local pathologist must review the slides again and the Clinical Coordinator should transcribe the histology data from the Histology Worksheet (HW) onto the FLINT HF form. The FLINT SD form needs to be completed; transcribe information from the Adult Database 2 SD. For items relating to slide sequence numbers, transcribe the slide numbers that were previously sent for the Adult Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the FLINT LT form must be completed; transcribe information from the Adult Database 2 LT form. Where the FLINT form asks for the duplicate LT label to be pasted onto the LT form, write in the label information from the Adult Database 2 LT form and write in the margin 'see Adult Database 2 LT form'. The Adult Database 2 SD and LT forms can remain in the data system.
- Retain all Adult Database 2 forms completed for the patient in the patient's NASH CRN file.

2.6. Guidelines for transferring NAFLD Adult Database 2 and FLINT patients

Patient registered in FLINT, but found to be ineligible, now wants to register in the NAFLD Adult Database 2

- The patient should be closed out of FLINT by completing and keying the FLINT RZ form to document the reason(s) the patient was found to be ineligible.
- Have the patient sign the Adult Database 2 consent form.
- Complete and key the Adult Database 2 RG form but do NOT issue a new patient ID and code.
- Blood for serum and plasma repository must be collected even if already banked for FLINT.
- Blood for genetics repository:
  - If blood was not already collected, have the patient sign the Adult Database 2 genetic consent, collect a sample, and complete the Adult Database 2 CG form.
  - If blood was already collected, do not send another sample unless the yield was unsatisfactory, but the CG form must still be completed.
- Interviews and questionnaires must be completed on the Adult Database 2 forms.
- If the same biopsy is used for the Adult Database 2 that was used for FLINT, the Clinical Coordinator should transcribe the histology data from the Histology Worksheet (HW) form onto the Adult Database 2 HF form. If slides were previously sent for FLINT, the Adult Database 2 SD form must be completed referencing the slide numbers for the slides that were sent (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the Adult Database 2 LT form must be completed. Where the Adult Database 2 LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the FLINT LT form and write in the margin 'see FLINT LT form'. The FLINT SD and LT forms can remain in the data system.
- Retain the FLINT forms in the patient's NASH CRN file.

These procedures are complicated. Please contact the DCC if you have questions or if you run into problems when trying to key forms or enroll/randomize a patient.

3. Certification

3.1. Certification overview

What is certification?

- It is a study-related requirement designed to identify the staff responsible for specific data items, data collection procedures, or decisions about eligibility.
- It is a managerial and quality assurance tool for the study.

Who and what does it apply to?

- It applies to:
  - FLINT staff
  - Each clinical center
- Certification for FLINT is required before any patient visits or data collection may occur; patients may not begin any screening examinations, sign any consent statements, or complete any study forms until the clinical site has been certified for the study.
- More than one staff member may be certified for a function and it is recommended that more than one staff member be certified for a function.

Why do we require it?

- Primary purpose is to help assure consistent conduct of the study over time, within and across clinics. The conduct of procedures should be similar across patients and in serial testing of the same patient over the duration of follow-up.
- Study procedures may vary from the usual practice of a participating clinical center, but it is important that methods be carried out in the same manner within and across clinical centers.
- It identifies the staff and sites that carry out study procedures and identifies to staff that they and their site are a part of the FLINT trial.
- It provides a mechanism for tracking who collected key data items or made key decisions.
- The certification process may help a clinic prepare for study activities by presenting the training, facility, and equipment needs in an organized fashion and requiring acquisition or completion of these items before study specific activities may begin.

3.2. Clinical center certification

General comments

- Each clinical center participating in FLINT must be certified.
- Completion of the Clinical Center Certification (CC) form will be required.
- IRB approval for the FLINT protocol and consents will be required.

Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol and who will staff the study.
- Guide a clinical center through the steps of getting ready for FLINT – provide a checklist of what needs to be in place before patient activities begin.

Requirements for certification of a site

- Complete the Clinical Center Certification (CC) form.
- Certify at least one person for each function that requires certification (a person may be certified for more than one function).
- Obtain IRB approval of the most current FLINT protocol and consent documents.
- Receive written notice of approval (email) from the Data Coordinating Center that the site is certified.
- Provide assurances that the study participants' protected health information will be kept confidential.
- Provide assurances that the linkable information will not be transmitted to the DCC.

3.3. Personnel certification

Staff functions requiring certification

- Clinical Coordinator
- Hepatologist
- Pathologist
- Data Entry Technician
- Radiologist
- Imaging personnel (optional)

Requirements

- Everyone:
  - Read the FLINT protocol
  - Complete the Knowledge Assessment (KA) form; this is a written general knowledge assessment about the Database (open book)
  - Complete the Personnel Certification (PC) form; this form identifies the functions applied for and provides an assurance of data confidentiality and integrity
- Additional requirements for Pathologist:
  - Be approved by David Kleiner and Elizabeth Brunt
- Additional requirements for Data Entry Technician:
  - Complete the Data Entry Certification/Decertification Request (DC) form
  - Complete the data system tutorial (personnel previously certified as Data Entry Technician do not need to complete the data system tutorial a second time)
- Additional requirements for Radiologist and Imaging personnel:
  - Be approved by Radiology Reading Center
  - Follow instructions indicated in the FLINT SOP Part VI: MRI Procedure Manual

Process

- Send required materials to the DCC
- The DCC will send written notice of approval for certification or pending certification
- Each staff member will be issued a Personnel Identification Number (PIN)

Staff PINs

- Each staff member certified for at least one function will be issued a PIN which will consist of 3 digits – the first digit will identify the clinical center and the next two digits will be a sequential number assigned by the Data Coordinating Center
- The PIN is used when completing forms
- The Data Entry Technician uses his/her PIN when signing on to the FLINT data system
- Staff can be certified for more than one function, but will have only one PIN

3.4. MRI qualification

The Radiology Reading Center (RRC), which is located in the Liver Imaging Group, Department of Radiology at the University of California, San Diego, is responsible for coordinating the FLINT MRI activities. Before you can begin the MRI component of the FLINT Trial, you must be certified by the RRC. Please note that the MRI Qualification and start-up process is separate from the FLINT certification and start-up. All of the forms and questionnaires listed below may be found in the FLINT SOP Part VI: MRI Procedure Manual.

Requirements

- Review the FLINT SOP Part VI: MRI Procedure Manual
- Have an IRB approved FLINT MRI Consent
- Key MRI personnel must have a Collaborative IRB Training Initiative (CITI) certificate (or equivalent)

Process

1) Complete MRI Qualification Questionnaires found in the FLINT SOP Part VI
   – FLINT MRI Center Regulatory Qualification Questionnaire
   – FLINT MRI Center Technical Qualification Questionnaires
2) Send the following documents to Lisa Clark at the RRC via email (liclark@ucsd.edu) or fax (619-471-0503)
   – FLINT IRB approval letter and MRI consent
   – MRI Qualification Questionnaires
   – CITI training certificates
3) Participate in a start-up call with the RRC
4) Arrange for the imaging of two volunteers*, complete the MRI Case Report Forms found in the FLINT SOP Part VI, and submit them to the RRC
   – FLINT MRI Data Transmittal CRF
   – FLINT MRI Radiologist Report CRF
   – FLINT MRI Adverse Event CRF
   *** Please note that the volunteers should not be patients or participants in the FLINT trial**

Contact Information

For more information regarding MRI certification, please review FLINT Trial SOP Part VI: MRI Procedure Manual. All questions concerning MRI procedure and MRI certification should be directed to the RRC staff.

Michael Middleton, MD PhD office: (619) 543-6766 cell: (858) 750-0878 msm@ucsd.edu	Lisa Clark, PhD phone: (619) 471-0513 fax: (619) 471-0503 liclark@ucsd.edu	Claude Sirlin, MD office: (619) 471-0513 cell: (619) 709-3341 csirlin@ucsd.edu

4. Human subjects

4.1. Background

Consent to participate in the FLINT Trial must be completed before screening for FLINT may begin. The patient must consent to procedures offered to and performed on him/her for screening, as well as those for the follow-up visits which the patient will face in the future.

The consent process is a dynamic process involving explanations, time to think, questions, clarifications, and advice that a patient may seek from relatives, friends, or anybody else considered relevant. We wish to inform the prospective participant as much as possible and as accurately as possible about what will be offered to him/her, how it will be done, what are the reasonable risks and benefits, what are the alternatives, and what is expected of the patient. We wish to answer patients' questions in a consistent and complete way.

The FLINT consent process has four major stages:

- The patient is asked to consent to screening and randomization into FLINT.
- The patient is asked to sign the HIPAA authorization to disclose protected health information.
- The patient is asked to consent to the collection, storage, and use of blood samples for genetic research, which is optional.
- The patient is asked to consent to an MRI assessment, which is optional.

4.2. Institutional review board process

Three prototype consent statements have been prepared for the FLINT trial:

- Consent for screening and enrollment in FLINT
- Consent for the collection, storage, and use of blood samples for current and future genetic research (optional)
- Consent for MRI assessment (optional)

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in FLINT. Each clinical center must send copies of the consent statements to be used at the clinical center, stamped with their IRB's seal, to the Data Coordinating Center prior to initiating patient activities in FLINT. Data Coordinating Center staff will review and compare the approved local consents to the prototype consents. Specific local additions to and editing of the prototypes may be required at individual institutions, but deletion of material and major rewording of text may need to be explained and justified. Once a consent form has been approved by an institution's IRB, it cannot be changed without the IRB's approval.

The study protocol and consent forms will be submitted to each clinical center's IRB and to the DCC's IRB. Additionally, each clinical center will submit to their IRB any data collection forms or recruitment materials to be used at their site. A clinical center may not initiate any patient contact about FLINT until the site has IRB approval for FLINT and the DCC has certified the site for initiation of FLINT patient activities. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines.

HIPAA authorization forms will be prepared by each clinical center according to local clinical center institutional requirements and guidelines.

4.3. Consent administration

FLINT

Patients referred to a clinical center for screening may have heard about FLINT, but their level of knowledge and expectations may differ. We wish to standardize the consent administration across clinical centers as much as possible. Administration of the FLINT consents involves two tasks:

(1) A FLINT staff member (hepatologist or clinical coordinator) must sit down with the patient and review the contents of the statement; explain the risks, benefits, and responsibilities of participation; review the alternatives to participation; and answer questions.

(2) A FLINT certified hepatologist or clinical coordinator must sign the consent statement, and in addition to the principal investigator, take overall responsibility for the patient's informed and voluntary consent.

Staff at each clinical center should be designated to carry out these tasks. The rationale for requiring that the consent statement be signed by a study physician is to help assure that the physician signing the consent is one who has a broad role in the study.

Generally, the consent statements should be offered to the patient to read through at least a day before signature is requested. The consent will then be reviewed with the patient by the staff member designated to obtain consent; the consenter may opt to read the statement to the patient, pausing to explain issues as needed. This activity should take place in a quiet, private, and relaxed setting in the clinical center.

The patient should sign the consent statement in the presence of the FLINT staff member after all questions have been answered and when the patient has asserted orally that he/she is ready to sign the consent. After the patient has signed and dated the consent, the patient should meet with a FLINT study physician for the physician to sign the consent statement. Ordinarily, this meeting should take place on the same day that the patient signed the consent statement. The physician should ask the patient to confirm his/her voluntary consent and query the patient about any questions or concerns the patient may have about participation. Both signatures on the consent form must be in a non-erasable ink pen. If the physician cannot meet with the patient on the same day that the patient signs the consent statement, the physician may sign on another day.

Consent for genetic research

The consent for collection and banking of blood for genetic research should be administered in the same way that the FLINT consent is administered, except that it should not be signed until the patient has been determined to be eligible for the FLINT trial. Patients who have already consented to collection and banking of blood for genetic research as part of the NAFLD Database 2 do not need to sign this consent again as part of the FLINT trial.

Consent for MRI research

The consent for participation of Magnetic Resonance Imaging (MRI) assessment should be administered in the same way that the FLINT consent is administered, except that it should not be signed until the patient has been determined to be eligible for the FLINT trial.

4.4. Time considerations for obtaining consent

- The **FLINT Consent and HIPAA authorization** must be obtained at the start of the initial screening visit (FLINT visit s); documents from the referring physician (if any) or from the NAFLD Adult Database 2 study should have been reviewed prior to the visit and the patient should be judged eligible for screening prior to the visit. Signature of this consent is required prior to sending the patient for any FLINT diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

- A patient may be given the consent statements to review prior to the initiation of the screening visit to meet patient needs with respect to review time. Whenever a consent is first given to a patient for review, it should be made clear to the patient that the consent should not be signed until requested by a FLINT staff member. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed FLINT procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in FLINT. Patients may request and should be given time to "think it over" at home and come back at a later time.

- The **FLINT Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for FLINT has been established during the screening visit or at the randomization visit. If the patient has already consented to genetic banking as part of the NAFLD Adult Database 2, the patient does not need to be presented this option as part of the FLINT trial and does not need to sign this consent. Signature of this consent is required prior to drawing blood for genetic research; a check for signature of this consent statement occurs on the Genetic Consent and Blood Collection Documentation (CG) form. Signature of this consent statement is not required for FLINT eligibility (i.e., the patient may choose not to participate in the genetic research component of FLINT).

- The **FLINT Consent for Magnetic Resonance Imaging Research** must be obtained after eligibility for FLINT has been established during the screening visit and prior to randomization. Signature of this consent is required prior to undergoing an MRI exam; a check for signature of this consent statement occurs on the MRI Consent and Report Form (MR). Signature of this consent statement is not required for FLINT eligibility (i.e., the patient may choose not to participate in the MRI research component of FLINT).

4.5. Consent handling

- Signed consent statements are important legal documents. These signed statements should be kept in the patient's FLINT clinical center file together with his/her other FLINT forms and documents. These forms are not part of the individual's institutional medical record, but part of his/her study record in the FLINT trial. Consent statements will be examined during site visits.

- Consents should be annotated with the patient's study identifiers (ID number and code).

- The FLINT consent statement is an "all or none" form. The patient either accepts it in its entirety and signs it, or does not. The patient must consent to the evaluation procedures, the follow-up evaluations, and the banking of his/her serum, plasma, and liver tissue specimens. If the patient refuses any part, the patient may not enroll in FLINT. If the patient was previously enrolled in the NAFLD Adult Database 2, the patient must also consent to the FLINT evaluation, follow-up, and banking procedures to enroll in the FLINT trial.

- The FLINT Consent for Genetic Research has been made a separate consent statement so that the patient can opt out of genetic research and still participate in the FLINT trial. If the patients previously consented to DNA banking as part of the NAFLD Adult Database 2 study, the patient will not need to sign a new consent unless the amount of blood was considered to be unsatisfactory.

- The FLINT Consent for Magnetic Resonance Imaging (MRI) Research has been made a separate consent statement so that the patient can opt out of MRI assessment and still participate in the FLINT trial.

4.6. Informing participants of changes to consent statement after randomization

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As new data become available during the conduct of FLINT, the consent statements may need to be changed to reflect the current assessment of risks and benefits to participants in the study.

Procedures for dissemination of revisions of consent statements from the DCC

- Changes deemed necessary will be made to the prototype consent statements.
- Revisions of the prototype consent statements will be distributed to sites via a numbered Policy and Procedure Memorandum (PPM) with instructions to submit the revised consent to their IRB

Procedures for reviewing changes to consent statements with participants

- Clinical center personnel will develop a chronology of IRB approved changes to the consent statements used at their site
- At each follow-up visit, staff will use the chronology of consent changes to review with the participant any changes to the consent since the last visit. This review does not require obtaining the participant's signature on a new consent statement, unless the local IRB requires obtaining a signature.
- Review changes to the consent statements with participants at follow-up visits.
- This review process is not intended to be a reaffirmation of consent. The clinical center, if required by their local IRB, may develop procedures for reaffirmation of consent.

4.7. HIPAA considerations

FLINT study staff have access to patient health information and to patient identifiers, such as name, address, and telephone number. Study records are to be kept in a secure place. Only people working on FLINT should have access to these records. However, these records could be reviewed to make sure that the study is being done as it should. People who may see medical records supporting study records are:

- Officials of your institution
- Your institution's research ethics committee
- Monitors from the NASH CRN Data Coordinating Center at the Johns Hopkins University, or other individuals selected by the NASH CRN Steering Committee to monitor the study
- Government officials from the Office of Human Research Protections, the National Institutes of Health, or the Food and Drug Administration

Each clinical center should take steps to protect patient privacy. The assigned patient ID number and code should be used to identify patients on forms and in the data files. Personal information such as name, address, and telephone number should be kept only at the clinical center where a patient completes visits.

People outside the clinical center who will receive FLINT study data include:

- The NASH CRN Data Coordinating Center at the Johns Hopkins University in Baltimore, Maryland (or its successor) to maintain the central study database.
- The NASH CRN Radiology Reading Center, located at the University of California, San Diego, to receive MRI image transfers for analysis as well as imaging data recorded onto CD/DVD and sent via Federal Express. The data sent to the MRI Center are anonymized – identified only by a NASH CRN number and code
- The NASH CRN Data and Safety Monitoring Board to review the FLINT data for performance and safety.
- The NIDDK Genetics Repository at Rutgers, the State University of New Jersey in New Brunswick, New Jersey (or its successor) to receive patients' blood to obtain DNA. The blood samples for a particular patient will be identified by the patient's study ID number and code, not by name.
- The NIDDK Biosample Repository at Fisher Bioservices in Germantown, Maryland (or its successor) to receive patients' plasma, serum, and liver tissue. The samples for a particular patient will be identified by the patient's study ID number and code, not by name.

- The NASH CRN investigators, as well as outside researchers, to analyze and report FLINT trial data. Patient identity will not be disclosed in any reports or publications resulting from the study. While FLINT is ongoing, the use of FLINT data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

Patient agreement to join the FLINT trial indicates that the patient also agrees to the use of study data as described above. If a patient does not agree to the described uses of study data, the patient may not participate in FLINT. The only exceptions are refusal to provide blood for genetic research or refusal of MRI procedure; patients may refuse to provide blood for genetic research or refuse the MRI procedure and still enroll in FLINT.

5. Study visits

5.1. Overview of visit schedule

The patient-related activities of the FLINT trial can be divided into 4 phases:

- **Screening for eligibility for enrollment** (1-2 visits over a maximum of 16 weeks)
  - s: Consent, baseline history, review of concomitant drugs, physical exam, AUDIT questionnaire on alcohol use, liver biopsy, fasting liver profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose and/insulin/PT and INR, HbA1c, pregnancy test, etiologic tests

    Skinner questionnaire on alcohol use, MRI (optional), quality of life interview, 2-hour OGTT, serum and plasma for banking (FGF-19, CK-18, bile acids, free fatty acids)

- **Randomization to treatment** (1 visit)
  - RZ: Consent and re-affirmation, review of concomitant drugs, pregnancy test, assignment to treatment group, dispense study drug

- **Follow-up treatment phase** (8 visits over 72 weeks)

  - f02: Follow-up medical history, review of concomitant drugs, review of adverse events, study drug adherence (may be conducted by phone)

  - f04: Follow-up medical history, review of concomitant drugs, review of adverse events, study drug adherence (may be conducted by phone)

  - f12: Medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, focused physical exam, fasting lipid profile, hepatic panel and GGT, fasting glucose and insulin, pregnancy test, serum and plasma for banking (including FGF-19, CK-18, free fatty acids)

  - f24: Medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, detailed physical exam, quality of life, fasting lipid profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose and insulin, HbA1c, pregnancy test, serum and plasma for banking (including FGF-19, CK-18, free fatty acids, bile acids)

  - f36: Medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, focused physical exam, fasting lipid profile, hepatic panel and GGT, fasting glucose and insulin, pregnancy test, serum and plasma for banking (including FGF-19, CK-18, free fatty acids)

f48: Medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, detailed physical exam, quality of life, fasting lipid profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose/insulin/fatty acids, HbA1c, pregnancy test, serum and plasma for banking (including FGF-19, CK-18, free fatty acids, bile acids)

f60: Medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, focused physical exam, fasting lipid profile, hepatic panel and GGT, fasting glucose and insulin, pregnancy test, serum and plasma for banking (including FGF-19, CK-18, free fatty acids)

f72: Medical history, review of concomitant drugs, review of adverse events, study drug adherence, detailed physical exam, MRI (optional), liver biopsy, quality of life, fasting lipid profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose and insulin, 2-hour OGTT, PT and INR, HbA1c, pregnancy tests, serum and plasma for banking (including FGF-19, CK-18, free fatty acids, bile acids)

- **Post-treatment 24-week washout**

  f96: Medical history, review of concomitant drugs, review of adverse events, detailed physical exam, quality of life, fasting lipid profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose and insulin, HbA1c, serum and plasma for banking (including FGF-19, CK-18, free fatty acids, bile acids), closeout

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
Screening (s) visit		
	RG	Registration (document consent, sociodemographics, assign IDs)
	BG	Baseline history
	PE	Physical examination (detailed exam)
	SD	Liver biopsy materials documentation
	HW	Liver biopsy histology worksheet
	HF	Liver biopsy histology findings (reading at clinical center)
	LT	Liver tissue banking
	AD	Alcohol Use Disorders Identification Test (AUDIT)
	LR	Laboratory results done during screening and follow-up visits
	LS	Laboratory tests done only during screening
	QF	SF-36v2 health related quality of life questionnaire
	CG	Genetic consent and blood collection documentation
	BP	Blood processing for plasma and serum
	LD	Lifetime drinking history (Skinner)
	MR	MRI consent and report form
	PL	Patient location (patient contact information)
Randomization (RZ) visit		
	RZ	Randomization checks
	RD	Study drug distribution and return
2 week follow-up (f02) visit		
	HI	Follow-up medical history
4 week follow-up (f04) visit		
	HI	Follow-up medical history
12 week follow-up (f12) visit		
	HI	Follow-up medical history
	LR	Laboratory results done during screening and follow-up visits
	PF	Focused physical examination
	RD	Study drug distribution and return
	BP	Blood Processing for Plasma and Serum

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
24 week follow-up (f24) visit		
	HI	Follow-up medical history
	LR	Laboratory results done during screening and follow-up visits
	RD	Study drug distribution and return
	PE	Physical examination (detailed exam)
	BP	Blood Processing for Plasma and Serum
	QF	SF-36v2 health related quality of life questionnaire
36 week follow-up (f36) visit		
	HI	Follow-up medical history
	LR	Laboratory results done during screening and follow-up visits
	PF	Focused physical examination
	RD	Study drug distribution and return
	BP	Blood Processing for Plasma and Serum
48 week follow-up (f48) visit		
	HI	Follow-up Medical History
	LR	Laboratory results done during screening and follow-up visits
	RD	Study drug distribution and return
	PE	Physical examination (detailed exam)
	BP	Blood Processing for Plasma and Serum
	QF	SF-36v2 health related quality of life questionnaire
60 week follow-up (f60) visit		
	HI	Follow-up medical history
	LR	Laboratory results done during screening and follow-up visits
	PF	Focused physical examination
	RD	Study drug distribution and return
	BP	Blood Processing for Plasma and Serum
72 week follow-up (f72) visit		
	BP	Blood Processing for Plasma and Serum
	HI	Follow-up medical history
	PE	Physical examination (detailed exam)
	LT	Liver Tissue Banking
	LR	Laboratory results done during screening and follow-up visits
	SD	Liver biopsy materials documentation
	MR	MRI consent and report form
	QF	SF-36v2 health related quality of life questionnaire

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
96 week follow-up (f96) visit		
	BP	Blood processing for plasma and serum
	HI	Follow-up Medical History
	PE	Physical examination (detailed exam)
	LR	Laboratory results done during screening and follow-up visits
	QF	SF-36v2 health related quality of life questionnaire
	CO	Close out

As needed: RC, PL, IE, DR, MV, CG, CR, LT, SD, SR, TN

5.3. Guide for screening visits

Before the patient arrives for the visit

- Abstract data from patient's medical records as needed
- Gather the most recent prior liver biopsy slides as available
- Apply labels to forms as appropriate
- Gather specimen collection and mailing materials (labels, tubes, shippers)
- If advisable, alert phlebotomy lab staff of need to obtain plasma and serum samples
- Confirm eligibility with respect to whatever data have been keyed

Procedures

- Obtain signed consent
- Obtain permission to abstract data from patient's medical records
- Obtain patient location information
- Initiate data collection for screening and baseline values
  - Physical exam and anthropometry
  - Interview for baseline history (responses may be modified or expanded upon chart review)
  - Laboratory testing
  - Alcohol use questionnaire
- If patient appears eligible at the close of screening visit
  - Schedule patient for second screening visit
  - Schedule patient for any needed etiologic tests
  - Schedule patient for biopsy, if appropriate
  - Schedule patient for MRI exam, if applicable
- Liver biopsy (pathologist should grade slides from most recent eligible biopsy and obtain 10 unstained slides if possible or arrange for biopsy if needed; if arranging for biopsy, prepare for collection of flash frozen liver tissue)
- MRI exam (radiologist should prepare images and complete forms as indicated in the FLINT SOP VI: MRI Procedure Manual)
- Complete quality of life questionnaire, life time drinking questionnaire, and additional testing
- Obtain consent for DNA banking (if available)
- Collect blood for genetic specimen banking (2 tubes) and biosample banking (3 tubes)
- Confirm eligibility (hand/eyeball review of unkeyed data)
- Explain to the patient that you will electronically confirm eligibility after keying the data collected at this visit but that since you believe the patient to be eligible, you would like to schedule the patient for randomization

5.3. Guide for screening visits

Data collection forms

- Forms completed for all patients
  - RG - Registration
  - PE - Physical Examination
  - BG - Baseline History
  - SD - Liver Biopsy Materials Documentation
  - HF - Liver Biopsy Histology Findings
  - HW - Liver Biopsy Histology Worksheet
  - LS - Laboratory Results – Tests Done Only During Screening
  - AD - Alcohol Use Disorders Identification Test (AUDIT)
  - LR - Laboratory Results - Screening and Follow-up
  - BP - Blood Processing for Serum and Plasma
  - CG - Genetic Consent Documentation (this form documents both consent and refusal)
  - QF - SF-36 Health Related Quality of Life Survey
  - LD - Lifetime Drinking History (Skinner)
  - MR- MRI Consent and Report

- Additional forms required under specific conditions
  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)
  - CR - Central Histology Review
  - RC - Rescreen in FLINT

- Forms completed for all patients

Forms for clinical center use only

- PL - Patient Location
- Medical records release (use local form)

After the patient leaves the clinical center

- Register patient on clinic data system
- Apply labels to forms as needed
- Set up FLINT chart for patient and file the materials generated at registration that will be used during visits
- Key completed data forms
- Package biopsy slides for sending to the DCC
- Batch ship flash frozen liver tissue specimen (if available) to NIDDK Biosample Repository by overnight delivery service
- Key data collection forms
- Run Randomization Task and re-check eligibility
- Package whole blood tubes for DNA banking for mailing and ship to Genetics Repository

5.3. Guide for screening visits

- Submit MRI data to the RRC
- Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates

5.4. Randomization visit

Procedures

- Randomization visit to be conducted as a visit separate from the screening visit
- Patient will be randomized to study drug assignment
- Requests for randomizations will be made by clinical centers using a web based application
- A randomization assignment will be issued only if the database shows that the patient is eligible, has signed the consent statement, and has had all required baseline data keyed to the database
- Patient is given the assigned study drugs with a number unique to the patient, instructed about starting the drugs and monitoring for adverse effects, and begins taking study drugs

Data Collection Forms

- RZ - Randomization Check
- RD - Study Drug Dispensing and Return

Comment

- The date of randomization visit is the date for reckoning all follow-up visits

5.5. Visit windows: randomization and follow-up

- **Randomization** must occur within 3 months (90 days) of date of liver biopsy and within 16 weeks (112 days) of registration

Visit	Window opens: weeks (days) after randomization	Window closes: weeks (days) after randomization	Ideal date
*f02	1 week (7 days)	3 weeks (21 days)	2 weeks (14 days)
*f04	3 weeks+1 day (22 days)	8 weeks (56 days)	4 weeks (28 days)
f12	8 weeks+1day (57 days)	18 weeks (126 days)	12 weeks (84 days)
f24	18 weeks+1day (127 days)	30 weeks (210 days)	24 weeks (168 days)
f36	30 weeks+1 day (211 days)	42 weeks (294 days)	36 weeks (252 days)
f48	42 weeks+1 day (295 days)	54 weeks (378 days)	48 weeks (336 days)
f60	54 weeks+1 day (379 days)	66 weeks (462 days)	60 weeks (420 days)
f72	66 weeks+1 day (463 days)	84 weeks (588 days)	72 weeks (504 days)
f96	84 weeks+1 day (589 days)	108 weeks (756 days)	96 weeks (672 days)

*f02 and f04 are safety visits and may be done by telephone interview.

5.6. Interim (unscheduled) visits or telephone contacts

- Unscheduled visits or telephone contacts may occur as needed. No time windows or minimum time separations are imposed for such visits or contacts.
- Data are not collected at interim visits (i.e., data forms are not completed) unless reporting a death, a serious adverse event, or liver biopsy.
- If a liver biopsy is performed for a FLINT patient at a time other than the baseline and f72 visit, complete the forms related to liver biopsy.

6. Study procedures

6.1. Assignment of study identifiers

What

- The NASH CRN uses 2 identifiers for patients
  - ID number (4 digits)
  - ID code (3 alphabetic characters)
- These identifiers help assure confidentiality of patient identity

Materials

- ID number and code labels received from the Data Coordinating Center
- Registration (RG) form

When

- Eligibility evaluation visit (visit s)

By whom

- Clinical Coordinator

Procedures

- Complete the FLINT Registration (RG) form; if the patient remains eligible at the close of the form, assign the ID number and code by peeling a label of the label sheet and affixing it to the specified item on form RG or note ID assigned previously in NASH CRN
- The patient will be known by these IDs for the duration of the NASH CRN, including participation in any other NASH CRN studies
- Key the Registration (RG) form into FLINT data system; this must be the first form keyed
- The Registration (RG) form should be keyed for each patient screened for FLINT, including patients already enrolled in the NAFLD Adult Database 2

Comments

- Once an ID number and its associated ID code are assigned, these IDs must be used by the patient for the duration of the NASH CRN and cannot be changed
- Do NOT reassign or reuse IDs assigned to patients found to be ineligible or who refuse enrollment

6.2. Baseline History (BG) Form

Who

- Complete for all FLINT patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries:
  - History of liver disease
  - Information on initial diagnosis of NASH
  - Liver biopsy history
  - Menstrual history (female patients)
  - Medical history (answer items based on information from all sources available to you)
  - Medication use currently and in the past 6 months to 1 year (12 months)
  - Willingness to use birth control methods

When

- Visit s (but given that you need to do chart review, this may take multiple visit days)

How

- Mix of interview data and data obtained by chart review
- Other questions on the BG form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

Definitions of hepatic events queried on Forms BG and HI

- The FLINT trial will be tracking the occurrence of clinically significant hepatic events. These include variceal bleeding, ascites, edema, hepatic encephalopathy, hepatopulmonary syndrome, hepatorenal syndrome or liver cancer. Any occurrence of these should be documented on the HI form. The following guidelines may be useful in defining these events:

  - **Ascites.** The results of abdominal paracentesis and testing of ascetic fluid is the gold standard for the diagnosis of ascites. However, the appearance of ascites on imaging such as ultrasound or CT scan in the setting of portal hypertension is highly suggestive.
  - **Hepatic encephalopathy.** There should be sufficient documentation of a reversible decrease in neurologic function, in the absence of hypoxia, acidosis, drug toxicity, and other metabolic and toxic insults.

- **Hepatopulmonary syndrome.** The diagnosis rests on documenting the presence of arterial deoxygenation and intrapulmonary vasodilation in patients with liver disease. Most commonly the presence of symptoms, such as the insidious onset of dyspnea, or the clinical findings of clubbing, cyanosis, or decreased oxygen saturation on pulse oximetry prompt an evaluation. Arterial blood gases, contrast echocardiography, and lung perfusion scanning are used in further evaluation of hepatopulmonary syndrome.
- **Hepatorenal syndrome.** Occurs in patients with advanced liver disease and portal hypertension characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of endogenous vasoactive systems. In the kidney there is marked renal vasoconstriction that results in low GFR, whereas in the extrarenal circulation there is predominance of arterial vasodilation, which results in reduction of total systemic vascular resistance and arterial hypotension.
- **Hepatocellular carcinoma.** Cirrhosis may predispose patients to develop hepatocellular carcinoma, the most common primary malignant hepatic neoplasm. Physical findings such as ascites, fever, portal hyptertension, and jaundice are associated with hepatocellular carcinoma. Rise in serum alkaline phosphatase and low alpha-fetoprotein (AFP) level and imaging studies for evaluation of liver lesions (tumor) may lead to a liver biopsy for confirmatory diagnosis.
- **Pedal edema.** Accumulation of excessive watery fluid in lower extremities will be evaluated by the investigator during physical examination.
- **Variceal bleeding.** The results of diagnostic testing including EGD, radionuclide imaging, or angiography, in the setting of portal hypertension should be confirmed.

6.3. Follow-up Medical History (HI) form

Who

- Complete for FLINT patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries/reviews
  - – Alcohol consumption since the last visit (AUDIT-C)
  - – Medical history diagnoses and procedures since the last visit
  - – Medication use since the last visit
  - -- Adverse events since the last visit

When

- Follow-up visits: f02, f04, f12, f24, f36, f48, f60, f72, f96. (Visits f02 and f04 are safety visits; that can be compared by telephone)

How

- Mix of interview data and data obtained by chart review
- Drinking items should be obtained by an interview with the patient
- Other questions on the HI form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

6.4. Physical examination (PE and PF forms)

Who

- All FLINT patients

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference (form PE only)
  - Hip circumference (form PE only)
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review (form PE only)
  - Chest and lungs
  - Heart
  - Abdomen abnormalities
  - Focused liver signs

When

- Detailed physical (form PE) at visit s, f24, f48, f72 and f96
- Focused physical (form PF) at visits f12, f36, f60
- Fasting is irrelevant for Forms PE & PF

How

- Ideally, use a stadiometer for height measurement.
- Ideally, use the Gulick II tape measure for waist and hip measurement; this device may be obtained from www.fitnessmart.com (608-735-4718, model 67019, listed at $36); it is manufactured by Country Technology Inc: 608-735-4718.

6.5. Height and weight measurements

Height

- Height may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a wall-mounted stadiometer with a horizontal measuring block (or fixed angle) is used; other height measuring devices are acceptable
- Follow the manufacturer's recommendation regarding method and frequency of calibration of the stadiometer
- The patient stands erect on the platform with his/her back parallel to the vertical mounted measure scale (but not touching the wall), looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane defined by the lower margin of the bony orbit (the bony socket containing the eye) and the most forward point in the supratragal notch (the notch just above the anterior cartilaginous projections of the external ear)
- The horizontal measuring block is brought down snugly, but not tightly, on the top of the head
- Record the height to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, raise the measuring block, ask the patient to return, and repeat the measurement

Weight

- Follow the manufacturer's recommendation regarding method and frequency of calibration of the scale
- Weight may be recorded in pounds or kilograms
- Two measurements are recorded
- Ideally, weight is measured in the morning after voiding and before breakfast; this should be possible in the FLINT since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's weight at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Patient should stand still in the middle of the scale platform with head erect and eyes looking straight ahead
- Record the weight to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, zero the scale, ask the patient to return, and repeat the measurement
- Patients who have limb amputations or who are wearing casts should have weight measured, but note this on the form on the margin (the notes may be keyed at data entry in the General Comments area of the keying)

6.6. Waist and hip circumference measurements

Waist

- Waist circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, waist circumference is measured in the morning after voiding and before breakfast; this should be possible in the FLINT since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's waist at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to bare his/her waist
- Wrap the tape once around the waist: the measure should be taken around the abdomen horizontally at the midpoint between the highest point of the iliac crest and lowest part of the costal margin in the mid-axillary line
- Mark the midpoint on both sides of the patient using a washable marker
- Patient may be asked to assist in passing the tape around the abdomen by holding the end of the tape in position
- When the tape is positioned in the horizontal plane at the correct height, the patient should be asked to keep his/her arms at the sides and breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape, retract the tape, and repeat the procedure
- If the tape cannot be made horizontal across the waist markings, default to the right hip and note this in the margin of the form

6.6. Waist circumference measurement

Hip

- Hip circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, hip circumference is measured in the morning after voiding and before breakfast; this should be possible in the FLINT since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's hip at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to adjust his/her clothing to allow measuring the hips over the patient's underwear
- Wrap the tape once around the hips over the underwear: the measure should be taken at fullest part of the hips (maximum extension of the bullocks)
- Patient may be asked to assist in passing the tape around the hip by holding the end of the tape in position
- When the tape is positioned correctly, the patient should be asked to keep his/her arms at the sides and breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape and repeat the procedure

6.7. Baseline and follow-up liver biopsy (SD and other forms)

Details of liver biopsy procedures, tissue banking, slide preparation, and shipment of slides to the DCC are discussed in the SOP Part IV, Liver Biopsy and NAFLD/NASH Histology Scoring System document.

- Baseline liver biopsy must be obtained within 90 days prior to randomization for all patients who have been found to be eligible for FLINT with respect to all other criteria.
- A follow-up liver biopsy should be obtained at the f72 visit for all patients enrolled in FLINT.
- Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy.
- The MRI exam will ideally be scheduled as close to the time of the biopsy as feasible.
  - Baseline MRI should be within 90 days of baseline biopsy and prior to randomization.
  - Follow-up/post-treatment MRI should be no more than 6 weeks before and 12 weeks after the f72 visit biopsy.
- A pregnancy test (for women of child-bearing potential) should be obtained prior to the liver biopsy and MRI exam.

Forms

- Occurrence of liver biopsy(s) done before screening and occurrence of liver biopsy during screening are queried on the Baseline Medical History (BG) form.
- Occurrence of a biopsy since the previous FLINT visit is queried on the Follow-up Medical History (HI) form.
- The Liver Biopsy Materials Documentation (SD) form must be completed to document the outcome of all biopsies obtained for FLINT (screening and follow-up) with regard to availability of tissue for banking and stained and unstained slides for scoring and archiving.
- If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed.
- If the biopsy was done prior to or during screening, then the local FLINT Study Pathologist must complete the Liver Biopsy Histology Worksheet (HW) and the Clinical Coordinator must complete the Liver Biopsy Histology Findings (HF) form.
- Central scoring of biopsies, shipment of slides to the DCC, and shipment of frozen liver tissue in RNA*later*Solution® to the Biosample Repository must be documented on the Central Histology Review (CR) form.
- The Histology Slide Transmittal Log (TS) form must be completed and accompany every shipment of slides sent to the DCC.
- The Specimen Shipment Log (SS) form must be completed and accompany every shipment of frozen liver tissue to the NIDDK Biosample Repository.

The hepatologist, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and each complete their sections of the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

6.8. Baseline and follow-up MRI (MR and other forms)

Details of MRI procedures, case report forms, and submission of data to the Radiology Reading Center (RRC) are discussed in the SOP Part VI, MRI Procedure Manual.

- Baseline MRI must be obtained within 90 days of liver biopsy and prior to randomization for all patients who have been found to be eligible for FLINT with respect to all other criteria
- A follow-up MRI should be obtained no more than 6 weeks prior to and no more than 12 weeks after the follow-up liver biopsy at the f72 visit for all patients enrolled in FLINT.
  - It is preferred that the MRI exam is done while the patient is still using the study drug.
  - This MRI should be the same time of day as the baseline MRI.
- The MRI exam will ideally be scheduled as close to the time of the biopsy as feasible.
- A pregnancy test (for women of child-bearing potential) should be obtained prior to the liver biopsy and MRI exam.

DCC Forms - entered into the NASH CRN data system

- The **MRI Consent and Report form (MR)** must be completed by the study hepatologist/radiologist and coordinator to document the occurrence of an MRI procedure obtained for FLINT (screening and follow-up) and transmittal of images to the RRC.

RRC Forms - submitted to the RRC via fax (619-471-0503) or email (liclark@ucsd.edu)

- **MRI Radiologist Report CRF** (see SOP VI Appendix B) is completed by the Study Radiologist after the images are reviewed. If there are any significant findings, the Study Hepatologist must be notified.
- **MRI Data Transmittal CRF** (see SOP VI Appendix A) is completed by the Clinical Coordinator
- **MRI Adverse Event CRF** (see SOP VI Appendix C) is completed by the Clinical Coordinator for each MRI that is performed, whether or not there are adverse events.

MRI Related Adverse Events

If there are adverse events related to the MRI procedure, the Clinical Coordinator should notify a Study Hepatologist and complete an Interim Event Report (IE) form for the DCC. More information regarding adverse events is discussed in Section 6.15 Adverse Event Reporting.

The Study Hepatologist, Clinical Coordinator, Study Radiologist, and other Imaging Personnel must work together to accomplish these tasks and each complete their sections of the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

6.9. Alcohol use questionnaires (AD, LD, other forms)

What / Who

- AUDIT (AD) form
- Skinner Lifetime Drinking History (LD) form
- Summary question on FLINT Randomization Checks (RZ) form
- Questions on interval alcohol consumption on Follow-up Medical History (HI) form
- Flash Card #9, Drink Equivalents, can be used with the alcohol questionnaires
- Flash Card #10, Patterns of alcohol intake, provides the interviewer with sample language for administering the LD form

Purpose

- At screening, obtain a detailed history of the patients alcohol consumption patterns from the onset of regular drinking
- Monitor alcohol use during follow-up

Who

- All FLINT patients

How

- Form AD is self-administered for patients, without help from spouse or family
- Form LD is interviewer administered

Comments

- At screening, the patient is never asked directly how much alcohol he/she drinks per week on average; clinic staff must judge this based on all information obtained from interview, chart review, discussions with those who know the patient etc; the clinic staff use their best judgement to answer the eligibility question on the FLINT Randomization Check (RZ) form
- The Clinical Coordinator should complete section A on page 1 of Form AD and record patient ID on subsequent pages before asking the patient to complete the form

6.10. Quality of life questionnaire (QF)

Purpose

- To evaluate correlations between self-reported quality of life and disease severity

What / Who

- All FLINT patients

When

- Visit s
- Follow-up visits f24, f48, f72, and f96

Procedure

- Clinical Coordinator should complete Part A and apply labels to subsequent pages as needed before giving the form to the patient to complete
- Self administered for patients, without help from spouse or family
- Clinical Coordinator should check returned forms for completeness before the patient leaves the clinical center

6.11. Laboratory measures (LS and LR)

Who

- All FLINT patients

What

- Form LS covers assessments collected only at screening:
  - Screening etiologic tests
  - Iron assessments
  - Ceruloplasmin measurement
  - Alpha-1 antitrypsin assessment
  - Autoantibody studies

- Form LR covers assessments collected during screening and follow-up
  - Hematology
  - Chemistries
  - Prothrombin time, INR, and HbA1c
  - Liver panel
  - Fasting lipids
  - Fasting glucose
  - Glucose tolerance test
  - Pregnancy

When

- Form LS: Visit S
- Form LR: All visits
- Given the extent of information recorded on form LS, you may still be completing it at visit s2 or drawing blood for tests at visit s2; use visit code s anyway
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

Instructions for Form LS

- Most of the tests on Form LS are intended to be obtained by chart review; time windows for the allowed age of tests are specified on the form
- Serological assessment to exclude viral causes of chronic liver disease is required for all patients
- Iron overload screening is required for all patients; hepatic iron index is recorded if available, but is not required
- HFE gene analysis is required only if the patient has an abnormality in an iron overload screening test, a family history of iron overload or hemochromatosis, or histological iron of greater than 3+
- Ceruloplasmin is required for patients age 40 or younger

- Alpha-1 antitrypsin assessment is required for all patients
- Autoantibody studies are required for all patients

Instructions for form LR

- The measures on form LR can also be obtained by chart review, both at screening and during follow-up; the time window for each type of assessment is specified on the form
- During follow-up, the time window for the assessment is "in the time window for the follow-up visit (check the patient's Visit time window guide)" – e.g., f48 has an acceptable calendar time period within which it may be done; if you can find a hematology assessment in the patient's chart that was done under the same conditions as required by the study and which provides the required values and was done within the time window for visit f48, you do not need to order another hematology at f48
- If the chart review tests are out of the time window or the test conditions can't be ascertained or differ from what is required, the measures have to be done at the study visit and can be charged to the study
- For baseline, the required time window is within 90 days of the liver biopsy.
- All laboratory test results are required during screening.

6.12. Plasma and serum collection for Biosample Repository (BP)

Purpose

- Collection of whole blood from the FLINT trial patients; when timed to coincide with a liver biopsy, blood should be collected ideally within ± 7 days and up to ± 90 days of the biopsy. Blood should not be collected in the 72 hour period after a biopsy
- Separation of plasma and serum at clinical center:
  - Screening visit and f72: ten 0.5 mL aliquots of plasma and twenty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials.
  - f12, f24, f36, f48, f60, and f96: ten 0.5 mL aliquots of plasma and ten 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials
- Store plasma and serum aliquots at -70° C prior to batch shipping to the NIDDK Biosample Repository at Fisher BioServices

Fasting Instructions

- Patient instructed to fast 12 hours (recommended) prior to blood draw; an 8-hour fast prior to blood draw is allowable

Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for heparin (green top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- Barcode scanner
- SS - Specimen Shipment log and Excel Spreadsheet
- NIDDK Biosample Repository shipper

When

- Visit s
- Followup visits (f12, f24, f36, f48, f60, f72, f96)
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator
- Person responsible for shipping to NIDDK Biosample Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

6.12. Plasma and serum collection for Biosample Repository

Equipment

Blood tubes/aliquot vials

- One 10 mL sodium heparin (green top) tube - *provided by clinical center*s
- One or two (depending on the visit) 10 mL SST (red-gray top) tubes - *provided by clinical centers*
- Up to thirty 2.0 mL cryogenic vials - *provided by clinical centers*
  - vials should be able to withstand -196 degrees C
  - vials should be self standing (flat bottom, not curved), externally threaded, 13.5 mm wide x 48.3 mm tall, with silicone washers

Labels

- Preprinted labels for whole blood collection tubes (10 mL heparin tube and 10 mL SST tubes) and preprinted labels for Form BP – *labels are printed at the clinical center via web-based data management system; use MACO ML-5000 1" x 1 ½" labels, 50 labels/page*
- Preprinted polypropylene labels for 2.0 mL cryogenic vials – *provided by the DCC*

Equipment

- Centrifuge
- -70° C freezer
- Swing out rotor
- 5 mL pipettes

Blood processing procedures

- Patient instructed to fast 12 hours prior to blood draw (recommended); an 8-hour fast prior to blood draw is allowable
- Collect whole blood into one 10 mL heparin (green top) tube for plasma
- During visit s and f72, collect whole blood into two 10 mL SST (red-gray top) tubes for serum.
- During other follow-up visits, collect whole blood into one 10 mL SST tubes for serum.
- If sample appears to have hemolyzed, do not aliquot. Re-draw blood

Plasma

- Collect blood into heparin (green top; Becton-Dickinson) tube. Ensure that heparin tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- Completely fill vacutainer tube
- Mix gently by inversion 5 times
- Within 30 minutes, centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Immediately after centrifugation, insert a 5 mL pipette below surface of plasma

- Remove the clear plasma while avoiding blood cells
- Transfer plasma into aliquots of 0.5 mL each into 10 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of plasma should be completed within 30 minutes

Serum

- Collect blood into serum separator (red-gray top) tubes. Ensure that SST tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- After filling, invert each SST tube gently at least 5 times
- Allow blood to clot for **at least 30 minutes** at room temperature
- Centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Transfer 0.5 mL of serum into 10-20 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of serum should be completed within two hours

Note: Separated serum and plasma may be stored at -20° C for up to one day before transfer to -70° C while ensuring samples remain frozen during the transfer.

Blood Processing for Plasma and Serum (BP) form

- Complete the Blood Processing for Plasma and Serum (BP) form
- Affix labels for the heparin plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

Shipping samples to the NIDDK Biosample Repository

- Specimens are to be batch-shipped monthly to Fisher BioServices on Monday, Tuesday, or Wednesday
- Aliquots will be stored locally at the clinical center at -70° C prior to shipping
- Ship specimens in the STP 320 Saf T Pak shipper (provided by the NIDDK Biosample Repository)
- Open the template Excel file used for shipments and scan each cryovial using the carcode scanner provided to your clinical center. The file should have the filename: NASHCRNsiteXXX_shipdate.xls. Replace the 'xxx' with your clinical center three digit site ID and replace 'shipdate' with the date of shipment.
- The Excel shipping file has column headings for barcode number: Site ID-Patient ID numbers; 3 letter patient code; date collected; specimen type: plasma, sera, liver tissue; volume; units of measure; study number; and visit code
- Record the Federal Express Airbill tracking number, at top of page 3, of the Specimen Shipping Log.
- Complete Section A. Center ID, shipment and study information and section B. Clinical Administrative information of the Specimen Shipment Log (SS). Enclose a printed copy of the Specimen Shipment Log and the Excel spreadsheet with each shipment of specimens.

- Keep a notebook of all original completed Specimen Shipment Logs (Form SS) and Excel spreadsheet so that you have a record of all shipments to the Biosample Repository
- Notify the Biosample Repository of the shipment via fax (301-515-4049) or email bio-niddkrespository@thermofisher.com) on the day the package is picked up by Federal Express. Include the tracking number in the subject line of the email.

Packaging Procedures

- Check that 1 absorbent pad is in the Saf T Pack Bio hazard plastic bag
- Insert frozen cryovial into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box may hold 81 cryovials
- Insert each cardboard box with cryovials into its own plastic bag and seal
- Place each plastic bag with specimen box into its own STP-710 Tyvek envelope and seal.
- Place each Tyvek envelope into STP-111 inner brown cardboard box. No more than 3 Tyvek envelopes containing boxes with cryovials can be placed into the STP-111 inner brown cardboard box. If shipping only 1 or 2 specimen boxes, fill the rest of the space inside the cardboard inner box with bubble wrap to prevent movement
- Tape the inner cardboard box closed before placing the styrofoam cooler
- Place cardboard box in upright position in bottom of styrofoam cooler
- Surround the STP-111 inner brown cardboard box with abut 8 kg of 2" blocks or nuggets of Dry Ice
- Fill excessive room left in the insulated freezer box with bubble wrap to stabilize specimens in transit
- Place the polystyrene lid onto the freezer box
- Place the "Empty Packaging" cover and shipping form, with Excel spreadsheet on the top of the cooler lid
- Place a completed Specimen Shipment Log (Form SS) on top of the cooler lid
- Close and seal outer cardboard box with tape

Labeling Shipper:

- Place a checkmark in the block on the outer cardboard box next to "BIOLOGICAL SUBSTANCE, CATEGORY B". Do not cover this marking with labels.
- Affix a label with your name and return address to the side of the box in the "Shipper:" block
- Affix the repository address label to the side of the box in the "Consignee:" block
- Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms
- Affix the "UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B" label to the right of the dry ice label
- Use the preprinted Federal Express air bill to ship specimens to the NIDDK Biosample Repository. Complete return address (leave "Sender Federal Express account number" blank). Section 6, Special Handling: Check "Yes, Shippers Declaration not required," check "Dry Ice" block and entry "1" x "8" kg. Section 7, Enter "1" under "Total Packages" and the total weight of 24 lbs. Place completed Federal Express Airbill on side of box adjacent to the labeled side. Call Federal Express at 1-800-463-3339; give them the account number in section 7 of the Airbill

  Do not write on exterior of box

  Do not ship frozen packages on Friday; the repository is closed on weekends

6.13. Genetic consent and blood collection documentation (CG)

Purpose

- Collection of whole blood from FLINT patients who consent for genetic research
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking
- Do not repeat genetic consent or blood draw for patients who have had blood drawn for genetic research as part of other NASH CRN studies, unless original yield was low.

Forms

- FLINT consent for genetic research
- Genetic Consent and Blood collection Documentation (CG) form
- NIDDK Genetics Blood Collection form

When

- Visit s (or as needed during follow-up due to a low yield [less than 50g] of DNA)
- Ship same day as whole blood collection

By whom

- Clinical Coordinator and Study Physician (to obtain consent)
- Phlebotomist (to obtain whole blood)
- Person responsible for shipping whole blood to NIDDK Genetics Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

Equipment

- Two 10 mL NaEDTA vacutainer tubes (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube labels and form CG labels - *provided by clinical centers (printed from web based data management system; clinical center provides MACO ML-5000 labels (1" x 1 ½ ", 50 labels per page, www.maco.com)*
- Shipper - *provided by NIDDK Genetics Repository*
  - One model 472 Thermosafe Safety Mailer (body and lid)
  - One 2 ½" x 9" pre-cut section of absorbent materials
  - Two 18" strips of red waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs
  - One pre-printed Fed Ex airbill with third party billing
  - One NIDDK Genetics Blood Collection form
  - Instructions for Blood Sample Collection form

6.13. Consent and specimen collection for Genetics Repository (CG)

Blood collection procedures

- Affix MACO tube label onto the tube and avoid covering the barcode label
- Collect blood into two 10 mL NaEDTA (purple top) tubes
- Invert each tube gently 6 times to mix blood with additives
- Keep blood at room temperature
- Check that patient ID information on preprinted tube labels matches information recorded onto the NIDDK Genetics Blood Collection form

Packaging procedures

- Ship whole blood at ambient room temperature same day to the NIDDK Genetics Repository
- Package the whole blood tubes in the body of the Safety Mailer (Model 472 Thermosafe Safety Mailer)
- Place absorbent material so it exactly covers cavity of the Safety Mailer
- Place lid of Safety Mailer over body and absorbent material and press down firmly so that lid closes properly. Reposition absorbent material so that it does not get caught between the lid and body
- Peel backing from two 18" long pieces of red waterproof tape and seal the Safety Mailer lid to the body; peel backing from second piece of tape and continue sealing the mailer, overlapping the first piece of tape about two inches on both ends
- Place the sealed Safety Mailer into the press-lock plastic bag. **Do not seal the bag**.
- Place the NIDDK Genetics Blood Collection form in the mailer box outside the plastic bag
- Slide the Safety Mailer and open press-lock bag into the corrugated carton
- Seal the press-lock bag and close carton using the locking tabs
- Place sealing tape (not included) over them

Shipping procedures

- Use the preprinted Federal Express shipping label, marked for *Priority Overnight Delivery*, to ship whole blood to the NIDDK Genetics Repository
- Outside cardboard box must have stamped “Diagnostic Specimen Packed in Compliance with IATA Packing Instructions 650"
- Call Federal Express, 1-800-Go-FEDEX (1-800-463-3339) for courier*
- Notify Dana Witt or Elva Peralta at the NIDDK Genetics Repository that blood is being shipped and provide the Federal Express tracking numbers and the NIDDK ID numbers. Notification may be via:
  - Web portal: http://rucdr.rutgers.edu/shippingblood
  - email: witt@biology.rutgers.edu
peralta@biology.rutgers.edu

  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498
- Ship whole blood to:
  Rutgers University/Cell Repository/NIDDK
  604 Allison Rd., Room C120A
  Nelson Laboratory
  Piscataway, New Jersey 08854-8000

Genetics Repository Web Portal System
(Rutgers University Cell and DNA Repository - RUCDR)

- Establishing a Username and Password

  http://rucdr.rutgers.edu/scripts/up.exe?AIMACTION=vnewaccountconiddk&enforce_color=ON&skey=10925637151082500795

- Go to the URL listed above and then just follow the directions on the top of the page. You can sign up for multiple NIDDK sites (if you are associated with more than one) at once. (Phlebotomists performing off-site draws will send a notice from http://rucdr.rutgers.edu/shippingblood.)

Logging in to the System
- The URL for the RUCDR Web Portal is http://rucdr.rutgers.edu. Click on the square for NIDDK to get to your login screen. Enter your newly created username and password. If you ever forget your username or password there is an option on this screen to "Retrieve Lost Password". You will need to remember what email address you used to create your account to use this function!

Announcement Board
- When you enter the web portal you will see announcements from the RUCDR. The dates of future holiday closings will be listed here.

Navigating the Web Portal
- Click the tabs on the top of the screen to access the different parts of the web portal. The functions accessible from each tab are listed below.

Request Functions
- From the "Request Functions" tab you can do two things: "Submit Request" or "Look Up Status of Request".

6.13. Consent and specimen collection for Genetics Repository (CG)

1. **Submit Request**
   - To get to these options, pick a function from the drop-down menu: Shipping Blood, Request Mailers, or Question.
   - Next, pick a site number from the drop-down menu.
   - Fill out the section of the form corresponding with the function you chose. Even if your function choice was not "Question" you can add information to any request in the textbox under the heading "Special Notes/Special Instructions/Questions".
   - *Good thing to know! If you choose "Shipping Blood" you can only enter one FedEx tracking number per submission, but if you have more than one sample in the box you can list all the NIDDK ID numbers separated by commas. As always, do not overpack the mailers and enclose a separate piece of paperwork for each sample.*
   - In Section 2: Attachments (a light grey area towards the bottom of the page) you can add a file.

2. **Look Up Status of Request**
   - You can search your recent requests to see their status in multiple ways. These are self-explanatory. If you just hit the search button without selecting any search criteria all the requests you have made will be shown.
   - There are 4 different status assignments a request can have:
     – Open
     – Assigned
     – Pending
     – Closed
   - **Open:** This status signifies that a request has been submitted, but is not yet assigned.
   - **Assigned:** This status signifies that an open request is assigned to a particular staff person.
   - **Pending:** This status signifies that a request has been assigned and a staff person is working on it, but hasn't yet completed the job.
   - **Closed:** When a request is completed the status is set to closed.

6.13. Consent and specimen collection for Genetics Repository (CG)

Self Help Resources

- This tab is a holding area for useful documents.

1. **FAQ** – If you have a question, hopefully it is already answered here.

2. **Download Center** – These instructions are here! Also, any paperwork enclosed with mailer kits is here in case you need to print off extras.

3. **View Announcements** – In case you missed the announcement page when you first logged in to the web portal you can read it again.

4. **Support Resources** – Links that may be of interest to visit.

Account Management

- From this tab you can "Modify Your Profile" or "Change Password".

Important Information Regarding Blood Shipments

- When a package is received, a mailer request is filled or a question is answered, you will receive an email from us and the status will be changed to "closed".

*Due to inconsistent Federal Express delivery around holidays and likelihood of closure of the Genetics Repository on holidays, do not schedule deliveries on the day before or the day of a national holiday. Check with Federal Express and with the Genetics Repository if there is any question about delivery availability or closure.

6.14. Study drug dispensing and return (RD)

Forms

- RD - Study Drug and Return form

Drug supply

- Obeticholic acid: 25 mg/capsules, (33 count/bottle) taken orally once a day (qd)
- Placebo: 25 mg capsules taken orally once a day (qd)

Distribution of study drug

- Study drug to be distributed at: Rz, f12, f24, f36, f48, and f60
- 3 month supply (3 bottles)

Checks on return of study drug

- Unused study drug to be returned by patient at: f12, f24, f36, f48, f60 and f72

By whom

- FLINT clinical coordinator or pharmacist

Ordering procedures at clinical center

- Inventory current drug supplies
- Study drug supplies are shipped to arrive within 2 working days of receipt of order
- Notify DCC if the supply falls below 12 bottles

Handling and disposal

- Unused portions of open bottles in the possession of patients should be considered contaminated and handled accordingly
- Returned capsules should be counted by the pharmacist or clinic coordinator and the number of capsules and the number bottles returned, should be recorded on the RD form
- Expired study drug, partially used study drug, and bottles of study drug returned by patients should be destroyed following your institution's procedures for disposal of investigational study drug
- Documentation should be recorded onto the FLINT Trial study drug accountability records to account for all returned study drug as well as its destruction per your institutional guidelines

Storage and stability

- Store at room temperature (68-77 degrees F) in a cool dry place

6.15. Adverse event reporting

Definitions (21 CFR 312.32 IND Safety Reporting)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or life-threatening suspected adverse reaction. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Reportable FLINT adverse events

- Adverse events should be recorded on the FLINT Follow-up Medical History (HI) and Interim Event (IE) data forms whether or not thought to be associated with FLINT or the study drugs.
- Any event threatening the integrity of the FLINT Trial or well-being of the participant (eg, suspected fraud) is a reportable event. We recognize that this category is not well-defined; however, it is included as a reminder that reportable events can have a broader scope than events that happen to a patient. Some examples include:
  (1) events that impact the patient's treatment or participation in FLINT;
  (2) adverse events that are recorded on the Follow-Up Medical History (HI) form;
  (3) adverse events that may or may not be related to study drug;
  (4) other events that clinical center staff feel should be reported;
  (5) when a follow-up report is needed for a previously completed IE form.
- Deciding whether an event is reportable to FLINT (ie, is in either of these categories) will be the responsibility of the Principal Investigator of the clinical center.
- The Data Coordinating Center will maintain a list of adverse events for reporting and review at Steering Committee meetings and DSMB meetings.

6.15. Adverse event reporting (IE)

CTCAE v3.0

- The NASH CRN uses the Common Terminology Criteria for Adverse Events (CTCAE v3.0) to specify and grade adverse events.
- This document is posted on the NASH CRN website (www.nashcrn.com – click on Studies and then click on FLINT)
- Use the CTCAE v3.0 to specify the Short Name for the adverse event and the severity grade for the adverse event. All adverse events should be assigned a severity grade 1-5.
- Adverse events Grade 3 or higher must be faxed to the DCC within 1 week for immediate review by the Safety Officer, Dr. Jeanne Clark.

Local reporting requirements

- Your clinical center's IRB has reporting requirements of its own regarding events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by FLINT. Regardless of what FLINT requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than FLINT, you may report events locally that you do not report to FLINT.
- It is possible that some FLINT patients will develop significant liver-related morbidity or mortality during the course of followup. This information is important and should be documented on the Followup Medical History (HI) form and Interim Event (IE) data form, but it may also be considered a reportable adverse event according to the local institutional guidelines.
- For more information please refer to FDA Guidance for Clinical Investigators, Sponsors, and IRB: Adverse Event Reporting to IRBs – Improving Human Subject Protection

6.16. Serious adverse event reporting

Definitions (21 CFR 312.32 IND Safety Reporting)

Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered **"SERIOUS"** if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the FLINT study drug caused the adverse event. For the purposes of IND safety reporting**, "REASONABLE POSSIBILITY"** means there is evidence to suggest a causal relationship between the FLINT study drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered **"UNEXPECTED"** if it is not listed in the most current obeticholic acid investigator brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Expedited review process

- Adverse events that are judged by the principal investigator to be **SERIOUS**, **UNEXPECTED** and have a **REASONABLE POSSIBILITY** of being caused by FLINT study drug should be recorded on the Serious Adverse Event/IND Safety Report (SR) form. The SR form should be entered into the data system and faxed to the DCC within 2 business days along with a narrative of the event and a copy of the clinical center's IRB report.

- The narrative should describe the serious adverse event and include history with the FLINT study drug, any comorbidities, concurrrent medications with doses, and any recent changes hospitalizations or ER visits and justification of how the serious adverse event is determined to be serious, unexpected, and have reasonable possibility of being caused by FLINT study drug as defined above and in 21CFR312.32.
- The Data Coordinating Center along with the Safety Officer, Dr. Jeanne Clark, will review the materials to make sure that the required information is included. At any time, the DCC may ask for revisions of the supporting documentation and further clarification of the serious adverse event until the amount of information is sufficient.
- The DCC will notify NIDDK of the event within 3 business days of receiving the SR form and submit a preliminary report for further review of the material. At any time NIDDK may ask for additional information or further clarification of the serious adverse event.
- If deemed necessary, NIDDK will notify Intercept Pharmaceuticals within 1 week.
- Not every SR form that is submitted will result in an expedited IND Safety Report to the FDA. The final decision will be the responsibility of the NIDDK (sponsor).
- If NIDDK determines that the serious adverse event requires an expedited IND Safety Report, they will notify the FDA within 7-15 business days of the SR form receipt at the DCC.
- Within 1 month of the initial SR form, the clinical center must submit to the DCC and the NIDDK a follow-up report when:
  (1) serious adverse event is resolved;
  (2) there has been a significant change in the patient's condition;
  (3) there has been a significant change in the physician's judgment about the serious adverse event;
  (4) periodic updates to report the details of the disposition of the serious adverse event.
- For more information, please refer to the FDA Final Rule: IND Safety Reporting requirements for Human Drug and Biological Products

Data Coordinating Center responsibilities

- The Data Coordinating Center will catalog all serious adverse events for reporting and review at Steering Committee meetings and DSMB meetings.
- The DSMB will review each serious adverse event report and provide comments to the NIDDK project officer and Steering Committee. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the serious adverse events and recommend any actions to the NIDDK.
- If the FDA determines that a change to the investigators brochure, IND or protocol is needed, the Data Coordinating Center will send a copy of the report to all clinical centers, with instructions to forward the report to their IRB. Copies of the report will also be sent to the NIDDK, Data and Safety Monitoring Board (DSMB), and Intercept Pharmaceuticals.

6.16. Serious adverse event reporting

Local reporting requirements

- When you receive a report from the Data Coordinating Center regarding occurrence of an event reportable to the FLINT trial at another NASH CRN clinical center, you must forward that report to your IRB. It may be that your IRB has no comment on events occurring elsewhere; nevertheless, the notification of your IRB is still a FLINT requirement.
- Your clinical center's IRB has reporting requirements of its own regarding serious adverse events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by FLINT. Regardless of what FLINT requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than FLINT, you may report events locally per your IRB guidelines.
- For more information, please refer to the FDA Guidance for Clinical Investigators, Sponsors, and IRB: Adverse Event Reporting to IRBs – Improving Human Subject Protection

6.17. Anticipated adverse events

Essentially all patients with NASH have metabolic syndrome or overt, previously diagnosed or undiagnosed Type 2 diabetes. Furthermore, advancement of metabolic syndrome and diabetes and emergence of the multitude diabetic complications are typically gradual, ongoing processes. Please consult the obeticholic acid investigator's brochure for a complete and current listing of anticipated adverse events

Cardiovascular, Cerebrovascular, and/or Peripheral Vascular:Angina, atherosclerosis, acute coronary syndrome, cardiac ischemia, myocardial infarction, cerebrovascular accident (CVA) or stroke (ischemic or hemorrhagic), cerebral ischemia, transient ischemic attack, claudication, decreased peripheral pulses, and abnormal ankle-brachial index.

Dermatologic: Acanthosis nigricans, foot ulcers, and stasis ulcers.

Endocrine: Hypo- and hyperglycemia, elevated hemoglobin A1C, insulin resistance, dyslipidemia, ketoacidosis, and hyperosmolar hyperglycemia.

Gastrointestinal: Gastric and intestinal dysmotility (related to autonomic neuropathy) and mesenteric ischemia (intestinal atherosclerosis).

Immunologic/Infectious: Infections including foot infections, urinary tract infections (including fungal infections and emphysematous urinary tract infections), superficial fungal infections (oral and/or esophageal candidiasis, onychomycosis, candidal intertrigo, and vulvovaginal candidiasis), zygomycosis (mucomycosis), malignant (necrotizing) external otitis, pyomyositis (primary bacterial infection of skeletal muscle characterized by the formation of one or more intramuscular abscesses), and necrotizing infections of the skin and fascia (which may necessitate surgery and amputations).

Neurologic: CVAs. Diabetic neuropathy and related signs and symptoms including numbness, tingling, decreased sensation, neuropathic pain, and sensory or autonomic neuropathy.

Ophthalmic: Diabetic retinopathy and related signs and symptoms including nerve-fiber layer infarcts (cotton-wool spots), intraretinal hemorrhages, hard exudates, retinal microvascular abnormalities (including microaneurysms, occluded vessels, and dilated or tortuous vessels), macular edema, macular ischemia, neovascularization, preretinal and vitreous hemorrhage (with or without subsequent fibrosis), and traction retinal detachment. Acute and/or chronic vision loss related to diabetic retinopathy may also occur.

Orthopedic: Charcot foot, bony infarcts, and amputations secondary to these complications, ulcers, or peripheral vascular disease.

6.17. Anticipated adverse events

Renal: Diabetic nephropathy and related signs and symptoms including micro- and macroalbuminuria, proteinuria, glucosuria, ketonuria, glomerular hyperfiltration, mesangial expansion, glomerular basement membrane thickening, and nodular glomerulosclerosis (Kimmelstiel-Wilson lesions). Acute and/or chronic renal failure (characterized by rising serum creatinine and BUN levels and/or electrolyte disturbances) and acute and/or chronic worsening of hypertension related to diabetic nephropathy may also occur. These complications may necessitate renal dialysis and renal transplantation

If such an event occurs, appropriate medical care should be provided immediately in the clinic. If a suspected anticipated event is reported by telephone at the time of the event or later, the participant should be evaluated in the clinic by medical staff or referred to an appropriate facility for evaluation and management.

6.18. Procedures for unmasking treatment assignment

- Treatment assignments are unmasked after all data collection for the FLINT trial has been completed (i.e., after completion of the 24 week post trial followup for all patients)
- Unmasking of study drugs will occur under the following conditions:
  - **Severe allergic reaction (Stevens-Johnson Syndrome)**: Study drugs will be stopped indefinitely. The patient, primary care provider (PCP), local principal investigator and pharmaceutical manufacturer may be unmasked.
  - **Pregnancy during the study**: Study drug will be stopped indefinitely, and the coded medication will be unmasked. The patient, PCP, and investigator will be notified of the assigned treatment and the associated risks of teratogenicity.
- In unforeseen situations where the clinical center principal investigator considers unmasking is in the best interest of the participant's health and well being, unmasking may be done after notifying the Executive Committee.
- The Data and Safety Monitoring Board will review all instances of unmasking that occur.

6.19. Procedures for missed or incomplete visits (MV)

Purpose

- Record data about missed or incomplete visits

Form

- Missed or Incomplete Visit (MV) form

When

- At close of a visit window for any missed follow-up visit or for any follow-up visit with specific forms not completed

By whom

- Clinical Coordinator

Procedures for missed or incomplete in-person visits

- For a missed visit:
  - Date of missed visit is the last date of the visit window
  - Indicate reason(s) for missed visit
- For an incomplete visit:
  - Date of incomplete visit is the date on which a partial set of procedures was performed
  - Indicate reason(s) for missed procedures

6.20. Procedures for patients lost to follow-up

Purpose

- Ascertain vital status of patient
- Document reason(s) patient did not attend visit
- Ascertain if patient is lost to followup

When

- Whenever patient misses a study visit and is difficult to contact

By whom

- Clinical coordinator

Search strategies

- Contact all persons identified on the Patient Location (PL) form
  - Telephone different times during the day/evening
  - Send letter via regular or certified registered mail to determine if patient is still at listed address
- Check current telephone directory for listings both for the patient and the patient's contacts specified on the PL form, e.g., next of kin, health care professionals
- Check post office for forwarding address; ask patient's contacts for forwarding address
- Check obituaries
- Check state vital records

6.21. Procedures for mortality closeout (DR)

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.22. Medical management of patients and side effects

To keep recommendations and care for participants in the study as uniform as possible, investigators should generally discuss with participants what is laid out in the FLINT SOP V: Standards of Care Documents for Adult patients with fatty liver disease.

Diabetes

Since patients with diabetes may be entered into the FLINT trial, is is likely that some will develop diabetic-related events. The criteria for diabetes is as follows (Diabetes Care, January 2010, 33: S11-S61) and patients should be treated according to guidelines set forth by the American Diabetes Association:

- Fasting blood glucose test: ≥126 mg/dL (7.0 mmol/L)
- Blood glucose level ≥ 200 mg/dL (11.1 mmol/L) after two hour OGTT (75 g load)
- Hemoglobin A1c measurement ≥ 6.5%

Pruritus

Pruritus was the most common AE seen in the clinical trials of Obeticholic Acid (OCA) in a PBC patient population at Virginia Mason Medical Center. We observed that the most severe cases of pruritus typically appear shortly (within the first 2 weeks) after starting therapy. Other patients with less severe pruritus reported its development following dose increases or during times of significant life stressors. We treated the pruritus with the following tiered approach:

Step 1: OTC treatment options: oil based lotions (such as Aveeno) or Benedryl: while these treatments do not seem to be a solution for the pruritus reported by most patients, they do seem to help those with mild pruritus that is mostly noticeable at bedtime or that is exacerbated by seasonal dry skin.

Step 2: Doxepin or hydroxyzine: improved pruritus symptoms in some of the more mild or intermittent cases.

Step 3: Cholestyramine: A bile acid sequestrant seemed to have the most benefit for patients reporting severe pruritus. Patients should be instructed to stagger the dosing of OCA (and/or UDCA) and cholestyramine by at least 4 hours to avoid binding of the PBC drugs.

Step 4: 'Drug holiday' or qod dosing: instruct the patient to stop taking OCA until the pruritus subsides to a manageable level. Then have the patient re-start by taking OCA every other day for a few weeks before returning to the original daily dosing.

Few adverse events related to study drugs are expected. Other potential adverse events are those related to blood draws, liver biopsy and MRI procedures. If such an event occurs, appropriate medical care should be provided immediately in the clinic and documented in the study chart.

6.23. Closeout and transferring into NAFLD Adult Database 2 Study (CO)

Purpose

- To close out a patient's participation in FLINT and document the patient's consent to join or re-enter the the NAFLD Adult Database 2

Form

- Closeout (CO) form

When

- The Closeout form should be completed at the f096 visit or at the close of the f096 window for all patients randomized in FLINT.

By whom:

- Clinical coordinator

Instructions

- Ask the patient if he/she consents to re-entering or enrolling in the NAFLD Adult Database 2

- Patients willing to re-enter or join the NAFLD Adult Database 2 should sign the most recent version of the NAFLD Adult Database 2 informed consent approved by your IRB (follow your institutional IRB guidelines for re-consenting participants previously enrolled in the NAFLD Database).

- Each consenting patient should be scheduled for a NAFLD Adult Database 2 follow-up visit approximately 12 months after the date of their FLINT f096 visit. For patients previously enrolled in the NAFLD Database, consult the patient's NAFLD Database visit schedule (time windows guide) generated at their enrollment and schedule the NAFLD Adult Database 2 visit that is open 12 months from the date of their FLINT f96 visit.

- For patients who were not previously enrolled in the NAFLD Database, a new NAFLD Adult Database 2 visit schedule (time windows guide) will be automatically generated when the FLINT Closeout form (CO) is keyed into the online database. The new visit schedule will use the FLINT randomization date as the effective date of enrollment into the NAFLD Adult Database 2. Schedule the participant approximately 12 months from their FLINT f96 visit for their f144 NAFLD Adult Database 2 follow-up visit.

- For FLINT participants who decline to participate in the NAFLD Adult Database 2; inform them that the study results and their treatment assignment will be available to them sometime after the close of the FLINT trial.

7. Forms management

7.1. Clinical center ID codes

Alphabetic IDs

- Alphabetic clinic IDs are used on forms, lists, and tables.
- Alphabetic clinical center IDs are based on the name of the institution with which the clinical center is affiliated.
- Assigned IDs:

Case Western Reserve University	CWRU
Cleveland Clinic Foundation	CCF
Cincinnati Children's Hospital	CINC
Columbia University	CU
Duke University	DUKE
Northwestern Univ: Children's Memorial Hospital	NWU
Johns Hopkins Children's Center	JHU
Indiana University	IU
Saint Louis University	SLU
Baylor University: Texas Children's Hospital	BCM
University of California, San Diego	UCSD
University of California, San Francisco	UCSF
California Pacific Medical Center	CPMC
Virginia Mason Medical Center	VMMC
Univ of Washington: Seattle Children's Hospital	UW
Virginia Commonwealth University	VCU
Mount Sinai Kravis Children's Hospital	MSCH

Numeric site IDs

- The NIDDK Genetics and Biosample Repositories use numeric IDs to identify the NASH CRN clinical centers.
- These will be used on the specimens (whole blood and plasma, serum, and liver tissue samples sent to the Genetic and Biosample Repositories, respectively).
- Assigned IDs (satellite centers should use site ID of parent clinic):

Case Western Reserve University	220
Columbia University	828
Duke University	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
Virginia Mason Medical Center	226
Virginia Commonwealth University	227

7.2. Patient identifiers

What

- Patient ID number
- Patient code

Patient ID number

- 4 characters, all numeric
- ID number labels will be distributed to clinics by the Data Coordinating Center
- The ID number for a patient will remain the same for the duration of the NASH CRN, even if the patient enters another NASH CRN study or if the patient fails screening and is subsequently re-evaluated – the ID never changes

Ranges of patient IDs assigned to clinics

Case Western Reserve University	CWRU/CCF/CINC	1001	-	1999
Columbia University	CU	9001	-	9999
Duke University	DUKE/NWU/JHU	2001	-	2999
Indiana University	IU	3001	-	3999
Saint Louis University	SLU/BCM	4001	-	4999
University of California, San Diego	UCSD	5001	-	5999
University of California, San Francisco	UCSF/CPMC	6001	-	6999
Virginia Mason Medical Center	VMMC/UW	7001	-	7999
Virginia Commonwealth University	VCU/MSCH	8001	-	8999

Patient code

- 3 character alpha code assigned by the Data Coordinating Center and printed on the ID number label
- Each patient code is unique across the NASH CRN

7.3. Visit ID code

- 1 to 3 character alpha-numeric code
- Determined by purpose of visit and timing with respect to visit windows
- Visit ID codes

s	Screening visits
Rz	Randomization
f02	2 weeks follow-up visit (approximately 14 days)
f04	4 weeks follow-up visit (approximately 1 month)
f12	12 weeks follow-up visit (approximately 3 months)
f24	24 weeks follow-up visit (approximately 6 months)
f36	36 weeks follow-up visit (approximately 9 months)
f48	48 weeks follow-up visit (approximately 12 months)
f60	60 weeks follow-up visit (approximately 15 months)
f72	72 weeks follow-up visit (approximately 18 months)
f96	96 weeks follow-up visit (approximately 24 months)
n	Unscheduled visit

7.4. General guidelines for forms completion

Ink

- Forms should be completed in blue or black ink that is dark enough to photocopy legibly.

Changing responses on forms

- If an error is made on the form, correct the response by marking through the response with one or two lines and writing the correct response next to or above the original response. The staff member making the correction should put their initials and the date in the margin by the correction. A brief explanation for the change should also be written in the margin; e.g., 'error', 'pt changed mind', 'wrong response checked'.
- Do not obliterate, erase, or white-out incorrect responses.
- The idea is to preserve an audit trail of the original response and subsequent changes to it.

Multipage forms

- The patient ID number should be written on the top right of every page of every form in the space provided -- protect yourself against ineffective staples and photocopying mishaps.

Miscellaneous

- All written responses should be printed legibly so the responses can be keyed to the database.
- Do not use abbreviations or short-hand that may not be easily understood or keyed in the written responses.
- Numeric data should be recorded in the units prescribed on the form and to the level of precision (number of digits) indicated on the form.
- All numbers should be right justified and leading and trailing zeroes should be recorded on the form where applicable (e.g., an age of 8 would be written and keyed as "08").
- All letter codes should be left justified with the remaining spaces left blank (e.g., a visit ID for the s visit code would be completed and keyed as "s").
- The clinical coordinator should review all responses for completeness and accuracy before signing off on the form.
- Wherever possible, forms should be completed in real time. Interviews and questionnaires should be completed on the actual data form.
- The data on some forms, such as the Laboratory Results form, will be transcribed from worksheets or lab reports, but the visit date on the form should correspond to the date the testing took place.
- Staple relevant lab reports and worksheets to the data form; if your lab reports are transferred to you electronically, print a paper copy of the report and staple the copy to the form.

Calculations

- All calculations should be performed using a calculator.
- Values should be rounded according to the NASH CRN data rounding rule (see section on data rounding rule, later in this chapter of the SOP).

7.5. Instruction box

- Each form includes an instruction box at the top of the first page. This instruction box gives the purpose of the form, when it should be completed, who administers the form, the respondent, and specific instructions for the form.

7.6. Form skips, stops, ineligibility symbols

Skip pattern

- Form navigation (skip pattern) instructions are indicated in **boldface**. Skips are designated by an arrow from that response to a box with the number of the next item to be completed.

Caution sign

- Cautions are designated by a triangle with enclosing a C

Stop sign

- Stops are indicated with an arrow from the response to a stop sign – instructions are given that must be fulfilled in order to continue with the form. For example, Form RG (Registration) asks if the patient has signed the consent form; if the response is "no", the form is stopped with the instructions that *'the consent form must be signed prior to continuing with screening'*.

Ineligibility sign

- Ineligible conditions are designated by an arrow from the response to the symbol:

Other

- Other special instructions are indicated on the form in *italics*. Some examples are:
  – *check only one*: only one of the listed responses should be checked
  – *check all that apply*: one or more of the listed responses may be checked
  – *specify*: a response should be printed on the line(s) provided

7.7. Headers and footers

- Data Collection Forms include headers and footers at the top and bottom of each page, respectively, which identify the form and the patient. The top right of the first page of each form has a space to check when the form is keyed [( )keyed]. The top right of subsequent pages is reserved for the patient ID number. The footers include the form abbreviation, form revision number and date, the form name, and the page number. For example:

FLINT Patient ID: __ __ __ __

Form RG FLINT
Revision 1 (07Jan11) RG - Registration Page 2 of 3

- The keyed box should be √ed when the form is keyed; the person keying the form should also date and initial the form by the keyed box.

- The patient ID number should be written on each page of the form.

7.8. Key fields

- The first 7 items of each form include the key fields which identify the clinical center, patient, visit and study

A. Clinical center, patient and visit identification
   1. Center ID: __ __ __ __
   2. Patient ID: __ __ __ __
   3. Patient code: __ __ __
   4. Date form completed: __ __ - __ __ __ - __ __
      day mon year
   5. Visit code: __ __ __
   6. Form & revision: __ __ __
   7. Study: FLINT 7

- The form and revision number will be printed on the forms in item 6; if a form is only used for one specific visit, the visit code will also be printed on the forms.
- When a form revision affects the data that are collected, the form revision number and date will change; if this occurs, older revisions of that form should no longer be used for data collection.
- If the form is revised without affecting the data collection - i.e., the wording of an item is revised - only the revision date of the form will be changed.

7.9. Missing data

- If a data item is missing and cannot be obtained when the form is completed or reviewed, write the appropriate code in the first left hand space of the empty data field:

  ? = data temporarily missing or inconsistent; to be collected or resolved in the near future; items keyed with a ? will need to be followed up on and resolved
  d = patient does not know the answer
  n = not applicable in this situation
  m = data missing
  r = patient refused

- When using any of the above codes, the entire data field does not need to be filled with the code (e.g., a missing height would be completed as m _ _ _._).

- If data are missing on the form, an explanation for the missing values should be written on the form and keyed to the database in the General Comments section of the keying.

- It is important to keep the number of missing data items at a minimum since resulting conclusions and paper publications depend on complete data, especially at the Baseline Visits.

- If an item is missing at the time the form is filled out, but is expected to be collected in the near future, record "?" rather than "M" code for the item on the form. The "m" code should only be recorded for data that are actually missing. The screening visit windows are broad enough to allow you to collect all data within the allotted time windows.

- If the data system will not accept a value, because it is out of range, please contact the DCC. In the meantime, record "?" rather than "m" on the form.

- If there is a valid reason that a required baseline laboratory value is missing, fax the LR or LS form to the DCC with the reason for the missing value.

- Participants cannot be enrolled until all "?"s keyed on the forms have been resolved.

7.10. Administrative sign off

- Each form contains a section for administrative sign off.
- These items include the Clinical Coordinator PIN and signature and the date the form was reviewed.
- Depending on the form, they may also include the PIN and signature of other staff.

It is the standard of practice with NIH funded studies to certify study physicians who assume responsibility for the accuracy and integrity of the sponsored studies. Coordinators are certified separately based on their professional qualifications and privileges even though the functions fulfilled may overlap functions of the study physician.

On the FLINT data collection forms that require the Physician's signature, the signature is the assurance that as the clinical center's principal investigator, they are assuming responsibility for the accuracy of the data recorded on the study form. This does not require that the study physician completes the forms or performs the procedures, but does require assumption of responsibility signified by signing the FLINT forms. This is also the standard of practice required by the FDA for case-report forms completion.

7.11. Handling forms

Form duplication

- The forms will be available on the NASH CRN website.
- You can print master copies from the website and then photocopy as needed or print as needed from the website – if you print copies ahead of time, do not print huge quantities as forms may be revised, especially in the early days of a study.
- The forms will also be available for printing from the data system.
- If a master copy gets frayed or faded, print a new master — always use clear copies for reproduction masters.

Form storage

- Forms for patients registered but not enrolled in FLINT should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is enrolled in FLINT will have a patient file – either a notebook or file folder which is kept in a locked room or locked filing cabinet. The patient file should contain all FLINT documents for the patient – consents, forms, appointment schedule, labels, and randomization materials. The forms should be arranged in the notebook or folder chronologically by visit. Tabs can be used to separate the visits.

7.12. Data rounding rules

To round data, examine the digits following the last position required on the form:

- If the first digit following the last data position required for the response is less than 5, leave the digit in the last data position required for the response unchanged, e.g., if you need to round to _._, then 4.73 rounds to 4.7 and 1.44 rounds to 1.4
- If the first digit following the last data position required for the response is 5 or more, round up the digit in the last data position required for the response, e.g., if you need to round to _._, then 4.78 rounds to 4.8 and 4.75 rounds to 4.8

When completing a calculation for FLINT, apply the rounding rule only at the last step, when required to record a quantity on the FLINT form.

7.13. Data audits and edits

Data audits

- The Data Coordinating Center will serve as the site monitor.
- The Data Coordinating Center will conduct periodic data audits as a quality control measure.
- Audits may be done by mail or on-site.
- During an audit, the forms will be reviewed to see if they were completed and keyed correctly; the forms will also be checked against the source documents to be sure that values were transcribed correctly.

Source documents include but are not limited to:

- Liver biopsy pathology reports
- MRI reports
- Laboratory test result reports
- Medical records for archival information
- Institutional drug accountability logs
- There are no source documents for questionnaires (the questionnaires are the original documents for the data collection)

Data edits

- Computerized data edits will be sent to the clinics periodically.
- The data edits check for consistency and questionable values in the database.

Changes resulting from audits or edits

- Changes made to the forms as a result of an audit or an edit should be marked "per audit" or "per edit" and should be dated and initialed.

8. Quality assurance

8.1. Site visits

Purpose

- Conduct an audit of selected patient data
- Review documentation and procedures for FLINT
- Tour facilities
- Discuss with clinical center personnel any problems that have occurred or that are expected to occur in conducting the study

The following regulatory and study documents should be available or accessible:

- IRB communications organized with original approval letters, revision approvals, annual renewals, serious adverse event forms, and any communications regarding concerns or special requests from clinical center review board
- Signed and dated consent and assent forms for all participants including the date and signature of a witness
- Documents including FLINT Protocol, PPMs, and SOPs
- Study forms for participants should be available for data audit

Participants

- At least two DCC personnel will attend the site visit. At least one person from another FLINT clinical center will also attend. Representatives from other resource centers associated with the NASH CRN may also attend.
- NASH CRN certified staff from the clinical center

Reviewed during site visit

- IRB documentation
  - Original approval
  - Annual renewals (if applicable)
  - IRB submissions
  - Approval for updated consent and assent forms and protocol

- Documents
  - Directory
  - SOPs
  - Forms Book
  - PPMs
  - Protocol

- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to follow-up

- Personnel
  - Certification status
  - Personnel changes
  - Backup plans for personnel in event of absence

- Clinical management
  - Adverse event reporting procedures
  - Study procedures
  - Clinical center coordination
  - Study drug storage and dispensing
  - Scheduling
  - Clinical center concerns or problems

- Participant files
  - Security
  - Organization
  - Consent statements

- Specimen shipment
  - Comparison of specimens expected and received
  - Shipping procedures and problems
  - Shipping supplies

- Protocol performance
  - Protocol deviations

- Forms and data management
  - Monthly form status reports
  - Source documentation
  - Data audit (selected patients)
  - Eligibility criteria
  - Adverse events
  - Death reports

- Previous site visit report
  - Action items follow-up
  - Data audit follow-up

Site visit followup

- A list of action items is compiled at the end of the site visit to identify items which require further action. The procedure for site visit action item follow-up is:
  - Action items will be listed at the end of the site visit report.
  - Clinical centers will be required to respond to action items within 30 days of receipt of the site visit report. Responses should be in writing and sent to the DCC.
  - The DCC will be required to respond to the action items within 30 days of the completion of the site visit report. The DCC will send a written report to the clinical center.

8.2. Performance monitoring

- The DCC will generate enrollment reports that will provide a count of participants enrolled at each clinical center.

- On approximately a quarterly basis, the DCC will generate reports summarizing the performance of all clinical centers. These reports will include information on enrollment and the percentage of expected visits for which documentation has been entered into the FLINT data system. Also, for those visits for which data have been entered, the report will show the percentage of missed visits, the completeness of data collection, the timeliness of data entry, and protocol deviations. Performance reports will be reviewed by the Steering Committee, and the Steering Committee will make decisions regarding actions to be taken in the event that a clinical center is performing poorly.

8.3. Data quality surveillance

General procedures

- Quality assurance of data accuracy will occur routinely through three main procedures: data entry checks, monthly checks for completeness and edits, and form audits.

- In addition, detection of problems may occur during data analysis. For example, in preparing reports for Steering Committee meetings, problems may be discovered. Outliers and unusual variations or patterns in the data are examined and may reveal problems.

- Quality assurance of data analysis is achieved by independent replication of key analyses within the DCC and review of reports by multiple individuals before distribution.

Data entry checks

- The data system will contain checks during the data entry process of range, logic, and consistency of items within forms.

- The data system will perform checks between forms to ensure that the same fields entered on different forms match.

- A double data entry system will be used for all forms.

Monthly check for completeness and edits

- On a monthly basis, after receipt of data transmittals from the clinical centers, the DCC will generate a database report of:
  - number of participants enrolled
  - missed visits
  - incomplete visits (missing or pending forms)
  - missed specimen collection or shipment
  - edits (see below)

- Edits are run on the database of the keyed forms monthly. Checks for missing, out-of-range, unusual and inconsistent values, cross-form checks and arithmetic errors are some of the types of checks performed. A listing of edits is sent to each clinical center for resolution. The clinical center must respond to each edit on the listing, make appropriate changes to the forms and database, compute documentation of each change, and file the documentation with the edited data collection form. Items that cannot be corrected (e.g., missing values, unusual measures) are entered into a database at the DCC. These items are excluded from future edits. A hard copy of the edits with each resolution should be kept in a notebook located at the clinical center.

8.3. Data quality surveillance

Forms audits

On a periodic (approximately monthly) basis the DCC selects and requests copies of forms for specific participants be sent by each clinical center to the DCC for auditing.

- Audited forms are compared with the database; discrepancies are noted and queried.
- Audited paper forms are also inspected for other problems, which are noted and queried.
- Each clinical center will be required to resolve discrepancies from the audit report and fax the resolutions to the DCC within 5 days.
- The DCC will generate a summary report of the audit discrepancies by clinical center to be distributed to all FLINT centers.
- Discrepancy rates over time by clinical center are reported to the Steering Committee.

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

FLINT

Standard Operating Procedures
Part IV:

Liver Biopsy
and
Histology Scoring System

August 2011

Contents

FLINT SOP IV: Biopsy and Histology Scoring

1. Overview

1.1. Philosophy

Nonalcoholic fatty liver disease (NAFLD) is defined by hepatic steatosis in the absence of known causes of liver disease and significant alcohol consumption. Nonalcoholic steatohepatitis (NASH) is a subset of NAFLD defined histologically by steatosis, spotty lobular inflammation, and distinctive pattern of zone 3 injury characterized by hepatocellular ballooning; Mallory's hyaline and perisinusoidal fibrosis (psf) may also be present. In some as yet poorly defined fraction of patients, there is progression of disease to cirrhosis and hepatocellular carcinoma. The NASH Clinical Research Network (NASH CRN) is a clinical research network sponsored by the NIDDK to study the natural history of NAFLD in both adult and pediatric patients and to carry out (1) treatment trials of NASH in adult patients and (2) treatment trials of NAFLD in pediatric patients. This document specifies the procedures for liver biopsy and histology scoring for the FLINT trial which will compare two treatments for adult patients with NASH. Procedures for other NASH CRN studies, including the NAFLD Database 2 and CyNCH trial, will be specified in separate manuals specific to the particular study.

The procedures specified by the NASH CRN for their studies are based on two concepts: one is the principle that a primary goal of the NASH CRN is to collect a database of specimens along with clinical and laboratory data, so that this information remains available for research after the NASH CRN has disbanded. The other concept behind these procedures is the necessity of a system of evaluation of NASH that can be utilized in evaluating efficacy in treatment trials. Because there are no serum markers or other diagnostic modalities that can accurately stage or grade the liver disease in these patients, a liver biopsy remains the standard against which all other methods will be compared. Every effort should be made to build a liver tissue archive and slide archive that are as complete as possible, so that what is accomplished will have value for the future as well as the present. With this principle in mind, study consent statements should include language that permits such approved uses of liver biopsy materials without the need for re-consent at a later date; patients who refuse access to their liver biopsies or who refuse to provide specimens for banking should not be enrolled in the NASH CRN.

1.1. Philosophy

Histologically confirmed NASH is an inclusion criterion for the FLINT trial. The baseline biopsy may have been done prior to screening (within specified time limits) or it may be done as standard of care as part of the screening procedure. FLINT patients will also have a followup biopsy after 72 weeks of treatment in the trial. Unscheduled biopsies also may occur after screening. Ideally, the FLINT trial will obtain a piece of liver tissue for banking and 10 unstained slides for archiving from each of these biopsies. However, because some of the biopsies evaluated for FLINT may not provide these materials (eg, not enough tissue is obtained or the paraffin block is exhausted), contingency plans have been developed, including requesting to borrow the institutional slides and returning them after central scoring has been completed.

It should be emphasized that a surgical pathology report alone is not sufficient for satisfying inclusion/exclusion criteria related to liver histology nor for comparison with other biopsy data. Tissue slides must be available for review and must be judged by the FLINT pathologist to be adequate for scoring according to the NASH CRN scoring protocol. However, a copy of the surgical pathology report must be obtained for all slides. The surgical pathology report serves two purposes: (1) to verify and document the identity of the patient whose tissue is on the slides that are being evaluated and (2) to provide the date of the biopsy.

1.2. Tasks and forms related to liver biopsy

The FLINT eligibility criteria include histologic evidence of NASH according to the NASH CRN protocol for histology scoring. The biopsy that is used to satisfy eligibility may be a historical biopsy (done in the 90 days prior to enrollment and the patient must not have used specified (antiNASH and antiobesity) medications in the 90 days prior to the biopsy) or it may be done prospectively under the care of the FLINT investigator as a screening procedure. Each randomized patient will have another biopsy after 72 weeks of treatment. In addition, a patient may have an interim biopsy as needed for standard of care. As a check to be sure that all biopsies that occur are caught, occurrence of a biopsy since the previous FLINT visit is queried on the Followup Medical History (HI) form.

Information about the biopsy procedure and materials is captured on the Liver Biopsy Materials Documentation (SD) form. Cautions about the use of proscribed medications in the 90 days prior to the biopsy used for eligibility screening are noted on the Baseline History (BG) form; lack of use of proscribed medications is confirmed on the Liver Biopsy Materials Documentation (SD) form. The SD form also documents the outcome of the biopsy with regard to availability of tissue for banking and availability of stained and unstained slides for scoring and archiving. If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed. If the biopsy was a screening biopsy (ie, done/evaluated to determine eligibility for FLINT), then the local FLINT Study Pathologist must complete the Liver Biopsy Histology Findings (HF) form. If the NASH activity score (NAS) for the screening biopsy is less than 4, then the patient is not eligible for FLINT. Other forms that the FLINT trial uses to document activities and materials related to liver biopsy are the

1.2. Tasks and forms related to liver biopsy

Central Histology Review (CR) form and logs for shipping tissue and slides (forms SS and TS). In summary, these seven forms (SD, LT, HW, HF, CR, TS, SS) are used to:

- Document what slides, if any, are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- If the biopsy is the screening biopsy, document lack of use of proscribed medications during the 90 days prior to the biopsy (form SD) and remind the clinical center that the screening biopsy cannot be older than 90 days at the time of randomization
- If liver tissue was obtained for banking, document collection of extra liver tissue and procedures for banking (form LT)
- Document local scoring of baseline biopsies (form HF)
- Document shipment of slides to the DCC (form TS)
- Document shipment of liver tissue in RNA*later*® Solution to the Biosample Repository (Form SS)
- Document scoring of baseline and followup biopsies by the NASH CRN Pathology Committee (form CR)

The FLINT hepatologist, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and complete the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

2. Obtaining liver biopsy materials for scoring for FLINT

2.1. Overview

Baseline (screening) liver biopsy specimens can be divided into two categories: (1) those performed prior to consent for screening, and (2) those performed as part of standard of care during the screening process, but after consent for screening for FLINT has been obtained. In the case of (1), we will try to obtain 10 unstained slides for the exclusive use by FLINT, but limited biopsy materials may require that the institution's biopsy slides be borrowed. In the case of (2), sufficient tissue should be collected to provide for the institutional pathology slides AND 10 unstained slides for FLINT exclusive use AND for banking a sample at the Biosample Repository. Followup liver biopsies should all be in category (2).

Baseline biopsies will be scored by the local FLINT Study Pathologist (to determine eligibility) and also centrally (after randomization), by the Pathology Committee. Biopsies obtained 72 weeks after randomization will be scored centrally only, by the Pathology Committee. Unscheduled biopsies will be read locally for standard of care and will also be scored centrally, by the Pathology Committee.

2.2. Baseline biopsies performed prior to consent for screening

Because these biopsies were obtained prior to consent for FLINT screening, investigators may have little control over variables such as fixation or biopsy size, but these considerations should not prevent investigators from trying to obtain unstained slides for FLINT purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

Upon learning that a patient had a recent biopsy and after checking that no proscribed medications were used in the 90 days prior to the biopsy, the Clinical Coordinator should obtain the original pathology materials: the surgical pathology report, the institution's H&E and Masson's trichrome slides, and the paraffin block so that 10 unstained slides may be obtained (see below for how to prepare and label those slides).

Upon receipt of the original pathology materials, the Clinical Coordinator should verify that all materials pertain to the FLINT patient they are said to and should annotate the surgical pathology report with the patient's NASH CRN ID number and code and black out the patient's name. The annotated report should be attached to the Liver Biopsy Materials Documentation (SD) form and a copy of the annotated report must be sent to the DCC when the biopsy slides are sent to the DCC. The Clinical Coordinator and/or Study Pathologist must determine the following so that form SD may be completed:

2.2. Baseline biopsies performed prior to consent for screening

- Confirm that no proscribed medications were used in the 90 days prior to the biopsy (baseline biopsy only)
- Determine the number and type of the institution's stained slides available for the local review of the biopsy and determine if any stained slides need to be sent to the DCC; if fewer than 2 unstained slides are available for sending to the DCC, the DCC must be sent the institution's stained slides
- If the DCC will be sent stained slides, label those stained slides with NASH CRN slide labels for stained slides (which are not the same as the labels for unstained slides); transcribe the slide sequence numbers (printed on the slide label) to form SD
- If the DCC will be sent stained slides, determine if FLINT is borrowing the stained slides from the institution or if FLINT is taking possession of the stained slides; if borrowing the stained slides, determine the date by which the slides need to be returned to the institution and note the source of the slides; record this information on form SD
- If unstained slides are obtained, label them with the NASH CRN slide labels for unstained slides (which are not the same as the labels for stained slides); transcribe the slide sequence numbers from the slide labels to form SD

The Study Pathologist should complete the FLINT Liver Biopsy Histology Findings (HF) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document. If the NASH activity score (NAS) is 3 or less the patient is ineligible for FLINT.

If there is no H&E stained slide or if there is no Masson's trichrome stained slide, the biopsy is insufficient for evaluation for entry into FLINT.

If only the H&E and Masson's trichrome slides are available (i.e., no unstained slides available), these should be reviewed locally. If the patient is found to be eligible, these slides will need to be sent to the DCC for central review by the Pathology Committee. Both of these slides must be available for central review for the patient to be found eligible for FLINT.

The FLINT trial should request that the slides be provided outright, with no arrangements to return the slides at the end of the trial. In the event that any of the slides from a prior biopsy need to be returned to the original laboratory, the first plan should be for those slides to be returned at the end of the trial. If that is not acceptable to the original pathology laboratory, the slides will be returned to the original pathology laboratory after the central review is completed.

Slides for patients who are not randomized in FLINT should be returned to the original pathology laboratory upon determination that the patient will not be randomized.

2.3. Baseline and followup biopsies performed after consent for screening - biopsy procedure

Each clinical center investigator will notify their FLINT Study Pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a FLINT patient so that when the block is initially cut for the local institution's requirements, the FLINT trial's additional 10 unstained slides can be cut at the same time. This will minimize the chances of loss of tissue with refacing the block.

In order to insure adequate material for histologic review, investigators should obtain needle core biopsies of at least 2.0 cm length using a 16 gauge or greater needle. If there is adequate tissue beyond 2.0 cm, the extra tissue may be stored in RNA*later*® Solution and banked at the Biosample Repository.

2.4. Preparation of slides

Biopsy tissue for slides should be placed in buffered formalin as soon as possible; investigators should use whatever formaldehyde solution is used by the site's pathology laboratory.

If this is a baseline biopsy, each clinical center will obtain locally stained H&E and Masson's trichrome slides as part of their standard institutional pathology materials; these will be read by the local FLINT Study Pathologist for the local evaluation (i.e., for completion of form HF).

For both baseline and followup biopsies, the local laboratory should cut 10 additional slides (additional to what is cut for the institutional pathology needs) at the same time as the institutional slides are obtained in order to decrease the chances of loss of tissue with refacing the block. The local NASH CRN Study Pathologist should advise on the selection of a maximum of 10 unstained slides to be submitted to the Histology Review Center at the DCC for banking if there are (1) more than 10 unstained slides available or (2) if the unstained slides were cut from different tissue blocks obtained from the same liver biopsy core(s). The NASH CRN Pathology Committee's recommendation is that all unstained slides submitted come from the same, single liver biopsy core when possible. Centers should be careful not to submit unstained slides with insufficient or fragmented biopsy tissue since it is not possible for DCC staff to sort those out before sending the slides for central staining.

Tissue should be placed on pre-cleaned, "charged" slides to allow for possible future use with immunohistochemistry. The specifications for the recommended brand of slides are:

SuperFrost Plus slides, Precleaned
Distributor: Fisher Scientific
Catalog No.: #12-550-15
Size: 25/75/1.0 mm
Estimated cost: $133.07 per gross (144 slides/gross); $1,118.24 per case of 10 gross
Tele: 1-800-766-7000

Coverslips should not be placed on the slides. Slides should be labeled with the patient specific preprinted NASH CRN labels for stained slides. The 10 (or as many that can be obtained up to 10) uncoverslipped, unstained slides will be forwarded to the DCC for the purposes of central staining, review, and archiving.

2.5. Labeling stained and unstained slides at the clinical center

Each of the slides (stained and unstained) sent to the DCC must be labeled with a NASH CRN slide label. Two types of slide labels are used in the NASH CRN:

(1) permanent labels are used for unstained slides; these labels can withstand the chemicals used during staining
(2) removable labels (overlabels) for stained slides that are borrowed from an institution.

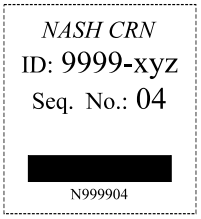
The requirements for the labeling scheme for slides are:

- Each slide must be uniquely identifiable
- The unique identifier for the slide has to be built from information that can be specified when the label is printed
- The slide label must be able to be read by human eyes
- Labels for unstained slides must be resistant to solvents used in the staining process and to over-labeling by interim processes (e.g., when sent for central staining)
- Labels for borrowed stained slides must be removable since those slides must be returned to the original pathology laboratory

The solution to these requirements is to use Brady labels in the format shown below: (1) labels for unstained slides and (2) labels for stained slides which are borrowed.

2.5. Labeling stained and unstained slides at the clinical center

(1)



(2)

Slide labels are provided to each clinical center in preprinted form, 1 set of labels per patient ID, by the DCC. Each set includes labels for unstained slides and labels for stained slides.

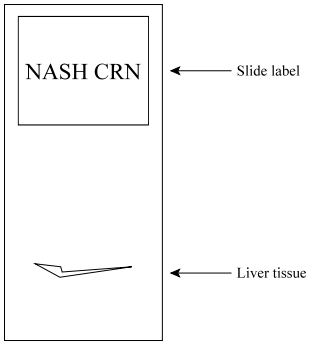
The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the life time of all NASH CRN studies). The sequence number on the labels for stained slides run from 81 to 90 (10 is the maximum number of stained slides that we think we will ever borrow for any one patient over the lifetime of all NASH CRN studies).

The slide labels include the following information:

- NASH CRN (printed on label)
- Patient ID number (4 digit number printed on label) and patient code (3 character alphabetic code printed on label)
- Slide sequence number (printed on label)
- Bar code constructed from N (for NASH CRN), 4 digit ID number, and 2 digit slide sequence number

Permanent labels should be positioned squarely at the top end of the slide, on the same side that the liver tissue is on and over the white part of the glass, with no edges hanging off the slide. Overlabels should be placed on the back of the slide, directly on the glass and opposite the existing

label. Both permanent labels and overlabels should be positioned so that the print is oriented vertically with the narrow end of the slide (see diagram below).



2.6. Liver tissue for banking at Biosample Repository

The extra piece of liver tissue (minimum 1-2 mm or greater) will be stored in RNA*later*® Solution as follows:

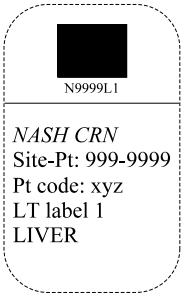
Labeling Procedures

- Apply pre-printed label provided by DCC to the cryogenic vial according to the following steps:

  - Attach the label to the vial when the vial is at room temperature
  - Leave the cap on the vial when labeling to ensure the inside of the vial remains sterile
  - Position the label on the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position; the label should not trail off the bottom of the vial or over the cap
  - While holding the vial in an upright position, affix the colored (white) portion of the label to the vial first
  - Wrap the clear tail around the perimeter of the vial; the end of the clear tail should overlap the colored portion of the label
  - Press firmly on the entire label; verify that all edges of the label adhere to the vial
  - When possible, allow newly labeled vials to set at room temperature for several hours prior to subjecting them to colder temperatures (24 - 48 hours is optimal)

2.6. Liver tissue for banking at Biosample Repository

- The liver vial labels have the following format:



- The vial used for banking extra liver tissue should be a 2.0 mL polypropylene cryogenic vial (13.5 mm wide x 48.3 mm tall) that is self-standing and externally threaded vials and silicone washers. This vial is designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

- Preferably within one minute and no more than five minutes after biopsy, place the liver tissue into the vial, pre-filled with approximately 1 mL of RNA*later*® Solution. If the sample is not placed in RNA*later*® Solution within 5 minutes discard the sample. Refrigerate the cryovial at 4° C overnight to allow thorough penetration of the liver tissue and then transfer to -70° C freezer for storage.

- RNA*later*® Solution may be ordered online at http://www.ambion.com/catalog/catnum.php?AM7020. The catalog number for 100 mL of Ambion RNA*later*® Solution cat#AM7020 and sells for $96.

- Complete the Liver Tissue Banking (LT) form; duplicate liver vial label should be attached to the LT form

- Complete the Specimen Shipment Log (SS) form. In the NASH CRN Excel shipment file under column J, enter "R"

- Batch ship cryovials to the NIDDK Central Biosample Repository on Monday, Tuesday or Wednesday; after refrigeration overnight at 4° C, store temporarily in -70° C freezer at the clinical center until the next batch shipment to Fisher Bioservices.

- Make sure you use the "Cryovial" and "LT form" labels from the same set (i.e.., with the same sequence number)

3. Development of the histology scoring system

3.1. Background

The NASH CRN has designed and validated a feature scoring system that can be used for the spectrum of NAFLD, both to correlate histologic features with clinical and physiologic measurements and to document changes in liver tissue observed over time in patients in natural history studies and clinical trials.

3.2. Methods and validation

Pathologists from the NIH and from the 8 clinical centers participating in the NASH CRN met in March 2002 to discuss the features to monitor and the criteria for scoring these features. Each study pathologist was asked to contribute cases of adult and pediatric liver biopsies. The cases were chosen from patients referred for consideration of NASH who had been histologically diagnosed as either diagnostic, borderline/suspicious, or not diagnostic of NASH. The cases were to have no confounding liver diseases. Subsequent to the March 2002 meeting and after criteria for the scoring system were established based on the discussions at the meeting, additional cases were requested from each center's pathologist. The original and newly received cases were anonymized and were assembled into what is called the anonymized study set. This set consists of 50 cases (32 adult, 18 pediatric) with H&E and Masson stains for each case.

During the summer and fall of 2002, the anonymized study set was circulated to each study pathologist for review to establish agreement for features and for diagnosis according to the three categories of definite, borderline, and not NASH. The adult cases were scored by each pathologist twice and the pediatric cases once.

Weighted group and pair-wise Kappa statistics were used to evaluate inter- and intra-rater agreement. Inter-observer agreement was assessed for both adult and pediatric cases and intra-observer agreement was determined using the adult cases.

Cochran's Chi-square test for trend and GEE logistic regression were used to analyze univariate and multivariate relationships between feature scores and the diagnosis of NASH. Adult cases were analyzed separately from pediatric cases.

3.2. Methods and validation

Fourteen features of NAFLD were evaluated in the scoring system. Four of the features were evaluated semi-quantitatively:

- Steatosis percent (0-3)
- Modified Brunt criteria for fibrosis (0-4); the modification, specifically, was subdivision of stage 1 into 1a, 1b, and 1c:
  - 1a: zone 3 perisinusoidal fibrosis that does not require a trichrome stain
  - 1b: zone 3 perisinusoidal fibrosis that does require a trichrome stain
  - 1c: portal fibrosis only

  The remainder of the fibrosis scoring used Brunt criteria.
- Lobular inflammatory foci/20x (0-3)
- Hepatocellular ballooning (0-2).

Location of steatosis was recorded but not scaled. An additional 9 features were recorded as either present or absent.

Weighted group kappa statistics for the inter-rater agreement on adult cases were 0.84 for fibrosis, 0.79 for steatosis, 0.56 for hepatocellular ballooning and 0.45 for lobular inflammation. Agreement on the diagnosis of NASH had a kappa of 0.61. Kappa values for intra-rater agreement were higher for all features, while inter-rater agreement on pediatric cases was generally lower. By multivariate analysis 5 features independently correlated with the diagnosis of NASH in adults: hepatocellular ballooning ($P<0.0001$), perisinusoidal fibrosis ($P=0.0009$), lobular inflammation ($P=0.002$), steatosis ($P=0.004$) and acidophil bodies ($P=0.02$).

In summary, we have devised a feature scoring system for NAFLD and NASH that has inter- and intra-rater agreement similar to other semiquantitative scoring systems for chronic liver disease. The pediatric NAFLD features were not as reproducible as those of adults, possibly due to the presence of varied patterns of injury. In accordance with the idea that NASH is a pattern of injury comprised of several features, this scoring system demonstrates that a firm diagnosis of NASH correlates with the degree of steatosis, characteristic pattern of fibrosis, lobular inflammation, and hepatocellular ballooning. Based on this evaluation, the NASH CRN will use this system to evaluate liver biopsies for features of NAFLD.

4. Evaluation at the clinical center (for forms HW and HF)

4.1. Introduction

The local site FLINT Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. FLINT patients must have histologically confirmed definite or probable steatohepatitis, with a NAS ≥4.

The local site FLINT Study Pathologist should make his/her evaluation using the locally stained H&E slide (all features except fibrosis) and Masson's trichrome slide (fibrosis only) and should complete the Liver Biopsy Histology Worksheet (HW) form. The information on the HW form will be transcribed to the study-specific HF form by the clinical coordinator. A copy of the HF form is included at the end of this document for your information; please obtain blank forms for completion for a patient from the study website (www.nashcrn.com).

4.2. Guidelines for features scored in the local evaluation

The following guidelines are provided for uniformity of reading among the FLINT pathologists. The system is based entirely on H&E stain for all lesions except fibrosis, and on Masson's trichrome stain for fibrosis.

4.2.1. Length of biopsy

The tissue is measured (mm) on the glass slide prior to reading the slide.

4.2.2. Steatosis grade (0-3; 4x or 10x)

0: <5%
1: 5 -33%
2: 34 - 66%
3: > 66%

In the recognition that (1) deciding on less than 5% can be challenging and (2) that this really is a significant cut-off for allowing patients into long-term follow-up studies such as the NASH CRN is conducting, we think it is best to err on the side of grade 1 rather than grade 0 when faced with a borderline case. Evaluation is best at 4x or 10x at most.

4.2.3. Steatosis location

Steatosis location has 4 choices for characterization:

Zone 3: distribution means not panacinar and not strictly zone 1
Zone 1
Azonal: this pattern is the random scattered macrosteatosis
Panacinar: implies severe steatosis that appears to involve the whole acinus equally, rather than the more random azonal pattern

4.2.4. Fibrosis stage (0-4; requires Masson's trichrome slide)

0: None
1a: Zone 3, perisinusoidal fibrosis (requires trichrome)
1b: Zone 3, perisinusoidal (easily seen on H&E; verified on trichrome)
1c: Portal/periportal only
2: Zone 3 and periportal, any combination
3: Bridging
4: Cirrhosis

4.2.5. Portal chronic inflammation (0-1)

0: None to minimal
1: Greater than minimal

4.2.6. Lobular inflammation (0-3; 20x)

Amount of lobular inflammation combines mononuclear, fat granulomas, and polymorphonuclear (pmn) foci.

0: None
1: < 2 / 20x mag
2: 2-4 / 20x mag
3: >4 / 20x mag

4.2.7. Hepatocellular ballooning (0-2)

0: None
1: Few
2: Many

4.2.8. Steatohepatitis diagnosis

At some point in your evaluation of the case, decide how you would sign this case at your institution, using whatever criteria you currently use to make a diagnosis of steatohepatitis. The question asked is "Is steatohepatitis present?" and the choices for response are:

0: No
1: Suspicious/borderline/indeterminate
2: Yes, definite steatohepatitis

This choice has to be made based on the H&E and Masson's trichrome slides, even if you have access to other stains (such as ubiquitin immunohistochemistry).

4.2.9. Exclusion of other liver disease and other features

- Primary biliary cirrhosis
- Wilson's disease
- Chronic cholestatic liver disease
- Inflammation suggestive of AIH, HCV
- Pigment suggestive of HH
- Globules suggestive of A1AT
- Hepatocellular changes suggestive of HBV
- Granulomas suggestive of sarcoid, PBC, infection

Diagnosis of primary biliary cirrhosis, Wilson's disease, bile duct obstructions, primary sclerosing cholangitis, autoimmune liver disease, HCV, hemochromatosis, A1AT deficiency, and HBV by the study physician are exclusionary; these diagnoses are marked with Caution symbols on the HF form.

4.2.10. NASH Activity Score (NAS)

The NASH Activity Score (NAS) is a composite score that is calculated from the steatosis grade (0-3), the lobular inflammation grade (0-3), and the hepatocellular ballooning score (0-2); the scores for these three components are summed. The NAS may range from 0 through 8. Patients with a NAS of 0-3 on screening are ineligible for FLINT. Patients are also ineligible for FLINT if any component of the NAS is less than 1.

4.2.11. Comments

This item is for any comments you want to share with the rest of the group on the case such as comments on unusual features or on a difficult scoring choice.

4.3. Unscheduled liver biopsy

Unscheduled biopsies (i.e., biopsies done after screening and at a followup time other than 72 weeks after randomization) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Forms HW and HF will not be completed for unscheduled liver biopsies, but the biopsy slides should be obtained and the SD form should be completed. Form CR will be completed upon central review by the Pathology Committee. The CR form will use visit code n.

5. Central pathology evaluation (for Form CR)

5.1. Procedures

Each pathology review session will include attendance by at least three Pathology Committee members and will include at least one of the co-chairs. Central reviews are planned for every 3-4 months. Review sessions will last 1 - 2 days. One or two DCC members will attend each central review to complete the Central Pathology Review (CR) form for each biopsy reviewed. These completed data forms will be keyed at the DCC.

Following central review, slides will be returned to storage at the DCC. Slides that are only loaned to the FLINT trial for a specified time will be returned to the clinical center at the required time. Slides will be packed and shipped for return to the clinical center as specified later in this manual.

5.2. Documentation of which slides were used for evaluation

The sequence numbers of the slides viewed will be recorded on the CR form for each case.

5.3. Guidelines for features scored in the central evaluation

The following features are evaluated both centrally and locally, and the guidelines to be used for the central evaluation of these features are the same as the guidelines used for the local evaluation:

- Steatosis grade
- Steatosis location
- Fibrosis stage
- Lobular inflammation
- Hepatocellular ballooning
- Cirrhosis diagnosis

Steatohepatitis diagnosis and amount of portal inflammation are scored both centrally and locally, but the scoring of these features for the central evaluation differs from the scoring for the local evaluation.

Guidelines for features scored only in the central evaluation or scored differently in the central evaluation from the local evaluation are described below.

5.3.1. Steatosis

5.3.2a Types of Macrovesicular steatosis

0: Predominantly large droplet macrovesicular steatosis
1: Mixed large and small droplet macrovesicular steatosis
2: Predominantly small droplet macrovesicular steatosis

5.3.2b Microvesicular steatosis, continguous patches

0: Absent
1: Present

The definition agreed on was the pathologists' definition of microsteatosis: foamy appearance to the cytoplasm, nucleus centrally located. Remember, this is not the small droplet type of "macro" steatosis that does not completely fill the cell. Microsteatosis typically needs ORO for confirmation in routine practice.

5.3.2. Microgranulomas seen (yes/no)

5.3.3. Large lipogranulomas seen (yes/no)

5.3.4. Portal chronic inflammation

0: None
1: Mild
2: More than mild

5.3.5. Ballooning

5.3.6a: Severe ballooning present

0: No
1: Yes

A score of "severe ballooning present" should be made if large, classical balloon cells are seen from low magnification in multiple areas throughout the biopsy. The biopsy should already have a score of "2" for ballooning to qualify for severe ballooning.

5.3.6b: Classical balloon cells present

0: No
1: Yes

Classical balloon cells are ones that are easily recognized at low to medium magnification, stand out from the surrounding parenchyma and have cytoplasm that is clumped. They may have Mallory-Denk bodies. A positive score requires only one classical balloon cell.

5.3.6. Pigmented macrophages (Kupffer cells)

0: Rare/absent
1: Many

5.3.7. Megamitochondria

0: Rare/absent
1: Many

5.3.8. Mallory - Denk bodies

0: Rare/absent
1: Many

5.3.9. Glyogen nuclei

0: Rare/absent
1: Present in patches

5.3.10. Glycogenosis of hepatocytes

0: Not present
1: Focal, involving less than 50% of the hepatocytes
2: Diffuse, involving more than 50% of the hepatocytes

5.3.11. Fibrosis

5.3.13a: Perisinusoidal fibrosis grade

0: No perisinusoidal fibrosis present
1: Perisinusoidal fibrosis present that requires a Masson stain to identify
2: Perisinusoidal fibrosis present that is visible on the H&E stain

Note that stage 1A fibrosis would automatically get a score of 1 and stage 1B fibrosis would automatically get a score of 2 on this scale.

5.3.13b: Predominant location of fibrosis (scored only if fibrosis stage is 1A, 1B, 1C, 2 or 3)

0: Fibrosis is more prominent around or between portal areas
1: No portal or central predominance to the fibrosis
2: Fibrosis is more prominent around or between central veins

Note that stage 1A and 1B fibrosis would automatically get a score of 2 and stage 1C fibrosis would automatically get a score of 0 on this scale.

5.3.12. Iron: hepatocellular grade (0-4)

0: Absent or barely discernible, 40x (skip hepatocellular iron distribution)
1: Barely discernible granules, 20x
2: Discrete granules resolved, 10x
3: Discrete granules resolved, 4x
4: Masses visible by naked eye

5.3.13. Iron: hepatocellular distribution

0: Periportal
1: Periportal and midzonal
2: Panacinar
3: Zone 3 or nonzonal

5.3.14. Nonhepatocellular iron grade (0-2)

0: None (skip nonhepatocellular iron distribution)
1: Mild
2: More than mild

5.3.15. Nonhepatocellular iron distribution

0: Large vessel endothelium only
1: Portal/fibrosis bands only, but more than just in large vessel endothelium
2: Intraparenchymal only
3: Both portal and intraparenchymal

5.3.16. Steatohepatitis diagnosis

99: Not NAFLD
0: NAFLD, not NASH
1a: Suspicions/borderline/indeterminate: Zone 3 pattern
1b: Suspicions/borderline/indeterminate: Zone 1, periportal pattern
2: Yes, definite

5.3.17. Comments

General comments on the biopsy can be recorded by the Pathology Committee.

6. Shipping slides to the Data Coordinating Center

6.1. Packing and shipping slides

The steps in shipping slides to the Data Coordinating Center are:

- Complete the Histology Slide Transmittal Log (Form TS), specifying the number of stained and unstained slides for each patient included in the shipment; specify the date and visit code for the slides for each patient included in the shipment; make a copy of the completed TS form for your clinic records and include the original in the shipment
- Attach a copy of the surgical pathology report for each slide set included in the shipment; make sure that each report is annotated with the patient's NASH CRN ID number and ID code and that the patient's name has been blacked out
- Packing
  - Pack slides in standard slide shipping boxes, which hold 25 slides (VWR International catalog number 82003-418, HS15989A (blue), 82003-420 (green); phone 800-932-5000)
  - Use tissue paper or something similar, inside the box, to prevent the slides from moving during shipment
  - Seal the box shut with tape
  - Wrap the box in bubble wrap
  - Place the bubble wrapped box inside a padded jiffy bag. Place the jiffy box and pathology reports and Form TS in a Federal Express box; stuff the Federal Express box as needed with newspaper or other packing material so that the bubble wrapped box does not move around
  - Ship second day arrival to:

    Pat Belt
    NASH CRN Data Coordinating Center
    615 North Wolfe Street, Room W5010
    Baltimore, MD 21205
    410-955-8175

    You may bill the shipment to the DCC's slide shipment Federal Express account (#2991-6250-8)
  - Notify Pat Belt to expect the shipment (email pbelt@jhsph.edu or fax 410-955-0932)

6.2. Receipt of slides at the Data Coordinating Center

When slides are received, staff at the Data Coordinating Center will:

- Notify the clinic that the shipment was received
- Review the shipment for consistency with the accompanying shipping log, for damage, for appropriate labeling and other quality control items
- Send slides for central staining as appropriate (currently, 3 stains are planned)
- Log the stained slides into the DCC slide inventory system and designate a storage location
- If the received slides are not adequate for evaluation, the institution's slides may be requested and used to evaluate a biopsy

6.3. Returning slides to the clinical center

- Log the slides out of the DCC slide inventory system
- Complete a shipping log for return of slides to the clinical center
- Pack up slides for return to the clinical center
- Notify the clinical center of the impending return of the slides
- Obtain confirmation of receipt of the returned slides from the clinical center

7. Appendices

Central Histology Review

Purpose: Record results of the NASH CRN Pathology Committee review of liver biopsy slides archived at the Histology Review Center.
When: Quarterly after the start of patient enrollment or more often as determined by the Pathology Committee.
By whom: Data Coordinating Center staff.
Instructions: Upon review of the liver biopsy slides by the NASH CRN Pathology Committee, the designated Data Coordinating Center staff member should complete the CR form. The CR form will be keyed by the Data Coordinating Center personnel.

A. Clinic, patient and visit identification

__ __ __ __ 1. Center ID

__ __ __ __ 2. Patient ID

__ __ __ 3. Patient code

__ __ / __ __ __ / __ __ 4. Date of central reading

__ __ __ __ 5. Visit code

c r 2 6. Form and revision

__ 7. Study: **6**=Database 2; **7**=FLINT

__ __ / __ __ __ / __ __ 8. Date of biopsy

B. Slide sequence number

9. Sequence number for

__ __ . . . a. H & E stained slide

__ __ . . . b. Masson's trichrome stained slide

__ __ . . . c. Iron stained slide

C. Adequacy of biopsy

__ __ 10. Biopsy length (mm)

__ 11. Tissue adequate: **0**=No ➜ Request original slides from submitting clinic; **1**=Yes

_____ 12. Followup with clinic (*Specify*):

D. Histology

H & E stain

13. Steatosis (assume macro, e.g., large and small droplet)

_____ . . . a. Grade: **0**=<5%; **1**=5-33%; **2**=34-66%; **3**=>66%

_____ . . . b. Location: **0**=Zone 3 (*central*); **1**=Zone 1 (*periportal*); **2**=Azonal; **3**=Panacinar

_____ . . . c. Type of macrovesicular steatosis: **0**=Predominantly large droplet; **1**=Mixed large and small droplet; **2**=Predominantly small droplet

_____ . . . d. Microvesicular steatosis, contiguous patches: **0**=Absent; **1**=Present

14. Inflammation

_____ . . . a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:
0=0; **1**=<2 under 20x mag; **2**=2-4 under 20 mag; **3**=>4 under 20 mag

_____ . . . b. Micrograunlomas seen: **0**=No; **1**=Yes

_____ . . . c. Large lipogranulomas seen: **0**=No; **1**=Yes

_____ . . . d. Amount of portal, chronic inflammation: **0**=None; **1**=Mild; **2**=More than mild

15. Liver cell injury

_____ . . . a. Ballooning: **0**=None ➜ **GOTO Item 15d**; **1**=Few; **2**=Many

_____ . . . b. Severe ballooning present: **0**=No; **1**=Yes

_____ . . . c. Classical balloon cells present: **0**=No; **1**=Yes

_____ . . . d. Acidophil bodies: **0**=Rare/absent; **1**=Many

_____ . . . e. Pigmented macrophages (*Kupffer cells*): **0**=Rare/absent; **1**=Many

_____ . . . f. Megamitochondria: **0**=Rare/absent; **1**=Many

_____ 16. Mallory-Denk bodies: **0**=Rare/absent; **1**=Many

_____ 17. Glycogen nuclei: **0**=Rare/absent; **1**=Present in patches

_____ 18. Glycogenosis of hepatocytes: **0**=Not present; **1**=Focal, involving less than 50% of the hepatocytes; **2**=Diffuse, involving greater than or equal to 50% of the hepatocytes

19. **Masson's trichrome stain**

_____ . . . a. Fibrosis stage: **0**=None ➜ **GOTO Item 20**; **1a**=Mild, zone 3 perisinusoidal (*requires trichrome*); **1b**=Moderate, zone 3, perisinusoidal (*does not require trichrome*); **1c**=Portal/periportal only; **2**=Zone 3 and periportal, any combination; **3**=Bridging; **4**=Cirrhosis

_____ . . . b. Perisinusoidal fibrosis grade: **0**=No perisinusoidal fibrosis present; **1**=Perisinusoidal fibrosis present that requires a Masson stain to identify; **2**=Perisinusoidal fibrosis present that is visible on the H&E stain

_____ . . . c. Predominant location of fibrosis: **0**=More predominance around or between portal areas; **1**=No portal or central predominance; **2**=More predominance around/between central veins

20. **Iron stain**

_____ . . . a. Hepatocellular iron grade: **0**=Absent or barely discernible, 40x ➜ **GOTO item 20c**; **1**=Barely discernable granules, 20x; **2**=Discrete granules resolved, 10x; **3**=Discrete granules resolved, 4x; **4**=Masses visible by naked eye

_____ . . . b. Hepatocellular iron distribution: **0**=Periportal; **1**=Periportal and midzonal; **2**=Panacinar; **3**=Zone 3 or azonal

_____ . . . c. Nonhepatocellular iron grade: **0**=None ➜ **GOTO item 21**; **1**=Mild; **2**=More than mild

_____ . . . d. Nonhepatocellular iron distribution: **0**=Large vessel endothelium only; **1**=Portal/fibrosis bands only, but more than just in large vessel endothelium; **2**=Intraparenchymal only; **3**=Both portal and intraparenchymal

_____ 21. Is this steatohepatitis? **99**=Not NAFLD; **0**=NAFLD, not NASH; **1a**=Suspicious/borderline/indeterminate: Zone 3 pattern; **1b**=Suspicious/borderline/indeterminate: Zone 1, periportal pattern; **2**=Yes, definite

_____ 22. Is cirrhosis present? **0**=No ➜ **GOTO item 25**; **1**=Yes

_____ 23. Is this cryptogenic cirrhosis: **0**=No ➜ **GOTO item 25**; **1**=Yes

24. Features suggestive of steatohepatitis etiology for cryptogenic cirrhosis:

_____ . . . a. Mallory-Denk bodies (*rule out cholate stasis*): **0**=Absent; **1**=Present

_____ . . . b. Perisinusoidal fibrosis away from septa: **0**=Absent; **1**=Present

_____ . . . c. Hepatocyte ballooning: **0**=Absent; **1**=Present

_____ . . . d. Megamitochondria: **0**=Absent; **1**=Present

_____ . . . e. Other notable findings: **0**=Absent; **1**=Present; Specify: _____

25. Other comments: _____

HW - Liver Biopsy Histology Worksheet

Purpose: Record results of histologic evaluation of slides from screening liver biopsy.

When: Whenever a biopsy is evaluated by the Study Pathologist for the NASH CRN.

By whom: Study Pathologist at the NASH CRN clinical center (this is not the form used for central reading) and Clinical Coordinator.

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome slide. Details for scoring liver biopsy can be found in the NAFLD Database 2 SOP IV. Upon completion of this form, the Study Pathologist should give the HW form to the Clinical Coordinator. The Clinical Coordinator should transcribe the information on the Liver Biopsy Histology Worksheet (HW) to the Liver Biopsy Histology Findings (HF) form for the study for which the patient is being evaluated; the worksheet should be stapled to the HF form. If the biopsy is being used for more than one NASH CRN study, the biopsy should only be read by the local pathologist once; the worksheet should be copied and attached to both HF forms.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of reading:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code: ____ ____ ____ ____

6. Form & revision: h w 1

B. Biopsy information

7. Date this biopsy was performed *(obtained from surgical pathology report):*

____ ____–____ ____ ____–____ ____
day mon year

8. What slides are to be used in this evaluation *(check all that apply)*

a. H & E: (1)

b. Masson's trichrome: (1)

c. Iron: (1)

9. Biopsy length: ____ ____
mm

C. NAFLD evaluation (use H & E and Masson's trichrome slides only)

10. Steatosis *(assume macro, e.g., large and small droplet)*

a. Grade:

< 5% (0)
5-33% (1)
34-66% (2)
> 66% (3)

b. Location:

Zone 3 (0)
Zone 1 (1)
Azonal (2)
Panacinar (3)

11. Fibrosis stage *(Masson's trichrome stain)*

0: None (0)
1a: Zone 3, perisinusoidal (requires trichome) (1)
1b: Zone 3, perisinusoidal (easily seen on H&E) (2)
1c: Portal/periportal only (3)
2: Zone 3 and periportal, any combination (4)
3: Bridging (5)
4: Cirrhosis (6)

12. Inflammation

a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:

0 (0)
< 2 / 20x mag (1)
2-4 / 20x mag (2)
> 4 / 20x mag (3)

b. Amount of portal, chronic inflammation:

None to minimal (0)
Mild (1)
More than mild (2)

13. Hepatocellular ballooning:

None (0)
Few (1)
Many (2)

14. Steatohepatitis diagnosis:

Not NAFLD (0)
NAFLD, but not NASH (1)
Suspicious/borderline/indeterminate, zone 3 pattern (1A) (2)
Suspicious/borderline/indeterminate, zone 1, periportal pattern (1B) (3)
Yes, definite steatohepatitis (4)

15. NAFLD activity score (NAS) *(sum of items 10a, 12a, and 13):* ____ 0-8

D. Exclusion of other liver disease

16. Is there evidence of primary biliary cirrhosis:

Yes (1) No (2)

17. Is there evidence of Wilson's disease:

Yes (1) No (2)

18. Features of chronic cholestatic liver disease *(check all that apply):*

a. Bile duct loss/infiltration/sclerosis: (1)
b. Florid duct lesions: (1)
c. Cholate stasis: (1)
d. Copper deposition: (1)
e. Other *(specify):* (1)

_____

f. None: (1)

19. Features of other forms of chronic liver disease *(check all that apply):*

a. Vascular lesions of ALD/B-C/OVD: (1)
b. Inflammation suggestive of AIH, HCV: (1)
c. Pigment suggestive of HH: (1)
d. Globules suggestive of A1AT: (1)
e. Hepatocellular changes suggestive of HBV: (1)
f. Granulomas suggestive of sarcoid, PBC, infection: (1)
g. Other *(specify):* (1)

_____

h. None: (1)

E. Evaluation of cryptogenic cirrhosis

20. Is cirrhosis present:

Yes (1) No (2)

23.

21. In your opinion, is this **cryptogenic cirrhosis** *(cirrhosis that fails to meet criteria for NAFLD and without evidence of other form(s) of chronic liver disease):*

Yes (1) No (2)

23.

22. Other features *(check all that apply):*

a. Mallory's hyaline (r/o cholate stasis): (1)

b. Perisinusoidal fibrosis away from septa: (1)

c. Hepatocyte ballooning: (1)

d. Megamitochondria: (1)

e. Other *(specify):* (1)

_____

f. None: (1)

F. Other comments

23. Other comments:

_____

_____

_____

_____

_____

G. Administrative information

24. Study Pathologist PIN: ____ ____ ____

25. Study Pathologist signature:

_____

FLINT

HF - Liver Biopsy Histology Findings

Purpose: Record results of histologic evaluation of slides from liver biopsy for eligibility.

When: Visit s.

By whom: Study Pathologist at the NASH CRN clinical center (this is not the form used for central reading) and Clinical Coordinator.

Instructions: The Study Pathologist should complete this form using the institution's H & E slide and if available, the institution's Masson's trichrome and iron slides. Upon completion of this form, the Study Pathologist should give the form to the Clinical Coordinator. If fewer than 3 unstained slides are available for the biopsy, the institution's H & E and Masson's trichrome slides must be sent to the DCC for central pathology review. If 3 or more unstained slides are available for the biopsy, only the unstained slides need to be sent to the DCC. The Study Pathologist should forward the stained slides (if needed) and up to 10 unstained slides to the Clinical Coordinator for forwarding to the Data Coordinating Center.

If Elig is checked for any item, the patient is not eligible for FLINT and the form should not be keyed. If C is checked for an item, use caution. If the Study Physician agrees with the diagnosis, the patient is ineligible for FLINT and the form should not be keyed.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Visit date:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code: s ____ ____

6. Form & revision: h f 1

7. Study: FLINT 7

B. Biopsy information

8. Date this biopsy was performed *(obtained from surgical pathology report):*

____ ____–____ ____ ____–____ ____
day mon year

9. What slides are to be used in this evaluation *(check all that apply)*

a. H & E: (1)

b. Masson's trichrome: (1)

c. Iron: (1)

10. Biopsy length: ____ ____
mm

C. NASH evaluation (use H & E and Masson's trichrome slides only)

11. Steatosis *(assume macro, e.g., large and small droplet)*

a. Grade:

< 5% (0) Elig ____

5-33% (1)

34-66% (2)

> 66% (3)

b. Location:

Zone 3 (0)

Zone 1 (1)

Azonal (2)

Panacinar (3)

12. Fibrosis stage *(Masson's trichrome stain)*

0: None (0)

1a: Zone 3, perisinusoidal (requires trichome) (1)

1b: Zone 3, perisinusoidal (easily seen on H & E) (2)

1c: Portal/periportal only (3)

2: Zone 3 and periportal, any combination (4)

3: Bridging (5)

4: Cirrhosis (6)

Elig ____

13. Inflammation

a. Amount of lobular inflammation: combines mononuclear, fat granulomas, and pmn foci:

0 (0)
Elig

< 2 / 20x mag (1)
2-4 / 20x mag (2)
> 4 / 20x mag (3)

b. Amount of portal, chronic inflammation:

None to minimal (0)
Greater than minimal (1)

14. Hepatocellular ballooning:

None (0)
Elig

Few (1)
Many (2)

15. Is steatohepatitis present:

No (1)
Elig

Suspicious/borderline/indeterminate (2)
Yes, definite (3)

D. Exclusion of other liver disease

16. Is there evidence of primary biliary cirrhosis:

Yes (* 1) No (2)
C

** Caution: Primary biliary cirrhosis is exclusionary*

17. Is there evidence of Wilson's disease:

Yes (* 1) No (2)
C

** Caution: Wilson's disease is exclusionary*

18. Features of chronic cholestatic liver disease *(check all that apply)*

a. Bile duct loss/infiltration/sclerosis: (* 1)
C

b. Florid duct lesions: (1)

c. Cholate stasis: (1)

d. Copper deposition: (1)

e. Other *(specify):* (1)

_____

f. None: (1)

** Caution: Bile duct obstruction and primary sclerosing cholangitis are exclusionary*

19. Features of other forms of chronic liver disease *(check all that apply)*

a. Vascular lesions of ALD/B-C/OVD: (1)

b. Inflammation suggestive of AIH, HCV: (* 1)
C

c. Pigment suggestive of HH: (* 1)
C

d. Globules suggestive of A1AT: (* 1)
C

e. Hepatocellular changes suggestive of HBV: (* 1)
C

f. Granulomas suggestive of sarcoid, PBC, infection: (* 1)
C

g. Other *(specify):* (1)

_____

h. None: (1)

** Exclusionary*

E. NAFLD Activity Score

20. NAFLD activity score (NAS)
(sum of items 11a, 13a, and 14)

(Note: each subscore must be 1 or more)

____
3-8

21. Is item 20 (NAS) 3 or less: ( ) Yes 1 ( ) No 2

C

F. Other comments

22. Other comments:

_____

_____

_____

_____

_____

G. Administrative information

23. Study Pathologist PIN: ____ ____ ____

24. Study Pathologist signature:

_____

25. Clinical Coordinator PIN: ____ ____ ____

26. Clinical Coordinator signature:

_____

27. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

FLINT

LT - Liver Tissue Banking

Purpose: To document collection of extra liver tissue and procedures for liver tissue banking.

When: Visits s and f72 when more than 2 cm of liver tissue are obtained during a biopsy. This form is expected when the Liver Biopsy Materials Documentation (SD) form says liver tissue was obtained for banking.

By whom: Clinical Coordinator, in consultation with study physician.

Instructions: Liver biopsy tissue should be obtained by a needle core biopsy (as opposed to a wedge biopsy) using a 16 gauge or greater needle. Whenever more than 2 cm of tissue are obtained during biopsy, place a 1-2 mm segment of liver tissue into a labeled 2.0 mL polypropylene cryovial pre-filled with approximately 1 mL of RNA*later*® Solution. Liver tissue should be placed in RNA*later*® Solution within one minute and no more than 5 minutes after biopsy. **Note: If the sample is not placed in RNA*later*® Solution within 5 minutes, discard the cryovial.** Refrigerate the cryovial at 4° C overnight to allow thorough penetration of the liver tissue and then transfer to -70° C freezer for storage. Batch ship cryovials monthly on dry ice to the NIDDK Biosample Repository located at Fisher BioServices.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code *(s or f72):* ____ ____ ____

6. Form & revision: l t 2

7. Study: FLINT 7

B. Liver biopsy/RNA*later*® Solution storage procedures

8. Date of biopsy:

____ ____–____ ____ ____–____ ____
day mon year

9. Was the liver tissue obtained from a needle core biopsy *(as opposed to a wedge biopsy):*

Yes (1) No (2)

10. Was liver tissue placed in RNA*later*® Solution preferably within 1 minute, but no more than 5 minutes after biopsy:

Yes (1) No (* 2)

13. ____

** Discard liver tissue*

11. Was liver tissue refrigerated at 4° C overnight, then transferred to freezer for storage:

Yes (1) No (2)

12. ____

a. If no, describe conditions of local storage:

_____

_____

C. Cryovial label

12. Attach duplicate cryovial label *(make sure you attach the duplicate of the label attached to the cryovial holding the liver tissue from this biopsy):*

D. Administrative information

13. Clinical Coordinator PIN: ____ ____ ____

14. Clinical Coordinator signature:

_____

15. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

FLINT

SD - Liver Biopsy Materials Documentation

Purpose: To document that the liver biopsy required for screening was obtained under the time and medication restrictions required by the protocol and to document whether liver tissue was obtained for banking (both screening and followup biopsies). The number and type of slides available for archival at the Data Coordinating Center is noted for both screening and followup biopsies. If slides cannot be archived at the Data Coordinating Center, the source of the slides and the time by which the slides must be returned to the clinical center are recorded.

When: Visits s, f72, and as needed for biopsies at interim times.

By whom: Clinical Coordinator in consultation with the Study Pathologist.

Instructions: This form provides information about the tissue and slides from the biopsy and alerts the DCC to expect completion of the Liver Tissue Banking (LT) form, receipt of tissue at the Biosample Repository, and receipt of slides from the biopsy at the Data Coordinating Center. It also provides a record of the source of the slides, the number and type of stained slides available for review at the clinical center, the need (if any) to borrow those stained slides or provision of those stained slides to the NASH CRN without requiring return of the slides, and the number of unstained slides to be provided to the NASH CRN. A copy of the original surgical pathology report for the biopsy must be obtained; the patient's name should be blacked out, the report should be annotated with the patient's NASH CRN ID number and code, and the annotated report should be stapled to the back of this form. The surgical pathology report documents the date of biopsy. The slides should be labeled using the labels provided by the DCC. For unstained slides use permanent labels with sequence numbers 01-60, the borrowed slides should be labeled with removable overlabels with sequence numbers 81- 90.

A. Center, patient and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date form initiated:

____ ____–____ ____ ____–____ ____
day mon year

5. Visit code: ____ ____ ____

6. Form & revision: s d 1

7. Study: FLINT 7

B. Surgical pathology report

8. Is a copy of the report annotated with the patient's NASH CRN ID number and code and with name blacked out attached to this form:

Yes (1) No (* 2) STOP

** Obtain a copy of the report, annotate it, and attach it. You can use one of the pathology labels to annotate the report.*

9. Biopsy information

a. Date of biopsy specified on the surgical pathology report:

____ ____–____ ____ ____–____ ____
day mon year

b. Lobe specimen obtained from *(check only one):*

Right (1)
Left (2)
Unknown (3)

C. Requirements for screening biopsy

10. Is this visit s:

Yes (1) No (2) **13.**

11. Is the date in item 9a within 90 days of the anticipated date of randomization:

Yes (1) No (* 2) Elig

** Biopsy date must be within 90 days of randomization.*

12. Were any proscribed medications (antiNASH medications or supplements or antiobesity medications, other than thiazolidinedione or vitamin E) used within 90 days of the date of the biopsy:

Yes (* 1) No (+ 2)

→ E.Hg

** Biopsy must be done when the patient has been free of proscribed medications (antiNASH medications or supplements and antiobesity medications) for at least 90 days prior to the date of the biopsy.*

+ Since this is the screening biopsy, the local Study Pathologist must complete the Liver Biopsy Histology Findings (HF) form for this biopsy.

D. Biopsy specimens and stained slides at the clinical center

13. Was a sample of liver tissue obtained for banking:

Yes (* 1) No (2)

** If Yes, complete the Liver Tissue Banking (LT) form*

14. What stained slides from the biopsy are available at the clinical center *(check all that apply)*

a. H & E stain: (1)

b. Masson's trichrome stain: (1)

c. Iron stain: (1)

E. Unstained slides to be sent to the DCC

15. Are unstained slides available for sending to the DCC:

Yes (1) No (2)

→ **18.**

16. How many unstained slides will be sent to the DCC: ____ ____

17. What are the slide sequence numbers for those slides *(from the NASH CRN labels on each slide - use permanent labels, sequence numbers 01-60):*

a. Slide sequence number ____ ____ (01-60)

b. Slide sequence number ____ ____ (01-60)

c. Slide sequence number ____ ____ (01-60)

d. Slide sequence number ____ ____ (01-60)

e. Slide sequence number ____ ____ (01-60)

f. Slide sequence number ____ ____ (01-60)

g. Slide sequence number ____ ____ (01-60)

h. Slide sequence number ____ ____ (01-60)

i. Slide sequence number ____ ____ (01-60)

j. Slide sequence number ____ ____ (01-60)

F. Stained slides to be sent to the DCC

(The institution's stained slides must be sent to the DCC only if fewer than 3 unstained slides will be sent to the DCC)

18. Are any stained slides to be sent to the DCC:

Yes (1) No (2)

→ **26.**

19. How many stained slides are to be sent to the DCC: ____

20. Sequence number of slides to be sent to DCC

a. Slide sequence number of H & E stain: ____ ____ (81-90)

b. Slide sequence number of Masson's trichrome stain: ____ ____ (81-90)

c. Slide sequence number of iron stain: ____ ____ (81-90)

d. Slide sequence number of other stain: ____ ____ (81-90)

21. Are any stained slides to be returned to the clinic:

Yes (1) No (2)

26.

22. How many stained slides are to be returned to the clinic: ____

23. List sequence numbers of those slides to be returned

a. Slide sequence number: ____ ____ (81-90)

b. Slide sequence number: ____ ____ (81-90)

c. Slide sequence number: ____ ____ (81-90)

d. Slide sequence number: ____ ____ (81-90)

24. When do the stained slides need to be returned to the clinical center *(check only one):*

Immediately after central review (1)

At the end of the NASH CRN funding period (2)

25. Which pathology department did these slides come from:

NASH CRN clinical center's pathology department (1)

26.

Other, *(specify):* (2)

_____
name

_____
address

_____
address

_____
address

_____
phone

Note: this is the FLINT trial record of the source of the slides i.e., where the clinical center should send the slides when they are received back from the DCC.

G. Administrative information

26. Clinical Coordinator PIN: ____ ____ ____

27. Clinical Coordinator signature:

_____

28. Date form reviewed:

____ ____–____ ____ ____–____ ____
day mon year

SS - Specimen Shipment Log

Purpose: To record information about contents of specimen shipment and receipt of specimens at the NIDDK Biosample Repository, Fisher BioServices.

When: Monthly. Ship on Monday, Tuesday, or Wednesday. Avoid shipments 2 days prior to weekends and holidays.

By whom: Clinical Coordinator or laboratory personnel who have received formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines.

Instructions to shipper: Complete one Specimen Shipment Log for every shipment of specimens sent to NIDDK Biosample Repository. Clinical Coordinator/laboratory personnel should record the Federal Express airbill number at the top of page 3, and record the date and number of specimens shipped. Attach a copy of the Specimen Shipment Log to the printed Excel file and place on top of the styrofoam cooler lid. **(Do not place the log inside of box with the dry ice).** Close and seal the cardboard box. Keep original Specimen Shipment Log and one copy of the Excel file in your study files. All shipments should be sent via Federal Express priority service (next day, AM delivery). Send an Email to bio-niddkrepository@thermofisher.com on the day package is picked up by Federal Express. Include the tracking number in the subject line and attach the Excel file.

Barcode scanning procedures:

The barcode scanner connects to your computer via the USB cable. No additional software is required to scan the cryovial labels. Use the provided template Excel file (NASHCRNsiteXXX_shipdate.xls) when scanning the cryovial barcodes.

The following summarizes the procedures for operating the barcode scanner and linking the data to your Excel file:

- Plug the scanner into a USB port on your computer,
- Open the template Excel file and place the cursor in the first cell under Barcode number.
- Hold the scanner approximately 4-8 inches from the cryovial barcode you wish to scan. (The scanner will emit a red light as it searches for the barcode and will emit a beep and a green dot when the barcode has been successfully read.)
- The scanner automatically enters the barcode into the proper field and immediately goes down to the next record.
- Scan all the cryovials you are shipping to the NIDDK Biosample Repository into the Excel spreadsheet.

Keep your small cryovial boxes in a container or tray filled with dry ice to prevent the specimens from thawing. Once all cryovials are scanned, place the cryovials back in the freezer or pack in shipper with dry ice as detailed under **Packing instructions** to prevent thawing.

For each cryovial barcode scanned, enter the following in the corresponding columns of the Excel file: B=Site ID-Patient ID numbers (xxx-9999); C=3 letter patient code (xyz); D=the date the sample was collected (mm/dd/yy format); E=the specimen type (S = serum; P = plasma, T = Tissue); F=for plasma and serum, the volume should be a standard 0.5 and the unit of measure (column G) will always be milliliters (mL); H=study number (7 = FLINT); I=Study visit code (s, f12, f24, f36, f48, f60, f72 or f96); and J=Conditions (enter an "R" in this column only for tissue cryovials containing RNA*later*®).

The template Excel filename is NASHCRNsiteXXX_shipdate.xls. You should replace the X's with your clinical center's three digit **site ID** and replace ship date with the date of shipment.
Do not manipulate the columns in the Excel file as the Repository can only upload your files in the format provided in the template.

Sending a shipment:

For every shipment of cryovials to the NIDDK Biosample Repository, fill out sections A and B of the Specimen Shipment Log (SS) form. Save the Excel spreadsheet with the correct site ID number and ship date at the end of the file name (Click on File, then click on Save As) and **print 2 copies**.
Staple one copy to a copy of the Specimen Shipment Log you send in the shipper to the NIDDK Biosample Repository. Staple the second copy to the original Specimen Shipment Log that you keep in your study files.

For each shipment, email the Excel spreadsheet to the Biosample Repository at bio-niddkrepository@thermofisher.com with the Fed Ex tracking number in the subject line of the email.

FLINT

Packing instructions:

Check that one absorbent pad is in the Saf T Pak Biohazard plastic bag.
Insert frozen cryovials into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box can hold 81 cryovials.
Insert each cardboard box with cryovials into its own plastic bag and seal.
Place each plastic bag with specimen box into its own STP-710 Tyvek envelope and seal.
Place each Tyvek envelope into STP-111 inner brown cardboard box. No more than 3 Tyvek envelopes containing boxes with cryovials can be placed into the STP-111 inner brown cardboard box. If shipping only 1 or 2 specimen boxes, fill the rest of the space inside the cardboard inner box with bubble wrap to prevent movement.
Tape the inner cardboard box closed before placing in the styrofoam cooler.
Place cardboard box in upright position in bottom of styrofoam cooler.
Surround the STP-111 inner brown cardboard box with about 8 kg of 2" blocks or nuggets of Dry Ice.
Fill excessive room left in the insulated freezer box with bubble wrap to stabilize specimens in transit.
Place the polystyrene lid onto the freezer box.
Place the "Empty Packaging" cover and shipping form on the top of the cooler lid.
Place a completed Specimen Shipment Log on top of the cooler lid.
Close and seal outer cardboard box with tape.

Labeling Shipper:

Place a checkmark in the block on the outer cardboard box next to "BIOLOGICAL SUBSTANCE, CATEGORY B". Do not cover this marking with labels.
Affix a label with your name and return address to the side of the box in the "Shipper:" block.
Affix the repository address label to the side of the box in the "Consignee:" block.
Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms.
Affix the "UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B" label to the right of the dry ice label.
Use the preprinted Federal Express air bill to ship specimens to the NIDDK Biosample Repository. Complete return address (leave "Sender Federal Express account number" blank). Section 6, Special Handling: Check "Yes, Shippers Declaration not required," check "Dry Ice" block and enter "1" x "8"kg. Section 7, Enter "1" under "Total Packages" and the total weight of 24 lbs. Place completed Federal Express Airbill on the side of box adjacent to the labeled side. Call Federal Express at 1-800-463-3339; give them the account number in section 7 of the Airbill.
Do not write on exterior of box.

Ship to: Heather Higgins (NIDDK Repository)
Fisher BioServices
20301 Century Blvd, Bldg 6, Suite 400
Germantown, MD 20874
Telephone: (240) 686-4703

Federal Express Airbill Tracking Number: __ __ __ __ __ __ __ __ __ __ __ __

Include this tracking number in the subject line of your email to bio-niddkrepository@thermofisher.com.

A. Center ID, shipment, and study information

1. Center ID: __ __ __ __

2. Sequential shipment number: __ __

3. Date specimens shipped:

__ __ - __ __ __ - __ __
day month year

4. Total number of plasma aliquots: __ __

5. Total number of serum aliquots: __ __

6. Total number of liver tissue cryovials: __ __

7. Study: FLINT 7

B. Clinic administrative information

8. Print information of person preparing shipment:

_____
name

_____
telephone number

_____
Email address

C. Comments (to be completed by clinical center)

D. Comments (to be completed by Biosample Repository)

FLINT

TS - Histology Slide Transmittal Log

Purpose: To inform the Data Coordinating Center of the shipment of histology slides (stained and unstained), and to record information about contents of slide shippers and status of slides received at the Data Coordinating Center. This form is also used by the Data Coordinating Center to inform clinical centers of the shipment of slides back to the clinical center.

When: Ship slides monthly (or more often, as needed).

By Whom: Clinical Coordinator responsible for slide shipping.

Instructions to Shipper:

- Complete one Histology Slide Transmittal Log for every shipment of slides
- Make a copy of the Histology Slide Transmittal Log for the clinical center's notebook of slide shipping logs
- Attach a copy of the surgical pathology report for each slide set included in the shipment. Make sure the report is annotated with the patient's NASH CRN ID number and patient code and that the patient identifiers are blacked out
- Place slides in interior slide box which holds up to 25 slides
- Place 1-2 sheets of tissue over the slides to help prevent shifting
- Surround slide box with bubble wrap and tape securely
- Place the slide box wrapped with bubble wrap into a card board shipping box (eg., DHL box, FedEx box)
- Insert a copy of the Histology Slide Transmittal Log into the shipping box (file a copy of the TS log at the Clinical Center/DCC)
- Secure the shipping box with tape
- Place the consignee and return address labels on the exterior shipping box
- Fax (410 955-0932) a copy of the Histology Slide Transmittal Log to the Data Coordinating Center/clinical center
- Ship by two day delivery service **with ability to track the shipment to:**
  NASH CRN Slide Coordinator, NASH CRN Data Coordinating Center, 615 North Wolfe Street, Room W5010, Baltimore, MD 21205, (410) 955-8175 (phone)

A. Center, date, shipping and study identification

1. Center ID: ____ ____ ____ ____

2. Date form completed:

____ ____ - ____ ____ ____ - ____ ____
day month year

3. Study: FLINT 7

4. Shipping destination *(check only one)*:

Data Coordinating Center (1)
Return to clinic (2)

5. Shipping service used *(check only one)*:

DHL (1)
FedEx (2)
Other, *(specify)* (3)

_____

6. Shipment tracking number:

_____

7. Person preparing shipment *(please print)*:

_____
please print

8. Comments *(to be completed by staff responsible for shipping slides. If applicable, record reason(s) for discrepancies between number of slides recorded for a patient on the SD form and the number of slides recorded for that patient on the TS form)*:

_____

_____

_____

B. Slide shipment information

Record specified information about slides shipped in items 9 through 25. Indicate slide number(s) of slides that are stained or unstained. Personnel receiving the shipment will fill in the Receipt code (column g) with all codes that apply. Codes for column h. are as follows: A=Satisfactory, B=Missing surgical pathology report, C=Slide(s) broken, D=Slide(s) not with shipment, E=Slide(s) not labeled, F=Slide(s) mislabeled, G=Other.

	a. Patient ID	**b.** Patient code	**c.** Date of biopsy (day-month-year)	**d.** surgical pathology report (y/n)	**e.** slide numbers(s) of stained slides	**f.** slide number(s) of unstained slides	**g.** Receipt codes (completed by staff member receiving and reviewing the shipment contents)
9.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
10.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
11.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
12.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
13.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
14.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
15.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
16.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
17.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
18.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
19.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
20.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
21.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
22.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
23.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
24.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____
25.	__ __ __ __	__ __ __	__ __ - __ __ __ - __ __	__	__ __-__ __	__ __-__ __	_____

C. Shipment review (to be completed by staff receiving shipment)

26. Other comments regarding contents of shipment received:

_____

_____

_____

27. Person receiving shipment (please print):

_____

28. Date shipment received and reviewed:

___ ___ - ___ ___ ___ - ___ ___
day month year

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

FLINT
Standard Operating Procedures

Part V:
Standard of Care for Adult Patients with Fatty Liver Disorders

4 February 11

Contents

FLINT Standard of Care SOP V

1. Introduction

The purpose of this document is to articulate a uniform set of practices to be applied by investigators in the NASH CRN to the care of patients with fatty liver disorders. As directed by the NASH CRN Steering Committee, these standards were developed so that patients with fatty liver disorders will receive uniform treatment across study sites, thereby reducing the extent to which care at a particular site will independently influence outcomes. These standards of care were derived by expert opinion, specifically the opinions of the NASH CRN investigators, and as such cannot and should not be construed to mean that they should be applied outside of the NASH CRN study sites or that the failure to apply these standards is a breach of community standards of care.

The NASH CRN Standard of Care Committee developed these standards in their initial draft form after the first meeting of the Committee on July 29, 2002, in Baltimore, MD. After review and revision, it was submitted to the CRN Steering Committee and approved in principal at its meeting on September 22, 2002, in Atlanta, GA. The document was revised for the continuation of the NASH CRN in 2009 and approved by the Steering Committee in 2009. Committee members are aware that application of the standards described here could be viewed as an intervention in itself. However, the committee also felt that these standards are essential to fulfill our obligation to provide the best care to the participating patients.

2. Specific recommendations

2.1. Dietary intake

a. Patients without diabetes will be instructed to follow the National Cholesterol Education Program (NCEP) Step 1 recommendations (Appendix 1). These recommendations will include specific discussions on total caloric intake, the amount and type of fat consumed, and the amount of carbohydrate consumed.
b. The importance of portion control will be discussed, especially in reference to eating at restaurants. Avoidance of calorie dense fast food and sugar sweetened beverages will be stressed.
c. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the American Diabetes Association (ADA) (Appendix 2).
d. Recommendations regarding the use of specific nutritional supplements are addressed below.
e. Dietary guidelines may not apply to all persons or situations.

2.2. Weight loss

a. Overweight subjects (BMI > 25 kg/m^2) will be given a goal of losing and sustaining the loss of 5-10% of body weight. This weight loss should be achieved at a rate of 1-2 lbs per week per NHLBI guidelines (Appendix 3).
b. Patients will be instructed not to fast as a means of achieving weight loss.
c. Alternative diet plans intended to promote weight loss will be considered individually based on nutritional completeness.

2.3. Alcohol consumption

Patients will be instructed that total abstinence from alcohol is advisable. The Committee acknowledges the paucity of data regarding a minimal safe dose of alcohol in individuals with liver disease and consumption limited to "ceremonial use" or even amounts up to 10 g per week (1 oz 80 proof liquor, 3.5 oz non-fortified wine, 8 oz beer) may be safe.

2.4. Exercise

Patients will be instructed to engage in a lifestyle that includes regular moderate exercise. The recommendations of the Institute of Medicine will be used: regular physical activity of at least one hour daily.

2.5. Preventive medicine

a. Vaccination for viral hepatitis. The Committee did not reach a consensus on recommendations regarding vaccination against hepatitis A and B. While arguments could be made in favor of both, local practices are variable and the failure to vaccinate is unlikely to bias the study. Therefore disparities in site-specific practices would have no impact on the studies of the NASH CRN.
b. Hepatocellular carcinoma (HCC) screening. Because recent data suggest that cirrhosis caused by NASH is associated with a similar risk of developing hepatocellular carcinoma as other major causes of cirrhosis, patients with cirrhosis should undergo regular surveillance testing for HCC. In view of the lack of consensus in the field regarding an optimal cost-effective screening strategy, screening methods will not be standardized across sites but will be in accordance with local standards.

2.6. Management of coexisting morbidities

a. Diabetes

i. Non diabetic NASH subjects who are diagnosed with diabetes after their oral glucose tolerance test may be enrolled in the study if their HbA1c is <9.5%. These subjects should be referred to their primary physicians with recommendation to use metformin instead of TZDs, sulfonylureas, exenatide or insulin.

The criteria for diabetes are:

1. Fasting blood glucose test: ≥126 mg/dL (7.0 mmol/L)
2. Blood glucose level of ≥ 200 mg/dL (11.1 mmol/L) after two hour OGTT (75g load).
3. Hemoglobin A1c measurement ≥ 6.5%

ii. Patients with controlled diabetes (hemoglobin A1c <7%) will be continued on their current treatment regimens.

iii. Patients with sub optimally controlled diabetes (hemoglobin A1c ≥7%) will receive a recommendation for follow-up with their primary physician for improved glycemic control.

b. Hypertriglyceridemia

Patients with fasting triglycerides > 200 mg/dL will be referred to their primary physicians for specific recommendations.

c. Hypercholesterolemia

i. Nondiabetic patients with fasting LDL cholesterol levels > 130 mg/dL will be referred to their primary physicians for specific recommendations.

ii. Diabetic patients with fasting LDL cholesterol levels > 100 mg/dL will be referred to their primary physicians for specific recommendations.

d. Hypertension

i. Nondiabetic patients with repeated systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg will be referred to their primary physicians for specific recommendations.

ii. Diabetic patients with repeated systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg will be referred to their primary physicians for specific recommendations.

e. Angina

Patients will not be specifically evaluated for coronary heart disease (CHD). A review of systems will be obtained and if symptoms suggestive of angina are elicited, patients will be referred to their primary physicians for specific recommendations.

f. Sleep apnea

Symptoms suggestive of sleep apnea (snoring, observed periods of apnea, disruptive sleep disturbances) will be specifically sought as part of the

review of symptoms. If these symptoms are present, patients will be referred to their primary physicians for a possible sleep study. The rationale for this assessment is that disrupted sleep will contribute to fatigue and the role of periods of hypoxia in the pathogenesis of liver injury is unknown.

g. Hyperandrogenism and polycystic ovary syndrome (PCOS)

Women with hirsutism (facial and/or chest hair) and non-menopausal menstrual irregularity (< 9 menstrual cycles in the past year) will be referred to their primary physicians or gynecologists to be evaluated for PCOS.

h. Occupational exposure to hepatotoxins

A history of ongoing exposure to volatile hydrocarbons will be sought. Patients with ongoing occupational exposure to hydrocarbons will be instructed to verify workplace compliance with Occupational Safety and Health Administration (OSHA) regulations.

2.7. Possibly helpful concomitant medication use

a. Vitamin E
   i. Recommendations regarding the use of vitamin E will be individualized and should not exceed 800 IU all natural vitamin E Daily. Start date of vitamin in relation to liver biopsy or date of randomization must be documented.

b. Ursodeoxycholic acid (UDCA; Actigall; Urso)
   i. UDCA will generally be stopped unless new data are published to indicate a significant benefit for patients with NASH.
   ii. A UDCA washout period of 3 months prior to liver biopsy or 3 months prior to randomization will be needed before entry into treatment trials.
   iii. UDCA may be continued in patients who have shown a significant improvement associated with treatment in liver histology or surrogate markers for NAFLD such as ALT or imaging studies.

c. Metformin
   i. Patient receiving metformin as a treatment for diabetes may remain on the drug.
   ii. Patients treated with metformin for a diagnosis of NAFLD or NASH may remain on the drug.
   iii. Patients receiving metformin as a treatment of other disorders of insulin resistance (e.g., PCOS) may remain on the drug.

d. Fibrates

Fibrates used to treat hypertriglyceridemia may be continued with dose escalations as clinically indicated.

e. Statins

Statins used to treat hypercholesterolemia may be continued with dose escalations as clinically indicated.

f. Thiazolidinediones (TZDs; pioglitazone, rosiglitazone)
    i. Patients receiving a TZD as a treatment for diabetes may remain on the drug.
    ii. Use of TZDs for NASH (non trial) will be at the discretion of the patient's physicians based on accumulating data regarding potential benefits and risks. Start date of T2Ds in relation to liver biopsy or date of randomization must be documented.

2.8. Possibly harmful concomitant medication use

a. Acetaminophen
    i. Acetaminophen should be restricted to < 3 grams in any given day in patients without cirrhosis and < 2 grams in any given day in patients with cirrhosis.
    ii. Repeated use of > 1.5 grams daily for more than 3 consecutive days should be discouraged.
    iii. A history of using over-the-counter medications that may contain acetaminophen will be obtained at each visit.

b. Tamoxifen

    A history suggesting the onset of NASH during tamoxifen use should lead to a discussion among the hepatologist, oncologist and patient regarding the risks associated with its continuation versus discontinuation. Additional options include use of an alternative estrogen receptor antagonist, although the risk of NASH posed by these agents is unknown.

c. Estrogens

    Estrogen use as oral contraception and hormone replacement therapy (HRT) will not be discouraged.

d. Amiodarone
    i. Amiodarone can be continued for life-threatening arrythmias.
    ii. The continued use of amiodarone for non-life threatening arrythmias (e.g., atrial fibrillation) will be discussed with the patient's primary physician or cardiologist.

e. Iron supplements
    i. Patients with iron deficiency will be allowed to continue the use of iron supplements until iron stores are sufficient.
    ii. In the case of ongoing blood loss (e.g., meno/metrorrhagia, refractory gastroesophageal reflux disease (GERD), portal hypertensive gastropathy), iron supplements will be continued as long as the transferrin saturation remains < 50%.
    iii. Patients having normal iron status (serum ferritin > 15 ng/mL or transferrin saturation > 20%) and taking iron supplements will be requested to discontinue the supplements.

2.9. Possibly helpful concomitant dietary supplement use

a. Multivitamins. A daily multivitamin with iron content < 20 mg daily will be allowed. (Many commonly used multivitamins contain small amounts of iron, typically < 20 mg each.)
b. Betaine use will neither be recommended nor discouraged.
c. S-adenosylmethionine use will neither be recommended nor discouraged.
d. Herbal supplements: Milk thistle use will neither be recommended nor discouraged.

2.10. Possibly harmful concomitant dietary supplement use

a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5,000 IU) should not be used.
b. Glucosamine use will be recorded but patients will not be given specific recommendations. Although hexosamines may have a role in causing insulin resistance, the effect of oral glucosamine on insulin sensitivity is unknown.
c. Herbal supplements
    i. St John's Wort has been associated with CYP 3A4 induction and should be discontinued if used and avoided if not used.
    ii. Ephedrin-containing products marketed for weight loss will be strongly discouraged because of potential adverse effects.
    iii. Other herbal remedies should be viewed as possible causes of liver injury and should be discontinued or avoided.

3. Implementation

The intention of the NASH CRN is to use these standards of care in the patients followed at all clinical centers. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. Implementing the standards in patients with only steatosis on a liver biopsy will be at the discretion of the hepatologist. The Committee believes that implementing these standards of care constitutes good clinical practice and does not constitute an intervention; moreover, data will not be collected regarding outcomes after implementation of these standards. As such, their implementation is not under the purview of local Institutional Review Boards.

Responsibility for implementation: The NASH CRN will continue to use uniform teaching materials to provide patients with the information detailed above. Local sites will be responsible for printing costs. The materials will be distributed to patients by their hepatologists or other health care worker such as nurses and nutritionists. Distribution of materials to patients will be documented in their medical records.

Compliance and reinforcement: Patients' adherence to the above guidelines will be established by the study personnel. The practices will be reviewed by the hepatologist or associated health care worker with the patient during the clinic visit. Patients will be given the opportunity to

take the teaching materials outlining the above recommendations at each hepatology clinic visit.

Frequency of follow-up: Patients will be seen at least once every 12 weeks by the hepatologist. The visit should include an interim history, review of symptoms, updated medication list, physical exam, lab work, and discussion of adherence to the standard of care recommendations.

4. Preparation and dissemination of materials needed to implement the standards of care

4.1. Physician summary of guidelines

a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care
b. Standard of care pocket guidelines (Appendix 5).

4.2. Patient brochures

a. Brochures that should be available for NASH CRN patients
   i. Healthy eating
   ii. Healthy weight loss
      1. BMI formula
      2. Goals
   iii. General NASH CRN brochure to cover most other recommendations
      1. Alcohol use
      2. Acetaminophen use
         a. Allowable amounts
         b. List of medications containing acetaminophen
      3. Supplemental iron use
      4. Vitamins
         a. Allowable vitamin E
         b. Allowable vitamin A
         c. Daily multivitamin
      5. Warnings about herbal remedies
      6. Symptoms to report
         a. Angina
         b. Sleep apnea
         c. Irregular menstruation, facial hair

4.3. Referring physician information

Implementation of the standards of care will occasionally require communication of findings such as newly diagnosed diabetes, hypertension, or hyperlipidemia. This communication will be in the form of a letter from the NASH CRN physician as part of standard medical care.

5. References

1. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Circulation. 2002 Dec 17;106(25):3143-421.

2. Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell JQ, Wheeler M; Nutrition principles and recommendations in diabetes. American Diabetes Association. Diabetes Care. 2004 Jan;27 Suppl 1:S36-46.

3. Diabetes Care, January 2010, 3:S11-S61

Appendix 1: NCEP Step 1 diet (standard recommendation)

The general dietary recommendations developed by the Institute of Medicine with the goal of promoting a healthy lifestyle will be reviewed for possible substitution for the NCEP guidelines below.

The following dietary recommendations were stated by the (National Cholesterol Education Program (NCEP)) in their monograph entitled *The Third Report of the Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*, or ATP III. The primary goal of these recommendations is to provide a diet that would reduce the risk of coronary heart disease in individuals with high LDL cholesterol levels. A secondary target of risk reduction, which was new to this version of the report, was the metabolic syndrome or insulin resistance.

Nutrient Composition of the Therapeutic Lifestyle Change Diet	
Nutrient	**Recommended Intake**
Saturated fat[1]	< 7% of total calories
Polyunsaturated fat	≤ 10% of total calories
Monounsaturated fat	≤ 20% of total calories
Total fat	25 - 35% of total calories
Carbohydrate[2]	50 - 60% of total calories
Fiber	20 - 30 g daily
Protein	Approximately 15% of total calories
Cholesterol	< 200 mg/day
Total calories[3]	Balance energy intake and expenditure to maintain desirable body weight/prevent weight gain

[1]Trans fatty acids are another LDL-raising fat that should be kept at a low intake.
[2]Carbohydrates should be derived predominantly from foods rich in complex carbohydrates including grains, especially whole grains, fruits, and vegetables.
[3]Daily energy expenditure should include at least moderate physical activity (contributing approximately 200 Kcal per day).

Appendix 2: American Diabetes Association (ADA) diet (for patients with type 2 diabetes)

Current evidence-based recommendations developed by the ADA are summarized below.

Carbohydrates
Choose whole grains, fruits, vegetables, low-fat milk
Amount of carbohydrate is more important than source
Non-nutritive sweeteners in usual doses

Fats
Limit to 10% or less of total calorie intake
Limit cholesterol to <300 mg per day

Obesity and Weight Loss
Modest weight loss by reduced calorie intake improves insulin resistance
Structured programs of lifestyle change can produce weight loss of 5-7% calorie
Exercise and behavior modification are useful adjuncts to reduction of calorie intake

Older Adults
Energy requirements decline with age
Encourage physical activity

Hypoglycemia
Glucose is preferred treatment

Hypertension
Reduced sodium intake reduces blood pressure
Modest weight loss reduces blood pressure

Appendix 3: NHLBI Step 1 diet (for weight reduction)

Source: The Practice Guide. Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. NHLBI, 2000, p 27. URL: http://www.nhlbi.nih.gov/guidelines/obesity/practgde.htm

Nutrient	Recommended intake
Calories[1]	Approximately 500 - 1,000 kcal/day reduction from usual state
Total fat[2]	30% or less of total calories
Saturated fatty acids[3]	8 - 10% of total calories
Monounsaturated fatty acids	Up to 15% of total calories
Polyunsaturated fatty acids	Up to 10% of total calories
Cholesterol[3]	< 300 mg/day
Protein[4]	Approximately 15% of total calories
Carbohydrate[5]	55% or more of total calories
Sodium chloride	No more than 100 mmol/day (approximately 2.4 g of sodium or approximately 6 g of sodium chloride)
Calcium[6]	1,000 to 1,500 mg/day
Fiber[5]	20 - 30 g/day

1. A reduction in calories of 500 to 1,000 kcal/day will help achieve a weight loss of 1 to 2 pounds/week. Alcohol provides unneeded calories and displaces more nutritious foods. Alcohol consumption not only increases the number of calories in a diet but has been associated with obesity in epidemiologic studies as well as in experimental studies. The impact of alcohol calories on a person's overall caloric intake needs to be assessed and appropriately controlled.
2. Fat-modified foods may provide a helpful strategy for lowering total fat intake but will only be effective if they are also low in calories and if there is no compensation by calories from other foods.
3. Patients with high blood cholesterol levels may need to use the Step II diet to achieve further reductions in LDL-cholesterol levels; in the Step II diet, saturated fats are reduced to less than 7 percent of total calories, and cholesterol levels to less than 200 mg/day. All of the other nutrients are the same as in Step I.
4. Protein should be derived from plant sources and lean sources of animal protein.

Appendix 3: NHLBI Step 1 diet (for weight reduction) (cont'd)

5. Complex carbohydrates from different vegetables, fruits, and whole grains are good sources of vitamins, minerals, and fiber. A diet rich in soluble fiber, including oat bran, legumes, barley, and most fruits and vegetables, may be effective in reducing blood cholesterol levels. A diet high in all types of fiber may also aid in weight management by promoting satiety at lower levels of calorie and fat intake. Some authorities recommend 20 to 30 grams of fiber daily, with an upper limit of 35 grams.
6. During weight loss, attention should be given to maintaining an adequate intake of vitamins and minerals. Maintenance of the recommended calcium intake of 1,000 to 1,500 mg/day is especially important for women who may be at risk of osteoporosis.

Appendix 4: Common acetaminophen-containing over-the-counter medications

The number of acetaminophen-containing preparations is quite large and an updated list of such preparations could not be found. The best approach to this issue will be to inquire about over-the counter (OTC) medication use as part of the medical history. The FDA convened a public advisory committee meeting on June 29, and June 30, 2009, regarding acetaminophen use in both OTC and prescription (Rx) products, the potential for liver injury, and potential interventions to reduce the incidence of liver injury. The FDA maintains information regarding the use of acetaminophen on their website: http://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm165107.htm

Appendix 5: Standard of care pocket guidelines

Guidelines For Patients

Dietary Intake
National Cholesterol Education Program Diet for non-diabetics (http://www.nhlbi.nih.gov/chd/)
American Diabetes Association Diet for type 2 diabetics (http://www.diabetes.org/home.jsp)
Avoid fast food and sugar-sweetened beverages

Alcohol Consumption
None is advised, but 1 glass of red wine per week may be safe

Weight Reduction (if BMI>25)
10% weight loss per year until target BMI is reached
Dietery consult if no weight loss with lifestyle changes

Exercise
3 hours of physical activity per week

Follow-Up Visits
At least once a year
Include interim history, ROS, updated medication list, physical exam, lab work, and discussion of adherence to the standard of care recommendations as appropriate

Guidelines for Other Medical Providers

Type 2 Diabetes
Goal of maintaining Hb A1c < 7%
Suggest insulin-sensitizing agent if meds needed

Hypertriglyceridemia (> 200 mg/dL)
Referral to primary care provider for management

Hypercholesterolemia
Nondiabetics: fasting LDL cholesterol >130 mg/dL or
Diabetics: fasting LDL cholesterol levels >100 mg/dL
Referral to primary care provider for management

Hypertension
Nondiabetics; repeated SBP > 140 mm Hg or DBP > 90 mm Hg or Diabetics; repeated SBP > 130 mm Hg or DBP > 85 mm Hg
Referral to primary care provider for management

Angina
Symptoms suggestive of angina
Referral to primary care provider for management

Sleep apnea
Symptoms suggestive of sleep apnea
Referral to primary care provider for management

Hyperandrogenism & PCO Syndrome
Women with hirsutism & non-menopausal menstrual irregularity (< 9 menstrual cycles in the past year)
Referral to primary care provider for management

Occupational Exposure to Hepatotoxins
Recommend workplace compliance with OSHA regulations be verified

Medications

Stop
Ursodeoxycholic acid when used for NAFL, but may be continued if documented treatment benefit
Case by Case
Amiodarone
Pioglitazone
Tamoxifen

May Use Or Continue With Appropriate Monitoring
Acetaminophen < 3 g on any given day
or <1.5 g daily if used > 3 consecutive days
Metformin when used for PCOS or diabetes
Fibrates when used for hypertryglyceridemia
Statins when used for hypercholesterolemia
Thiazolidinediones when used for diabetes
Estrogens

Supplements

Stop
Iron if ferritin > 15 ng/mL or iron saturation > 20%
St. John's Wort
Ephedrine-containing products
Most other herbal supplements

May Use Or Continue With Appropriate Monitoring
MVI with < 20 mg iron daily
Vitamin E upto 800 IU per day
Vitamin A < 5000 U daily

No Recommendation
Betaine
S-adenosylmethionine
Milk thistle
Glucosamine

Disease Progression

Consider staging disease every 3-5 years with liver biopsy or a validated non-invasive marker

Monitor for any signs and symptoms of liver decompensation if patient has advanced fibrosis on liver biopsy