NASH CRN

Nonalcoholic Steatohepatitis

Clinical Research Network

Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcocholic Steatohepatitis: a Multicenter, Randomized, Double Masked, Placebo-Controlled Trial

(PIVENS)

Standard Operating Procedures

Part I: Clinical Center Operations

27 March 2007

PIVENS SOP Part I: Clinical Center Operations

Contents

PIVENS SOP Part I: Clinical Center Operations

1.Design overview

1.1. Design synopsis

Title	Piogliatazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis: A Multicenter, Randomized, Double-Masked, Placebo-Controlled Trial (PIVENS)
Sponsor	NIDDK
Type of study	Phase III randomized clinical trial
Objective	To evaluate whether 96 weeks of treatment with either pioglitazone or vitamin E lowers NASH activity as determined from hepatic histology in nondiabetic adults with NASH compared to treatment with placebo
Study design	Multicenter, double-masked, double-dummy, placebo-controlled study with 3 parallel groups
Treatment groups	Group 1: Pioglitazone (30 mg q.d.) and vitamin E-placebo (q.d.) Group 2: Vitamin E (800 IU, natural form, q.d.) and pioglitazone-placebo (q.d.) Group 3: Pioglitazone-placebo (q.d.) and vitamin E-placebo (q.d.)
Study duration	Up to 6 months screening prior to randomization, including at least 3 months of drug washout for those using antiNASH, antidiabetic, or anti-obesity or nonstable doses of stations or fibrates in the 3 months medications prior to baseline liver biopsy or in the 3 months prior to randomization 96-week treatment period 24-week washout period
Sample size	240 (80 per group)
Number of clinics	8

Inclusion criteria

- Histological evidence of NASH activity based on standardized scoring of a liver biopsy obtained no more than 6 months prior to randomization
- Age 18 years or older at initial screening interview

Exclusion criteria

- Significant alcohol consumption more than 20 g/day for females and more than 30 g/day for males on average, either currently or for a period of more than 3 consecutive months in the 5 years prior to screening
- Inability to reliably quantify alcohol intake
- Clinical or histologic evidence of cirrhosis
- Evidence of other forms of chronic liver disease
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Fasting plasma glucose of 126 mg/dL or greater at initial screening or at the oral glucose tolerance test done at the second screening visit
- Serum creatinine of 2.0 mg/dL or greater
- 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, other known hepatotoxins) for more than 2 consecutive weeks in the 2 years prior to screening
- Use of antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitinides, metformin, thiazolidinediones) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Use of antiNASH drugs (thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, probiotics) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Use of a non-stable dose of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin) or fibrates (clofibrate, fenofibrate) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Known intolerance to thiazolidinediones or vitamin E

- Vitamin E supplementation of greater than 100 IU/day
- Inability to safely obtain a liver biopsy
- History of diabetes mellitus
- History of total parenteral nutrition in the year prior to screening
- History of bariatric surgery or currently undergoing evaluation for bariatric surgery
- History of biliary diversion
- Known positivity for antibody to Human Immunodeficiency Virus
- Known heart failure of New York Heart Association class 2, 3, or 4
- Active, serious medical disease with likely life-expectancy less than 5 years
- Active substance abuse, such as alcohol or inhaled or injection drugs, in the year prior to screening
- Women of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
- Women: breast feeding
- Men of childbearing potential: unwillingness to use an effective form of birth control during the trial
- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of the study
- Failure to give informed consent

1.1. Design synopsis

Outcome measures	***Primary:***	Improvement in NASH activity defined from change in standardized scoring of liver biopsies at baseline and after 96 weeks of treatment
	Secondary:	- Change in composite histology score - Change in fibrosis score - Change in serum aminotransferase levels - Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference, triceps skin fold thickness, body composition) - Change in insulin resistance (assessed by HOMA) - Change in serum vitamin E levels - Change in cytokines, leptin, fibrosis markers, and lipid profile - Change in HR-QOL scores
Randomization	Centrally administered randomization stratified by clinical center 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, hypoglycemia, pregnancy, new onset diabetes, and any other outcomes or events identified as safety-related	

1.2. Data collection schedule

Assessment/Procedure	Screening visits			Follow-up visits Weeks from randomization															
	S1	S2	RZ	2	4	8	12	16	24	32	40	48	56	64	72	80	88	96	120
Consent	X	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Medical history (Baseline/Interim)	B	.	.	.	I	I	.	I	I	I	.	I	.	I	I	I	.	I	I
AUDIT, Skinner alcohol question.	A	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Interim alcohol questionnaire	.	.	.	.	X	X	.	X	X	X	.	X	.	X	X	X	.	X	X
Review of concomitant drugs	X	.	.	.	X	X	.	X	X	X	.	X	.	X	X	X	.	X	X
Review for adverse effects	X	.	.	X	X	X	.	X	X	X	.	X	.	X	X	X	.	X	X
Drug dispensing/return	.	.	X	.	X	X	.	X	X	X	.	X	.	X	X	X	.	X	.
Review of study drug adherence	.	.	.	X	X	X	.	X	X	X	.	X	.	X	X	X	.	X	.
Physical exam (Detailed/Focused)	D	.	.	.	F	F	.	F	D	F	.	D	.	F	F	F	.	D	F
DEXA scan for body fat	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X	.
Liver biopsy	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X	.
Block 98 nutrition questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Functional activity questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
HR-QOL (SF-36)	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Liver symptom questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
OGTT with insulin and C-peptide	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Labs																			
Fasting glucose	X	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.	.	.	.
Fasting lipid profile	X	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
CBC	X	.	.	.	.	.	.	.	X	.	.	X	.	.	.	.	.	X	X
Metabolic panel	X	.	.	.	.	.	.	.	X	.	.	X	.	.	.	.	.	X	.
Hepatic panel	.	X	.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GGT and prothrombin time	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
HbA1c	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
Urine pregnancy test (females)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Microalbuminuria	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Serum, plasma for banking	.	X	.	.	.	.	.	X	.	X	.	X	.	X	.	X	.	X	.
Serum vitamin E (banked serum)	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
DNA for banking	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Note: **Detailed (D) physical** includes measurement of height, weight, waist, and hips; vital signs (temperature, heart rate, blood pressure); triceps skin fold thickness; 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, fetor). **Focused (F) physical** includes measurement of height and weight; vital signs (temperature, heart rate, blood pressure); examination for scleral icterus and pedal edema and auscultation of heart and lungs.
Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, globulin, total protein.
Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
Fasting visits: S1, S2, 16, 24, 32, 48, 64, 72, 80, 96, and 120.
Safety visits: 2, 12, 40, 56, and 88.

1.3. Whole blood draw schedule: mL of blood to be drawn at screening and followup visits

	Study visit (wk)																		
Procedure	**s1**	**s2**	**2**	**4**	**8**	**12**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**	**120**	**Total**
Fasting glucose	5	.	.	.	.	.	.	5	.	.	.	.	.	5	.	.	.	.	15
Fasting lipid	5	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	5	20
GTT w/insulin	.	25	.	.	.	.	.	.	.	.	25	.	.		.	.	25	25	100
CBC	5	.	.	.	.	.	.	5	.	.	5	.	.	.	.	.	5	5	25
Metabolic panel	5	.	.	.	.	.	.	5	.	.	5	.	.	.	.		5	.	20
Hepatic panel	.	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	85
GGT, PT/INR	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
HbA1c	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
Plasma: TGF-β	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
Serum: fibrosis	.	10	.	.	.	.	.	.	.	.	10	.	.	.	.	.	10	.	30
Serum: Vit E	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
Serum: banking	.	30	.	.	.	.	20	.	20	.	30	.	20	.	20	.	30	.	170
Genetics	.	.	20	.	.	.		.	.	.	.	.	.	.	.	.	.	.	20
Other screening	40	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	40
Total	60	90	25	5	5	5	25	20	25	5	105	5	25	10	25	5	105	40	**585**

All PIVENS study visits except for randomization, 2-week, 4-week, and 8-week, 12-week, 40 week, 56-week, and 88-week follow-up visits are fasting visits and need to be scheduled for early morning. Fasting is defined as nothing by mouth except water in the 12 hours prior to blood draw.

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 three groups for 96 weeks of treatment:

Group 1: Pioglitazone (30 mg q.d.) and vitamin E-placebo (q.d)
Group 2: Vitamin E (800 IU, natural form, q.d.) and pioglitazone-placebo (q.d.)
Group 3: Pioglitazone-placebo (q.d.) and vitamin E-placebo (q.d.)

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

In order to qualify for inclusion in the trial, patients must satisfy the following inclusion criteria:

1. Based on a liver biopsy obtained no more than 6 months prior to randomization (patient must not have used antiNASH medications in the 3 months prior to the biopsy), histological evidence of NASH as defined by a NASH activity score, which is **EITHER** (1) a composite score of 5 or greater (where each component score for steatosis, hepatocyte ballooning, and lobular inflammation is 1 or more) as judged by the local NASH CRN pathologist with a finding of possible (defined as suspicious/borderline/indeterminate) or definite steatohepatitis as judged by the local NASH CRN pathologist, **OR** (2) a composite score of 4 (where each component score for steatosis, hepatocyte ballooning, and lobular inflammation is 1 or more) as judged by the local NASH CRN pathologist and a finding of definite steatohepatitis as judged by central review.

2. Patient must be 18 years of age or older as of the initial screening interview.

Exclusion criteria

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

1. Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 5 years 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) or inability to reliably quantify alcohol consumption
2. Clinical or histological evidence of cirrhosis as judged by study physician (clinical cirrhosis is defined by presence of any of the following: albumin less than 3 g/dL, INR greater than 1.3, conjugated bilirubin greater than 2 mg/dL, varices, or ascites)
3. 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 the presence of anti-nuclear antibody (ANA) of greater than 1:80 and consistent liver histology; or history of autoimmune hepatitis as judged by study physician
   - Primary biliary cirrhosis as defined by elevation of alkaline phosphatase greater than upper limit of normal and anti-mitochondrial antibody (AMA) of greater than 1:80 and consistent liver histology

- Known primary sclerosing cholangitis and suggestive liver histology
- Wilson's disease as defined by ceruloplasmin below the limits of normal and consistent liver histology
- Alpha-1-antitrypsin deficiency as defined by a suggestive liver histology (confirmed by alpha-1 antitrypsin level less than normal 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

4. Serum alanine aminotransferase (ALT) greater than 300 U/L
5. Fasting plasma glucose of 126 mg/dL or greater at initial screening or at the oral glucose tolerance test done at the second screening visit
6. Serum creatinine of 2.0 mg/dL or greater
7. 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, or other known hepatotoxins) for more than 2 consecutive weeks in the 2 years prior to screening
8. Use of antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitinides, metformin, thiazolidinediones) in the 3 months prior to liver biopsy or the 3 months prior to randomization
9. Use of antiNASH drugs (thiazolidinediones,vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, probiotics) in the 3 months prior to liver biopsy or the 3 months prior to randomization
10. Use of antiobesity drugs in the 3 months prior to liver biopsy or the 3 months prior to randomization
11. Use of a non-stable dose of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin) or fibrates (clofibrate, fenofibrate) in the 3 months prior to biopsy or the 3 months prior to randomization
12. Known intolerance to thiazolidinediones or vitamin E
13. Vitamin E supplementation of greater than 100 IU/day
14. Inability to safely obtain a liver biopsy
15. History of diabetes mellitus
16. History of total parenteral nutrition in the year prior to screening
17. History of bariatric surgery (jejunoileal bypass or gastric weight loss surgery) or currently undergoing evaluation for bariatric surgery
18. History of biliary diversion
19. Known positivity for antibody to Human Immunodeficiency Virus
20. Known heart failure of New York Heart Association class 2, 3, or 4
21. Active, serious medical disease with likely life-expectancy less than 5 years

21. Active substance abuse, such as alcohol or inhaled or injection drugs, in the year prior to screening
22. Women of childbearing potential: positive urine pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
23. Women: Breast feeding
24. Men of childbearing potential: unwillingness to use an effective form of birth control during the trial
25. Any other condition which, in the opinion of the investigator would impede compliance or hinder completion of the study
26. Failure to give informed consent

2.2. Run-in period

Patients must not have used any prescription or over-the-counter medication or herbal remedy taken with an intent to improve or treat NASH or liver disease or obesity or diabetes for the 3 months prior to liver biopsy and for the 3 months prior to randomization. AntiNASH agents include: thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, and probiotics. Antidiabetic agents include but are not limited to: insulin, biguanides, sulfonylureas, metformin and thiazolidinediones. These agents are not to be used during screening nor for the duration of the trial (except in the form of assigned study treatment or treatment for new onset diabetes) .

Any over-the-counter medication or herbal remedy that is being taken with an intent to improve hyperlipidemia will not be allowed for at least 3 months prior to randomization nor after randomization. Patients will be allowed to continue on prescription anti-hyperlipidemic agents. Patients will be interviewed in a detailed fashion at screening, and at clinic visits after randomization to document the absence of such use. If using a statin or fibrate medication, the patient must have been on a stable dose in the 3 months prior to liver biopsy and must have been on a stable dose in the 3 months prior to randomization.

2.3. ICD-9-CM codes for medical conditions pertaining to eligibility and surveyed on medical history forms

The PIVENS eligibility determination may require data abstraction from patient charts and administrative records, in addition to administering interviews and ordering study specific tests. In addition, the patient's status relative to a long list of medical conditions is queried on the Baseline Medical History (BG) form and on the Followup Medical History (HI) form. The International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) codes for these conditions are specified below as an aid to data abstraction.

Viral hepatitis (070)

Malignant neoplasm of liver and intrahepatic bile ducts (155)

Malignant neoplasm of gallbladder and extrahepatic bile ducts (156)

Acquired hypothyroidism (244)

Diabetes mellitus (250)

Ovarian dysfunction (256)
- Polycystic ovaries (256.4)

Disorders of carbohydrate transport and metabolism (271)
- Glycogen storage disease (271.0)

Disorders of lipoid mechanism (272)
- Pure hypercholesterolemia (272.0)
- Pure hyperglyceridemia (272.1)
- Mixed hyperlipidemia (272.2)
- Other and unspecified hyperlipidemia (272.4)
- Lipodystrophy (272.6)

Gout (274)

Disorders of mineral metabolism (275)
- Disorders of iron metabolism (275.0)
  - Hemochromatosis, iron overload
- Disorders of copper metabolism (275.1)
  - Wilson's disease

Disorders of fluid, electrolyte, and acid-base balance (276)
- Acidosis (276.2)
- Fluid overload, retention (276.6)

Other metabolic disorders (277)
- Cystic fibrosis (277.0)
- Amyloid degeneration of liver (277.3)
- Disorders of bilirubin excretion, Gilbert's syndrome (277.4)
- Alpha-1 antitrypsin deficiency (277.6)
- Dysmetabolic syndrome X (277.7)

Obesity (278)
- Obesity, unspecified (278.0)
- Morbid obesity (278.01)
- Localized adiposity (278.1)

Disorders involving the immune mechanism (279)
- Autoimmune hepatitis, not elsewhere classified (279.4)

Coagulation defects (286)
- Acquired coagulation factor deficiency due to liver disease (286.7)

Schizophrenic disorders (295)

Affective psychoses (296)
- Major depressive disorder, bipolar affective disorder

Neurotic disorders (300)
- Anxiety states (300.0)
- Obsessive-compulsive disorders (300.3)

Personality disorders (301)

Epilepsy (345)

Muscular dystrophies and other myopathies (359)

Hypertensive disease (401-405)
- Essential hypertension (401)

Ischemic heart disease

Acute myocardial infarction (410)
Other acute and subacute forms of ischemic heart disease (411)
Old myocardial infarction (412)
Angina pectoris (413)
Other forms of chronic ischemic heart disease (414)

Ill-defined descriptions and complications of heart disease (429)
Cardiovascular disease, unspecified (429.2)
Arteriosclerotic cardiovascular disease, cardiovascular arteriosclerosis

Cerebrovascular disease
Transient cerebral ischemia (435)
Acute, but ill-defined, cerebrovascular disease (436)
Apoplexy, cerebral seizure, cerebrovascular accident, stroke
Other and ill-defined cerebrovascular disease (437)

Atherosclerosis (440)

Disease of capilleries (448)
Nevus, non-neoplastic (448.1)
Spider nevi, **spider angiomata**, or vascular (arterial) spider is a common skin stigma in cirrhosis usually forming on the skin above the level of diaphragm attachment. It consists of a central arteriole, from which many small vessels radiate. The central arteriole is sometimes elevated, and its pulsation can be felt. Similar nevi also develop in the shoulder and upper arm, but they usually lack of the central arteriole.

Portal vein thrombosis, portal obstruction (452)

Budd-Chiari syndrome, hepatic vein thrombosis (453)

Esophageal varices with bleeding (456.0)

Acute and subacute necrosis of liver (570)
Acute hepatic failure
Acute or subacute hepatitis, not specified as infective
Necrosis of liver
Parenchymatous degeneration of liver

Chronic liver disease and cirrhosis (571)
Chronic hepatitis (571.4)
Cirrhosis of liver without mention of alcohol (571.5)

Biliary cirrhosis (571.6)
Other chronic nonalcoholic liver disease (571.8)
Unspecified chronic liver disease without mention of alcohol (571.9)

Liver abscess and sequelae of chronic liver disease (572)
Abscess of liver (572.0)
Portal pyemia (572.1)
Hepatic coma (572.2)
Hepatic encephalopathy
Hepatocerebral intoxication
Portal-systemic encephalopathy
Asterixis: An abnormal tremor consisting of involuntary jerking movements, especially in the hands, frequently occurring with impending hepatic coma.
Portal hypertension (572.3)
Hepatorenal syndrome (572.4)
Other sequelae of chronic liver disease (572.8)
Hepatopulmonary syndrome

Other disorders of liver disease (573)
Chronic passive congestion of liver (573.0)
Hepatitis, unspecified (573.3)
Toxic (noninfectious) hepatitis

Cholelithiasis (574)

Other disorders of gallbladder (575)

Other disorders of biliary tract (576)

Diseases of pancreas (577)

Intestinal malabsorption (579)
Postoperative blind loop syndrome (579.2)
Short bowel syndrome

Nephritis, nephrotic syndrome, and nephrosis (580-589)

Hydronephrosis (591)

Calculus of kidney and ureter (592)

Other disorders of kidney and ureter (593)

Other conditions in the mother classifiable elsewhere, but complicating pregnancy (648)
Abnormal glucose tolerance in pregnancy, **gestational diabetes** (648.8)

Erythematous conditions (695)
Unspecified erythematous condition, **erythema** (695.9) Redness of the palmar skin caused by dilatation and congestion of the capillaries. **Palmar erythema** represent extensive arteriovenous anastomoses in the thenar and hypothenar eminences and digital pads of the fingers.

Other diseases of skin (701)
Acquired **acanthosis nigricans** (701.2) An eruption of velvety wartlike growths accompanied by hyperpigmentation mainly in the skin of the neck.

Diffuse diseases of connective tissue
Systemic lupus erythematosus (710.0)
Rheumatoid arthritis (714.0)

Disorders of muscle, ligament, and fascia (728)
Muscular calcification and ossification (728.1)
Contracture of palmar fascia (728.6)
Other disorders of muscle, ligament, and fascia (728.8)
Interstitial myositis (728.81)

Other disorders of soft tissues (729)
Myalgia and myositis, unspecified (729.1)

Other congenital anomalies of digestive system (751)
Anomalies of gallbladder, bile duct, and liver (751.6)
Congenital cystic disease of liver (751.62)

General symptoms (780)
Sleep disturbances (780.5)
Sleep disturbance, unspecified (780.50)
Insomnia with **sleep apnea** (780.51)
Hypersomnia with **sleep apnea** (780.53)
Other and unspecified **sleep apnea** (780.57)

Symptoms involving skin and other integumentary tissue (782)

Edema (782.3) An accumulation of an excessive amount of watery fluid in cells, tissues, or serous cavities.

Jaundice, unspecified (782.4) Yellowish discoloration of the whites of the eyes, skin, and mucous membranes caused by deposition of bile salts in these tissues. Jaundice is a sensitive indicator of liver dysfunction. As a sign and symptom, jaundice and hyperbilirubinemia are among the frequently used "liver function" tests.

Symptoms concerning nutrition, metabolism, and development (783)

Abnormal weight gain (783.1)

Polyphagia (783.6)

Other symptoms involving abdomen (789)

Abdominal pain (789.0)

Hepatomegaly (789.1)

Splenomegaly (789.2)

Ascites (789.5) Lymph fluid that has leaked into the peritoneal cavity, is one of the principal clinical manifestations cirrhosis and portal hypertension.

Nonspecific findings on examination of blood (790)

Abnormal glucose (790.2)

Impaired fasting glucose, elevated fasting glucose (790.21)

Impaired glucose tolerance test (oral), elevated glucose tolerance test (790.22)

Abnormal non-fasting glucose (790.29)

Nonspecific elevation of levels of transaminase or lactic acid dehydrogenase (790.4)

Other nonspecific findings on examination of blood (790.9)

Abnormal coagulation profile (790.92)

Nonspecific abnormal findings on radiological and other examination (793)

Abdominal area, including retroperitoneum (793.6)

Nonspecific abnormal results of function tests (794)

Liver, abnormal liver scan (794.8)

2.4. New York Heart association (NYAA) classification for heart failure

A functional and therapeutic classification for prescription of physical activity for cardiac patients.

- **Class I:** Patients with no limitation of activities; they suffer no symptoms from ordinary activities.
- **Class II:** Patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
- **Class III:** Patients with marked limitation of activity; they are comfortable only at rest.
- **Class IV:** Patients with severe limitation who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

2.5. 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 rescreened at a later time as follows:

- An ineligible liver biopsy – the participant may be rescreened after 12 months at the discretion of the investigator
- Age < 18 years – the participant may be rescreened after his or her 18th birthday
- Ineligibility determined on measurements of albumin, INR, conjugated bilirubin, ALT, fasting glucose, and creatinine – the participant may be rescreened after 6 months at the discretion of the investigator
- Pregnancy – the participant may be rescreened 3 months following end of pregnancy and breast feeding at the discretion of the investigator
- Unwilling to participate – the participant may be rescreened after 3 months at the discretion of the investigator

2.6. Co-enrollment in NAFLD Database

- When a NAFLD Database patient enrolls in PIVENS, the visit schedule and requirements of the trial take precedence over the requirements for the NAFLD Database – Database requirements are suspended for the duration of the participant's time in the treatment trial. The PIVENS trial protocol should provide instructions, but if you cannot find the answer to your question, call the Data Coordinating Center.

- NAFLD Database patients may enroll in the PIVENS trial after being enrolled in the NAFLD Database for more than three months.

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

2.7. 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 PIVENS and the randomization assignment and materials needed in follow-up will be generated (i.e., labels, visit time window); this task will categorize each patient into one of three treatment groups:
  - Pioglitazone and vitamin E placebo
  - Vitamin E and pioglitazone placebo
  - Vitamin E placebo and pioglitazone placebo

Overriding eligibility criteria

- Requests overriding eligibility criteria must be made in writing to the DCC (direct the request to Aynur Ünalp-Arida); the request must specify the eligibility criteria for which override is requested and the request must be justified; the request must come from the principal investigator of the clinical center
- The DCC may require agreement to the override from other NASH CRN investigators
- Override requests require time to review and the review process will not be shortened

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
- The "time zero" for reckoning the time windows specified on the patient's PIVENS visit time window guide is the date of randomization

2.8. Roll over into NAFLD Database after completion of PIVENS (form CO)

- Patients who complete participation in PIVENS should resume participation in the NAFLD Database (if previously enrolled in the Database) or be invited to join the NAFLD Database (if not previously enrolled)

- The Closeout form should be completed at the f120 visit (or at the close of the f120 visit window) for all patients randomized in PIVENS.

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

- Patients willing to re-enter or join the NAFLD Database should sign the most recent version of the NAFLD Database 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 Database follow-up visit approximately 6 months after the date of their PIVENS f120 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 Database visit that is open 6 months from the date of their PIVENS f120 visit (this visit will be f144 or f192).

- For patients who were not previously enrolled in the NAFLD Database, a new NAFLD Database visit schedule (time windows guide) will be automatically generated when the PIVENS Closeout form (CO) is keyed into the online database. The new visit schedule will use the PIVENS randomization date as the effective date of enrollment into the NAFLD Database. Schedule the participant approximately 6 months from their PIVENS f120 visit for their f144 NAFLD Database follow-up visit.

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

3. Certification

3.1. Certification overview

What is certification?

- It is an internal (i.e., related to the study) procedure designed to identify the staff responsible for specific data items or 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:
  - PIVENS staff
  - Each clinical center
- Certification for PIVENS 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 PIVENS 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 PIVENS must be certified for that participation
- Completion of the Clinical Center Certification (CC) form will be required
- IRB approval for the PIVENS 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, who will staff the study
- Guide a clinical center through the steps of getting ready for PIVENS – 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 PIVENS protocol and consent documents
- Receive written notice of approval (email) from the Data Coordinating Center that the site is certified

3.3. Personnel certification

Staff functions requiring certification

- Clinical Coordinator
- Study Physician
- Pathologist
- Data Entry Technician

Requirements

- Everyone
  - Read the PIVENS 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)

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 PIVENS data system
- Staff can be certified for more than one function or more than one study (NAFLD Database, PIVENS, TONIC), but will have only one PIN

4. Human subjects

4.1. Background

Consent to participation in the PIVENS Trial must be completed before screening for PIVENS may begin. The patient must consent to procedures offered to and performed on him/her for screening, as well as to 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 PIVENS consent process has three major stages:

- The patient is asked to consent to screening and randomization into PIVENS
- The patient is asked to consent to the collection, storage, and use of blood samples for genetic research
- The patient is asked to sign the HIPAA authorization to disclose protected health information

4.2. Institutional review board process

Two prototype consent statements have been prepared for the PIVENS trial:

- Consent for screening and enrollment in PIVENS
- Consent for the collection, storage, and use of blood samples for current and future genetic research

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in PIVENS. 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 PIVENS. 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, consent forms, and data collection 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 recruitment materials to be used at their site. A clinical center may not initiate any patient contact about PIVENS until the site has IRB approval for PIVENS and the DCC has certified the site for initiation of PIVENS 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

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

(1) A PIVENS staff member 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 PIVENS study physician (i.e., a PIVENS certified hepatologist) must sign the consent statement, taking 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 PIVENS 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 PIVENS 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 PIVENS consent is administered, except that it should not be signed until the patient has been determined to be eligible for the PIVENS trial. Patients who have already consented to collection and banking of blood for genetic research as part of the NAFLD Database do not need to sign this consent again as part of the PIVENS trial.

4.4. Time considerations for obtaining consent

- The **PIVENS Consent and HIPAA authorization** must be obtained at the start of the initial visit (PIVENS visit s1); documents from the referring physician (if any) or from the NAFLD Database should have been reviewed prior to the visit and the patient judged eligible for screening prior to the visit. Signature of this consent is required prior to sending the patient for any PIVENS diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

- The **PIVENS Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for PIVENS has been established, at PIVENS visit s2 or at randomization visit. If the patient has already consented to genetic banking as part of the NAFLD Database, the patient does not need to be presented this option as part of the PIVENS 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 Blood Collection for DNA (BC) form. Signature of this consent statement is not required for PIVENS eligibility (i.e., the patient may choose not to participate in the genetic research component of PIVENS).

- A patient may be given the consent statements to review prior to the initiation of visit s1 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 PIVENS staff member. The consents may be mailed to the patient prior to PIVENS visit s1. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed PIVENS procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in PIVENS. Patients may request and should be given time to "think it over" at home and come back at a later time.

4.5. Consent handling

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

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

- The PIVENS 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 PIVENS. If the patient is enrolled in the NAFLD Database, the patient must also consent to the PIVENS evaluation, follow-up and banking procedures to enroll in the PIVENS trial.

- The PIVENS 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 PIVENS trial.

4.6. Informing participants of changes to consent statement after randomization

As new data become available during the conduct of PIVENS, 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 followup 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. Consenting roll over patients from NAFLD Database

If the patient previously enrolled in the NAFLD Database

- Consent as for a new PIVENS patient

If the patient previously consented to DNA banking as part of the NAFLD Database

- Patient does not need to sign new consent for genetic banking as part of PIVENS

4.8. HIPAA considerations

PIVENS 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 PIVENS 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
- Members of the Data and Safety Monitoring Board (DSMB) to monitor overall progress of the study
- Government officials from the Office of Human Research Protections or 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 PIVENS 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 Data and Safety Monitoring Board to review the PIVENS 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) will 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) will 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 PIVENS trial data. Patient identity will not be disclosed in any reports or publications resulting from the study. While PIVENS is ongoing, the use of PIVENS data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

Patient agreement to join the PIVENS 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 PIVENS. The only exception is refusal to provide blood for genetic research; patients may refuse to provide blood for genetic research and still enroll in PIVENS.

5. Study visits

5.1. Overview of visit schedule

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

- Screening for eligibility for enrollment (2 visits over a maximum of 24 weeks)
- Randomization to treatment (1 visit)
- Treatment phase (15 visits over 96 weeks)
- Post-treatment washout phase (1 visit at 120 weeks)

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
Screening		
s1	RG	Registration (document consent, sociodemographics, assign IDs)
	BG	Baseline history
	PE	Physical examination (detailed exam)
	SD	Liver biopsy materials documentation
	HF	Liver biopsy histology (reading at clinical center)
	LT	Liver tissue banking (if SD form says liver tissue was obtained for banking)
	CR	Central histology review
	AD	AUDIT (alcohol questionnaire)
	LR	Lab tests done during screening and follow-up (hematology, hepatic, clinical chemistry)
	LS	Lab tests done only during screening (etiologic tests)
	PL	Patient location (patient contact information)
s2	BB/BD	Block Food questionnaire, and documentation of completion of the food questionnaire
	PA	Physical activity
	QF	SF-36 quality of life questionnaire
	CG	Genetic consent documentation
	BC	Blood collection for DNA
	BP	Blood processing for serum and plasma
	DX	DEXA scan report
	LQ	Symptoms of liver disease
	LR	Lab tests done during screening and follow-up
	LD	Lifetime drinking history (Skinner)
Randomization		
Rz	EC	Eligibility checklist
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
2 week follow-up visit		
f002	LR	Lab tests done during screening and follow-up (hematology, hepatic, clinical chemistry, HbA1c if needed)
	PL	Patient location (update as needed)
4 week follow-up visit		

Phase/ Visit	Form abbr	Procedure
f004	HI	Follow-up medical history (interim history – includes info re: adverse effects, alcohol use)
	PF	Focused physical examination
	LR	Lab tests done during screening and follow-up (hematology, hepatic, clinical chemistry, HbA1c if needed)
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
8 week follow-up visit		
f008	HI	Follow-up medical history
	PF	Focused physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
12 week follow-up visit		
f012	LR	Laboratory results
	PL	Patient location (update as needed)
16 week follow-up visit		
f016	BP	Blood processing for plasma and serum
	HI	Follow-up medical history
	PF	Focused physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
24 week follow-up visit		
f024	HI	Follow-up medical history
	PE	Physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return

Phase/ Visit	Form abbr	Procedure
32 week follow-up visit		
f032	BP	Blood processing for plasma and serum
	HI	Follow-up medical history
	PF	Focused physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
40 week follow-up visit		
f040	LR	Laboratory results
	PL	Patient location (update as needed)
48 week follow-up visit		
f048	BB/BD	Block food questionnaire, and documentation of completion of the food questionnaire
	BP	Blood processing for plasma and serum
	HI	Follow-up medical history
	LQ	Symptoms of liver disease
	PA	Physical activity
	QF	SF-36 quality of life questionnaire
	PE	Physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
56 week follow-up visit		
f056	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
64 week follow-up visit		
f064	BP	Blood processing for plasma and serum
	HI	Follow-up medical history
	PF	Focused physical exam
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
72 week follow-up visit		

Phase/ Visit	Form abbr	Procedure
f072	HI	Follow-up medical history
	PF	Focused physical examination
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
80 week follow-up visit		
f080	BP	Blood processing for plasma and serum
	HI	Follow-up medical history
	PF	Focused physical examination
	LR	Laboratory results
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return
88 week follow-up visit		
f088	LR	Laboratory results
	PL	Patient location (update as needed)
96 week follow-up visit		
f096	BB/BD	Block food questionnaire, and documentation of completion of the food questionnaire
	BP	Blood processing for plasm and serum
	CR	Central histology review
	DX	DEXA scan report
	HI	Follow-up medical history
	HF	Liver biopsy histology findings
	LQ	Symptoms of liver disease
	LT	Liver tissue banking
	PA	Physical activity
	PE	Physical exam
	LR	Laboratory results
	SD	Liver biopsy materials documentation
	PL	Patient location (update as needed)
	RD	Study drug dispensing and return

Phase/ Visit	Form abbr	Procedure
120 week follow-up visit		
f120	HI	Follow-up medical history
	PF	Focused physical examination
	BB/BD	Block food questionnaire and documentation of completion of food questionnaire
	PA	Physical activity
	QF	SF-36 questionnaire
	LQ	Symptoms of liver disease
	LR	Lab tests done during screening and follow-up
	PL	Patient location (update as needed)
	CO	Closeout form

5.3. Guide for visit s1

Procedures

- Obtain signed consent
- Obtain permission to abstract data from patient's medical records
- 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
  - 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)
  - Alcohol use questionnaire
- Obtain patient location information
- If patient appears eligible at the close of visit s1
  - Schedule patient for visit s2
  - Schedule patient for any needed etiologic tests
  - Schedule patient for biopsy if appropriate

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
  - LS - Laboratory Results – Tests Done Only During Screening
  - AD - AUDIT
  - LR - Laboratory results screening and follow-up

- Additional forms required under specific conditions
  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)
  - CR - Central Histology Review (this form does not have to be keyed for patient to enroll in PIVENS)

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 PIVENS chart for patient and file the materials generated at registration that will be used at visit s2
- 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

5.4. Guide for visit s2

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 (45 or more samples per patient)
- Confirm eligibility with respect to whatever data have been keyed

Procedures

- Complete diet, activity, and quality of life questionnaires
- Obtain consent for DNA banking (if available)
- Collect blood for genetic specimen banking (2 tubes) and biosample banking (5 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

Data collection forms (form abbreviation)

- Forms completed for all patients
  - BD - Food Questionnaire Documentation
  - BP - Blood Processing for Serum and Plasma
  - CG - Genetic Consent Documentation (this form documents both consent and refusal)
  - LR - Laboratory Results - Tests Done During Screening and Follow-up
  - Block 98 Food Questionnaire (use the printed booklet provided by the DCC)
  - PA - Physical Activity
  - QF - MOS 36-Item Short-Form Health Survey
  - LD - Lifetime drinking history
  - DX - DEXA Scan Report
  - LQ - Symptoms of liver disease
- Additional forms for patients who consent to blood draw for DNA extraction
  - BC - Blood Collection for DNA

Forms for clinical center use only

- Check for updates to Patient Location (PL)

5.4. Guide for visit s2

After the patient leaves the clinical center

- 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
- Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates
- Hold food questionnaires for batch mailing to the DCC

5.5. Randomization visit

Procedures

- Randomization visit to be conducted as a visit separate from s2
- 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

- EC - Eligibility checklist
- RD - Study Drug Dispensing and Return

Comment

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

5.6. Visit windows: randomization and follow-up

- **Randomization** must occur within 6 months (183 days) of date of biopsy
- ***f002**: window runs from 1 week through 3 weeks, ideal date is 2 weeks (14 days) after randomization date
- **f004**: window runs from (3 weeks+1 day) through 6 weeks, must be at least 1 week after f02; ideal date is 4 weeks (28 days) after randomization date
- **f008**: window runs from (6 weeks+1 day) through 10 weeks, must be at least 2 weeks after f04; ideal date is 8 weeks (56 days) after enrollment date
- ***f012**: window runs from (10 weeks+1 day) through 14 weeks, must be at least 2 weeks after f08; ideal date is 14 weeks (98 days) after randomization date
- **f016**: window runs from (14 weeks+1day) through 20 weeks, must be at least 4 weeks after f012; ideal date is 16 weeks (112 days) after randomization
- **f024**: window runs from (20 weeks+1 day) through 28 weeks, must be at least 4 weeks after f016; ideal date is 24 weeks (168 days) after randomization
- **f032**: window runs from (28 weeks+1 day) through 36 weeks, must be at least 4 weeks after f024; ideal date is 32 weeks (224 dys) after randomization
- ***f040**: window runs from (36 weeks+1 day) through 44 weeks, must be at least 4 weeks after f032; ideal date is 40 weeks (280 days) after randomization
- **f048**: window runs from (44 weeks+1 day) through 52 weeks, must be at least 4 weeks after f040; ideal date is 48 weeks (336 days) after randomization
- ***f056**: window runs from (52 weeks+1 day) through 60 weeks, must be at least 4 weeks after f048; ideal date is 56 weeks (392 days) after randomization
- **f064**: window runs from (60 weeks+1 day) through 68 weeks, must be at least 4 weeks after f056; ideal date is 64 weeks (448 days) after randomization
- **f072**: window runs from (68 weeks+1 day) through 76 weeks, must be at least 4 weeks after f064; ideal date is 72 weeks (504 days) after randomization

- **f080**: window runs from (76 weeks+1 day) through 84 weeks, must be at least 4 weeks after f072; ideal date is 80 weeks (560 days) after randomization

- ***f088**: window runs from (84 weeks+1 day) through 92 weeks, must be at least 4 weeks after f080; ideal date is 88 weeks (616 days) after randomization

- **f096**: window runs from (92 weeks+1 day) through 108 weeks, must be at least 4 weeks after f088; ideal date is 96 weeks (672 days) after randomization

- **f120**: window runs from (108 weeks+1 day) through 132 weeks, must be at least 12 weeks after f096; ideal date is 120 weeks (840 days) after randomization

***f002, f012, f040, f056** and **f088** are safety visits, i.e., if the patient can get his/her blood drawn locally for hepatic panel, he/she does not need to come to the clinic.

5.7. 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 or a serious adverse event or liver biopsy
- If a liver biopsy is performed for a PIVENS patient at a time other than the baseline and f096 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 s1)

By whom

- Clinical Coordinator

Procedures

- Complete the PIVENS 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 PIVENS data system; this must be the first form keyed
- The Registration (RG) form should be keyed for each patient screened for PIVENS, including patients already enrolled in the NAFLD Database

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 PIVENS patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries:
  - Family history of liver disease
  - Information on initial diagnosis of NASH
  - Liver biopsy history
  - Weight history
  - Tobacco cigarette smoking 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 2 years
  - Willingness to use birth control methods
- Flash Card # 9, Weight Pattern over past 5 Years, is used with Form BG

When

- Visit s1 (but given that you need to do chart review, this may not be finished until visit s2)

How

- Mix of interview data and data obtained by chart review
- The smoking interview should be an interview with the patient
- 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 PIVENS 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 PIVENS patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries/reviews
  - Alcohol consumption since the last visit
  - Tobacco cigarette smoking since the last visit
  - Medical history diagnoses and procedures since the last visit
  - Medication use since the last visit

When

- Follow-up visits: f004, f008, f016, f024, f032, f048, f064, f072, f080, f096, f120. (Visits f002, f012, f040, f056 and f088 are safety visits; patient does not need to come to clinic if can get blood drawn elsewhere for hepatic panel.)

How

- Mix of interview data and data obtained by chart review
- The smoking interview should be 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 PIVENS patients

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference
  - Hip circumference
  - Triceps skinfold
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review (limited review for focused physical examination (PF) form)
  - Skin, including grading of acanthosis nigricans
  - Head, eyes, ears, nose, throat
  - Neck
  - Lymphatic
  - Chest and lungs
  - Heart
  - Abdomen
  - Liver and spleen
  - Extremities
  - Genitourinary/pelvis (may be skipped)
  - Nervous system (may be skipped)

When

- Detailed physical (form PE) at visit s1 and at f024, f048, and f096
- Focused physical (form PF) at all other visits but f002,f012, f040, f056 and f088 (safety visits w/o any physical exam)
- 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.
- Ideally, use Harpenden skin fold calipers in the performance of triceps skin fold measurements; this device may be obtained from www.bodytrends.com (800-549-1667, listed at $419.99). Other less expensive calipers are also available.

6.5. Height measurement

- 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

6.6. Weight measurement

- 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 PIVENS 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)

6.7. Waist circumference measurement

- 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 PIVENS 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 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

6.8. Hip circumference measurement

- 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 PIVENS 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 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 abdomen 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

6.9. Tricep skin fold measurement

- Tricep skin fold measurement should be recorded in millimeters
- Two measurements are to be taken and recorded
- Ideally a Harpenden skin fold caliper will be used; the accuracy of measurement is dependent upon accuracy of the equipment used, the correct selection and location of the skinfold sites, the proper technique in taking the measurements, and the experience of the tester
- To measure tricep skinfold, measure a vertical fold on the posterior midline of the upper arm, over the triceps muscle, halfway between the acrosion process, and olecranon process. The elbow should be extended and the arm relaxed
- Measurement should be taken on dry skin. Moist skin is harder to grasp and can influence the measurement
- Instruct the rest subject to keep muscles relaxed during test
- Take all measurements on the right side of the body. An exception might be where a deformity or missing limb would necessitate using the left side
- Mark the skinfold site using a pen. Use a tape measure to accurately find the mid points
- The skinfold should be firmly grasped by the thumb and index finger, using the pads at the tip of the thumb and finger. Gently pull skinfold away from the body
- The calipers should be placed perpendicular to the fold on the site marked, dial up at approximately 1 cm below the finger and thumb. While maintaining the grasp of the skinfold. Try to visualize the location of the true double fold of skin thickness, and place calipers there
- A minimum of two measurements should be taken at each site. If repeated tests vary by more than 1 mm, repeat the measurement. If consecutive measurements become increasingly smaller, the fat is being compressed; test the site a little later

6.10. Baseline and follow-up liver biopsy

- A baseline liver biopsy should be obtained prior to randomization for patients who:
  - have been found to be eligible for PIVENS with respect to all other criteria, or
  - who have not had a liver biopsy within 6 months of randomization, or
  - whose previous liver biopsy is of inadequate quantity, or
  - was done within three months of using anti-NASH medication
  - have used antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitides, metformin, thiazolidinediones) in the 3 months prior to liver biopsy or the 3 months prior to randomization
  - have used anti-NASH drugs (thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, probiotics) in the 3 months prior to liver biopsy or the 3 months prior to randomization
  - have taken a non-stable dose of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin) or fibrates (clofibrate, fenofibrate) in the 3 months prior to biopsy or the 3 months prior to randomization
- A follow-up liver biopsy should be obtained at the f096 visit for all patients enrolled in PIVENS
- 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

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

What / Who

- AUDIT (AD) form
- Skinner Lifetime Drinking History (LD) form
- Summary question on Eligibility Checklist (EC) form
- Questions on interval alcohol consumption on Follow-up Medical History (HI) form
- Flash Card #7, Drink Equivalents, can be used with the alcohol questionnaires
- Flash Card #8, 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 PIVENS 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 Eligibility Checklist (EC) form
- The Clinical Coordinator should complete section A on page 1 of Form AD and apply labels to subsequent pages before asking the patient to complete the form

6.12. Liver symptom questionnaire (form LQ)

What / Who

- LQ - Symptoms of Liver Disease, all PIVENS patients

Purpose

- To obtain the patient's view of his/her liver disease symptoms

When

- Visit s2
- At follow-up visits f048, f096, and f120

How

- Self-administered, without help from spouse or family
- Clinical Coordinator must be available to answer questions and to review form for completeness

6.13. Diet questionnaire (forms BB and BD)

Who/What

- Block 98 Food Questionnaire (form BB)
- Food Questionnaire Documentation (BD) form
- Portion size illustration (part of the Block Food Questionnaire booklet)

Purpose

- To determine patient's usual eating habits over the past year or so, including all meals or snacks, at home or in a restaurant or carry-out.
- To assess food frequency and quantity over the preceding year

When

- Visit s2
- Follow-up visits f048, f096, and f120

Procedure

- Use #2 pencil and fill in the bubble areas completely
- Before giving the booklet to the patient to complete, the Clinical Coordinator must:
  - Affix the Food Questionnaire ID label in the area where the patient is instructed to complete his/her name
  - Mark the patient's four digit ID # in the bubble area of the front page of the booklet; fill in from the left leaving remaining 5 bubbles blank
  - Mark the date in the bubble area of the front page of the booklet – THIS IS VERY IMPORTANT TO DO CORRECTLY – when the analysis is returned by the Block staff, date is the item which will distinguish screening from follow-up questionnaires
  - Mark the patient's gender in the bubble area of the front page of the booklet
  - Mark the patient's age at last birthday in the bubble area of the front page of the booklet
- Provide the patient with:
  - #2 pencil
  - Booklet
  - Portion size illustration
- Instruct the patient on completion of the booklet
  - Patient enters his/her best estimate of height and weight
  - Patient enters his/her best estimate of food eaten in the past year, frequency of eating a food, and portion size
  - Patient not to skip any foods and to mark "NEVER" if did not eat a certain food
- Remain available to answer questions as patient completes the booklet

- Review the completed booklet for completeness and color in any bubble areas that are partially or lightly completed
- Complete the Food Questionnaire Documentation (BD) form
- Put the completed booklet in a box (or other collection site) at the clinical center to hold for batch mailing to the DCC

Mailing completed questionnaires to the DCC

- Batch mail monthly using a mailing service that tracks packages
- Use the Transmittal Log for Food Questionnaires (TB) as a shipping list for the batch shipment
- Address the batch to:

Food Questionnaire Coordinator
NASH CRN Data Coordinating Center
615 North Wolfe Street, Room 5010
Baltimore, MD 21205
410-955-8175

Comments

- The Block questionnaire booklets are obtained from the DCC; contact Laura Miriel (lmiriel@jhsph.edu) to obtain additional copies
- The diet analysis provided by the Block group will be sent to the DCC

6.14. Physical activity questionnaire (form PA)

Purpose

- To evaluate correlations between level of activity and disease or mechanisms of disease

What / Who

- PA - Physical Activity, all PIVENS patients

When

- Visit s2
- Follow-up visits f048, f096, and f120)

How

- PA - self-administered, without help from spouse or family
- Clinical Coordinator should train patient in form completion
- Clinical Coordinator should complete pages 2 and 4

6.15. Quality of life questionnaire (QF)

Purpose

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

What / Who

- All PIVENS patients

When

- Visit s2
- Follow-up visits f048, f096, and f120

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

6.16. Laboratory measures (forms LS and LR)

Who

- All PIVENS patients

What

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

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

When

- Form LS: Visit s1
- 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 s1 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
- 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

6.16. Laboratory measures (forms LS and LR)

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., f048 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 f048, you do not need to order another hematology at f048
- 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 3 months of the screening visit date

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

Purpose

- Collection of whole blood from all PIVENS patients
- Separation of plasma and serum at clinical center: five or six 0.5 mL aliquots of plasma and forty 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

Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for citrate CTAD (blue top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- SS - Specimen Shipment log
- NIDDK Biosample Repository shipper

When

- Visit s2
- Follow-up visits f016, f040, f064 and f080, and f096
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator

Equipment

Blood tubes/aliquot vials

- One 4.5 mL CTAD (blue top) tube - *provided by clinical centers*
- Four 10 mL SST (red top) tubes - *provided by clinical centers*
- 45 or more 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, 12.5 mm wide x 49 mm tall, with silicone washers
  - one model/vendor that may be used is:
    Model # CV200-2
    Cyrogenic Storage Systems and Supplies
    243 Lawyers Road, NW
    Vienna, VA 22180
    703-319-8247
    877-738-8247
    703-938-9351 (fax)

Labels

- Preprinted labels for whole blood collection tubes (4.5 mL CTAD 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
- Collect whole blood into one 4.5 mL CTAD (blue top; Becton-Dickinson) tube for plasma
- Collect whole blood into four 10 mL SST (red top) tubes for serum
- Blood for plasma and serum to be centrifuged, aliquoted, and frozen within one hour

Plasma

- Collect blood into CTAD (blue top; Becton-Dickinson) tube
- 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 5-6 labeled 2.0 mL cryovials
- Freeze at -70° C immediately

Serum

- Collect blood into serum separator (red 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 40 labeled 2.0 mL cryovials
- Freeze at -70° C immediately

6.17. Plasma and serum collection for Biosample Repository

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 CTAD plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

6.18. Specimen collection for Genetics Repository (BC)

Purpose

- Collection of whole blood from PIVENS 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 research consent or blood draw for patients who have had blood drawn for genetic research as part of the NAFLD Database

Forms

- PIVENS consent for genetic research
- Genetic Consent Documentation (CG) form
- Blood Collection for DNA (BC) form
- NIDDK Genetics Initiative Phlebotomy (GP) form

When

- Visit s2 (or randomization visit or f002)
- 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

Equipment

- Two 10 mL NaEDTA vacutainer tubes (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube labels and form BC 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
  - One roll waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs

Blood collection procedures

- 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 Initiative Phlebotomy form
- Phlebotomist to sign and date the section: To Be Completed by Phlebotomist on the NIDDK Genetics Initiative Phlebotomy 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)
- Tear off one section of absorbent materials along perforates and place it 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 Initiative Phlebotomy 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
- Affix the UN3373 BIOLOGICAL SUBSTANCE, CATEGORY "B" label to the outside cardboard box
- Call Federal Express, 1-800-Go-FEDEX (1-800-463-3339) for courier*
- Notify Dana Witt or Elva Peralta 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=109256371510825007955

- 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".

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 Bloods" 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.

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.

6.19. Study drug dispensing and return (RD)

Forms

- RD - Study Drug and Return form

Drug supply

- Pioglitazone and placebo pioglitazone: 30 mg/tablet, 50 tablets/bottle taken orally once a day (qd) with morning meal. Distributed to patients in a white HDPE plastic bottle
- Vitamin E and placebo vitamin E: 800 IU/soft gel, 50 soft gels/bottle taken orally once a day (qd) with morning meal. Distributed to patients in a white HDPE plastic bottle

Distribution of study drug

- Study drug to be distributed at: Rz, Weeks 4, 8, 16, 24, 32, 48, 64, 72, and 80

Checks on return of study drug

- Unused study drug to be distributed by patient at: Weeks 4, 8, 16, 24, 38, 48, 64, 72, 80 and 96

By whom

- PIVENS 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
- Fax or mail completed Clinical Drug Request form to the DCC:

Handling and disposal

- Unused portions of open bottles in the possession of patients should be considered contaminated and handled accordingly
- Returned tablets and softgels should be counted by the pharmacist or clinic coordinator and the number of tablets/softgels and the number of P series and E series bottles returned, should be recorded on the RD form
- All unused E-series drugs, tablets returned by patients should be disposed per the drug disposal policy of your institution
- All unused P-series drugs, tables returned by patients should be returned to the drug distribution center at McKesson Bioservices

Storage and stability

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

6.20. Adverse event reporting

Definitions

- **Adverse event** is defined as any untoward medical occurrence that may present itself during treatment with a pharmaceutical product or clinical procedure and which may or may not have a causal relationship with the treatment. Adverse events include any unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants. The term "unanticipated problem" includes both new risks and increased rates of anticipated problems.

- **Serious adverse event** is defined as an adverse event occurring at any time during the study that results in death, life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Other events may also be considered a serious adverse event if, based on medical judgement, the event jeopardized the patient to the point of requiring medical or surgical intervention to prevent the occurrence of any of the conditions for a serious adverse event listed above.

- **Unexpected adverse event** is defined as any adverse event with specificity or severity that is not consistent with the risk information in the PIVENS protocol, current study drug investigator brochure, or current study drug package insert.

- **Associated with the use of the drug** means that there is a reasonable possibility that the adverse experience may have been caused by the study drugs.

Reportable PIVENS adverse events

- Adverse events will be recorded on PIVENS data forms whether or not thought to be associated with PIVENS or one of the study drugs.
- Any event threatening the integrity or well-being of the PIVENS trial (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.
- Deciding whether an event is reportable to PIVENS (ie, is in either of these categories) will be the responsibility of the Principal Investigator (PI) of the center. The study chair, the NIDDK project officer, and staff at the Data Coordinating Center are available for consultation.

CTCAE v3.0

- A subset of adverse events are reported on the Serious Adverse Event (AN) form
- 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 Documents and then click on General Documents)
- Use the CTCAE v3.0 to specify the Short Name for the adverse event and the severity grade for the adverse event.

Clinical center responsibilities regarding reportable PIVENS events that occur at your clinical center

- You must notify the Data Coordinating Center about occurrence of events judged reportable to the PIVENS trial as follows: If an event has occurred that you judge is reportable to PIVENS, complete the Serious Adverse Event (AN) form. Key this form to the PIVENS data system. Also send it to the Data Coordinating Center with a narrative description of the event and your subsequent course of action -- describe what happened, the actions taken in response to the event, and the relationship of the event to the PIVENS drugs or procedures. Please refer to the patient by his/her NASH CRN patient ID number and code; do not use the patient's name.

- The Data Coordinating Center should receive copies of all correspondence with a clinic's IRB regarding events judged reportable to the PIVENS trial. Because the PIVENS trial is a multicenter study, events judged reportable to the Database at one clinical center must be reported to the IRB of each participating center.

Data Coordinating Center responsibilities

- The Data Coordinating Center will send a copy of each report received about an event judged reportable to the PIVENS trial to all clinical centers, with instructions for each to forward the report to their IRB. Copies of the report will also be sent to the NIDDK, Chairman's Office, and chair of the NASH CRN Data and Safety Monitoring Board (DSMB).

- The Data Coordinating Center will maintain a list of such events for reporting and review at Steering Committee meetings and DSMB meetings.

Clinical center responsibilities with respect to events that occur at other clinical centers

- When you receive a report from the Data Coordinating Center regarding occurrence of an event reportable to the PIVENS 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 PIVENS requirement.

6.20. Adverse event reporting

Local reporting requirements

- Your institution'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 PIVENS. Regardless of what PIVENS requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than PIVENS', you may report events locally that you do not report to PIVENS.

6.21. Adverse event reporting management

- Few adverse events such as hepatotoxicity, hypoglycemia or hyperglycemia, and ovulation related to PIVENS study drugs are expected. Other potential adverse events are those related to blood draws for the study, such as hematoma, cellulitis, phlebitis, and arterial puncture; or liver biopsy procedures.

- If such an event occurs, appropriate medical care should be provided immediately in the clinic.

- If a suspected adverse event is reported by telephone at the time of the event or later, the participant should be evaluated in the clinic by medical staff (preferable) or referred to an appropriate facility for evaluation and management.

- All such events should be documented in the study chart.

- It is possible that some PIVENS patients will develop significant liver-related morbidity or mortality during the course of followup. While this information is important and should be documented on the Followup Medical History (HI) form, it may also be considered a reportable adverse event according to the local institutional guidelines.

6.22. Serious adverse event reporting (AN)

- Serious adverse events must be reported upon discovery at the clinical center
- Complete and key a Serious Adverse Event (AN) form describing the severity and details of the adverse event
- FAX a memo summarizing the circumstances of the event and the current status of the patient, along with the AN form to the Data Coordinating Center and to the NIDDK project officer within one working day of the discovery of the serious adverse event
- Notify the Data Coordinating Center and the NIDDK project officer of the serious adverse event by telephone or confirmed e-mail within one day
- If the serious adverse event is unexpected AND associated with a study drug, then the NIDDK project officer will notify the FDA and the pharmaceutical manufacturer no later than 15 days from the discovery of the serious adverse event (no later than 7 days if the serious adverse event is fatal or life threatening)
- The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form
- The DSMB will review each serious adverse event report (Form AN) and provide comments to the NIDDK project officer within one week of receipt of the report. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the serious adverse event and recommend any actions to the NIDDK project officer
- The clinical center must submit to the Data Coordinating Center and the NIDDK project officer a follow-up report when the adverse event is resolved or if there has been a significant change in the patient's condition or in the physician's judgment about the event since the previous AN form was filed within one month (and periodic updates if needed) to report the details of the disposition of the serious adverse event

6.23. Procedures for unmasking treatment assignment

- Treatment assignments are unmasked after all data collection for the PIVENS 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), and the investigator will 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.
  - **Development of hepatotoxicity**: Study drug will be discontinued according to the guidelines outlined in the PIVENS protocol. The patient, PCP, and the investigator will be unmasked.
  - **New onset diabetes**: The patient will be referred to their PCP and managed according to the stepped care approach defined in the PIVENS protocol. The patient and PCP may be unmasked to the study medication but the investigator will continue to be masked.
- 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.

6.24. 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

6.25. 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

6.26. Procedures for mortality closeout

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.27. Medical management of patients (standard of care)

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 Standards of Care Documents for adults. These can be found in PIVENS SOP Part V.

6.28. Transferring patients from PIVENS to the NAFLD Database (form CO)

Purpose

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

Form

- Closeout (CO) form

When

- The Closeout form should be completed at the f120 visit (or at the close of the f120 visit window) for all patients randomized in PIVENS.

By whom:

- Clinical coordinator

Instructions

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

- Patients willing to re-enter or join the NAFLD Database should sign the most recent version of the NAFLD Database 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 Database follow-up visit approximately 6 months after the date of their PIVENS f120 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 Database visit that is open 6 months from the date of their PIVENS f120 visit (this visit will be f144 or f192).

- For patients who were not previously enrolled in the NAFLD Database, a new NAFLD Database visit schedule (time windows guide) will be automatically generated when the PIVENS Closeout form (CO) is keyed into the online database. The new visit schedule will use the PIVENS randomization date as the effective date of enrollment into the NAFLD Database. Schedule the participant approximately 6 months from their PIVENS f120 visit for their f144 NAFLD Database follow-up visit.

- For PIVENS participants who decline to participate in the NAFLD Database; inform them that the study results and their treatment assignment will be available to them sometime after the close of the PIVENS 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
Duke University	DUKE
Indiana University	IU
Saint Louis University	SLU
University of California, San Diego	UCSD
University of California, San Francisco	UCSF
University of Washington	UW
Virginia Commonwealth University	VCU

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

Case Western Reserve University	220
Duke University	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
University of Washington	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	1001	-	1999
Duke University	DUKE	2001	-	2999
Indiana University	IU	3001	-	3999
Saint Louis University	SLU	4001	-	4999
University of California, San Diego	UCSD	5001	-	5999
University of California, San Francisco	UCSF	6001	-	6999
University of Washington	UW	7001	-	7999
Virginia Commonwealth University	VCU	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

- 2 to 4 character alpha-numeric code
- Determined by purpose of visit and timing with respect to visit windows
- Visit ID codes

s1	Screening
s2	Screening and baseline data collection
Rz	Randomization
f002	2 weeks follow-up visit (approximately 14 days)
f004	4 weeks follow-up visit (approximately 1 month)
f008	8 weeks follow-up visit (approximately 2 months)
f012	12 weeks follow-up visit (approximately 3 months)
f016	16 weeks follow-up visit (approximately 4 months)
f024	24 weeks follow-up visit (approximately 6 months)
f032	32 weeks follow-up visit (approximately 8 months)
f040	40 weeks follow-up visit (approximately 10 months)
f048	48 weeks follow-up visit (approximately 12 months)
f056	56 weeks follow-up visit (approximately 14 months)
f064	64 weeks follow-up visit (approximately 16 months)
f072	72 weeks follow-up visit (approximately 18 months)
f080	80 weeks follow-up visit (approximately 20 months)
f088	88 weeks follow-up visit (approximately 22 months)
f096	96 weeks follow-up visit (approximately 24 months)
f120	120 weeks follow-up visit (approximately 30 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; use pencil only for Block questionnaires.

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 s1 visit code would be completed and keyed as "s1 ").
- 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, not retroactively. 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 Database 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

- Each page of each form includes headers and footers 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:

PIVENS Patient ID: __ __ __ __

Form RG PIVENS
Revision 0 (17 Feb 04) 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: PIVENS 2

- 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.

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

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 PIVENS should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is enrolled in PIVENS 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 PIVENS documents for the patient – consents, forms, appointment schedule, labels, 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 PIVENS, apply the rounding rule only at the last step, when required to record a quantity on the PIVENS 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
- DEXA (Dual Energy X-ray Absorptiometry) scan 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 PIVENS
- 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 PIVENS 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 PIVENS 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 CC.
  - 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 PIVENS 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, 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.

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 PIVENS centers
- Discrepancy rates over time by clinical center are reported to the Steering Committee

PIVENS SOP IV: Biopsy and Histology Scoring

Contents

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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) a treatment trial of NASH in adult patients and (2) a treatment trial of NAFLD in pediatric patients. This document specifies the procedures for liver biopsy and histology scoring for the PIVENS trial which will compare 3 treatments for adult patients with NASH. Procedures for other NASH CRN studies, including the NAFLD Database and TONIC 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.

Histologically confirmed NASH is an inclusion criterion for the PIVENS 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. PIVENS patients will also have a followup biopsy after 96 weeks of treatment in the trial. Unscheduled biopsies also may occur after screening. Ideally, the PIVENS 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 PIVENS 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.

1.1. Philosophy

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 PIVENS 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 PIVENS 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 6 months prior to screening and the patient must not have used specified (antiNASH, antidiabetic, selected statins, fibrates, antiobesity) medications in the 3 months prior to the biopsy) or it may be done prospectively under the care of the PIVENS investigator as a screening procedure. Each randomized patient will have another biopsy after 96 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 PIVENS 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 3 months 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 PIVENS), then the local PIVENS Study Pathologist must complete the Liver Biopsy Histology Findings (HF) form. If the NASH activity score (NAS) for the screening biopsy is 4, then two additional readings of the biopsy for confirmation that steatohepatitis is present are required; these additional readings are documented on the Steatohepatitis Determination - 1st Reading (HS) and Steatohepatitis Determination- 2nd Reading (HT) forms. Instructions for obtaining the two readings are given on the IP form. Other forms that the PIVENS trial uses to document activities and materials related to liver biopsy are the Central Histology Review (CR) form and logs for shipping frozen tissue and slides (forms SS and TS). In summary, these nine forms (SD, LT, HF, HS, HT, IP, 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)

1.2. Tasks and forms related to liver biopsy

- If the biopsy is the screening biopsy, document lack of use of proscribed medications during the 3 months prior to the biopsy (form SD) and remind the clinical center that the screening biopsy cannot be older than 6 months at the time of randomization
- If liver tissue was obtained for banking, document how that tissue was obtained and frozen (form LT)
- Document local scoring of baseline biopsies (form HF)
- Document central determination of steatohepatitis baseline biopsies for patients with a local NAS score of 4 (forms HS, HT, and IP)
- Document scoring of baseline and followup biopsies by the NASH CRN Pathology Committee (form CR)
- Document shipment of slides to the DCC (form TS)
- Document shipment of flash frozen liver tissue to the Biosample Repository (Form SS)

The PIVENS 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 PIVENS

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 PIVENS has been obtained. In the case of (1), we will try to obtain 10 unstained slides for the exclusive use by PIVENS, 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 PIVENS 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 PIVENS Study Pathologist (to determine eligibility) and also centrally (after randomization), by the Pathology Committee. Central determination of steatohepatitis is also required in some cases (see description later in this SOP). Biopsies obtained 96 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 PIVENS 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 PIVENS 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 biopsy in the 6 months prior to screening and after checking that no proscribed medications were used in the 3 months 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 PIVENS 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 3 months 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 PIVENS is borrowing the stained slides from the institution or if PIVENS 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 PIVENS 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 PIVENS. If the NAS is 4, central confirmation of the finding of steatohepatitis is required for eligibility. If the NAS is 5 or greater, local evaluation of the biopsy is sufficient to establish eligibility for PIVENS.

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 PIVENS. However, the patient may be eligible for the NAFLD Database under criteria that do not require histologic evidence of disease (e.g., the NAFLD Database includes patients with suspected disease as well as those with histologically confirmed disease).

If only the H&E and Masson's trichrome slides are 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 PIVENS.

The PIVENS 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.

2.2. Baseline biopsies performed prior to consent for screening

Slides for patients who are not randomized in PIVENS should be returned 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 PIVENS Study Pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a PIVENS patient so that when the block is initially cut for the local institution's requirements, the PIVENS trial's additional 10 unstained slides can be cut at the same time and the chances of loss of tissue with refacing the block are minimized and so that liver tissue can be flash frozen for banking.

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 or greater gauge needle. If there is adequate tissue beyond 2.0 cm, the extra tissue may be flash frozen 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 PIVENS Study Pathologist for the local evaluation (i.e., for completion of form HF). If necessary, the H&E and trichrome slides may have to be sent for central confirmation of steatohepatitis.

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:

2.4. Preparation of slides

SuperFrost Plus slides, Precleaned

Distributor:	Fisher Scientific
Catalog No.:	#12-550-15
Size:	25/75/1.0 mm
Estimated cost:	$65.96 per gross (144 slides/gross); $660.78 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.

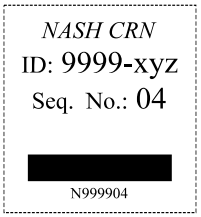
The requirements for the labeling scheme for slides are:

- 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 eye
- 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.

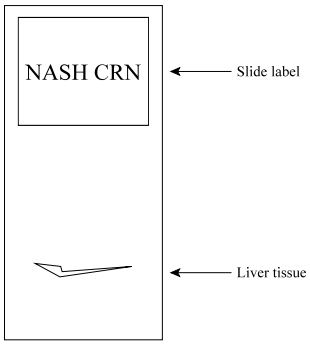
The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the life time of all NASH CRN 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
- Labels for borrowed slides also have “Overlabel” printed on the label

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 flash frozen as follows:

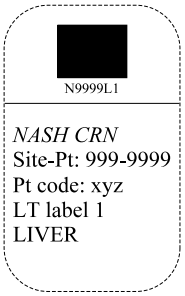
- Place extra liver tissue in a 2.0 mL cryogenic vial which is made of polypropylene and self-standing with externally threaded vials and silicone washers (13.5 mm wide x 48.3 mm tall); they are designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

- 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; the inside of the vial is sterile and should remain so
  - Apply the label to 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 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:



- Within 5 minutes of obtaining, place the liver tissue into the labeled vial; place the capped, labeled vial into the portable Barnstead/Thermolyne Thermo stainless steel Dewar liquid nitrogen flask (supplier: Fisher Scientific, catalog number 11-670-25C, 22.9 cm high; 800-766-7000)
- Complete the Liver Tissue Banking (LT) form
- Complete the Specimen Shipment Log (SS) form
- Ship cryovials to the Biosample Repository on Monday, Tuesday or Wednesday; store temporarily in -70° C freezer or in a liquid nitrogen freezer at the clinical center until the next batch shipment to McKesson.

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.

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 Form HF)

4.1. Introduction

The local site PIVENS Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. PIVENS patients must have histologically confirmed steatohepatitis.

The local site PIVENS 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 Findings (HF) form. 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 PIVENS 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. 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.

4.2.2. 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

4.2.3. 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

4.2.4. 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

4.2.5. Portal chronic inflammation (0-1)

0: None to minimal
1: Greater than minimal

4.2.6. Hepatocellular ballooning (0-2)

0: None
1: Few
2: Many

4.2.7. 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).

4.2.8. Exclusion of other liver disease and other features

- Primary biliary cirrhosis
- Wilson's disease
- Chronic cholestatic liver disease
- Vascular lesions of ALD/B-C/VOD
- Inflammation suggestive of AIH, HCV
- Pigment suggestive of HH
- Globules suggestive of A1AT
- Hepatocellular changes suggestive of HBV
- Granulomas suggestive of sarcoid, PBC, infection
- Evidence of cirrhosis
- Other features
  - Mallory's hyaline (rule out cholate stasis)
  - Perisinusoidal fibrosus away from septa
  - Hepatocyte ballooning
  - Megamitochondria

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.

4.2.9. 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 3 components are summed. The NAS may range from 0 through 8. Patients with a NAS of 0-3 on screening are ineligible for PIVENS. Patients with a NAS of 4 must have the diagnosis of steatohepatitis confirmed by central review prior to randomization in PIVENS. Patients with a NAS of 5 or more at screening are eligible for PIVENS based solely on local scoring of the slides.

If there are no ineligibility items checked on the Liver Biopsy Histology Findings (HF) form for a baseline biopsy and the NAS is 4, then you must arrange for additional readings of the biopsy prior to

randomization (it is okay to send a biopsy with caution items checked for review). Two of the three pathologists must agree that steatohepatitis is definitely present for the patient to be eligible for PIVENS. The steps for obtaining these readings are:

- Complete section A of the Steatohepatitis Determination - 1st Reading (HS) form
- Complete section A of the Steatohepatitis Determination - 2nd Reading (HT) form
- Complete your address and identification on the IP form (Instructions for Obtaining two Additional Pathology Readings)
- Label the slides with the removable overlabels. Put the overlabel on the back of the slide at the top of the slide opposite (underneath) the existing label. This will not de-identify the slide but will identify the slide as belonging to the NASH CRN.
- Alert Dr. Kleiner and Dr. Brunt to the need for readings for NAS=4 and verify that one of them is available to do the 1st reading (email kleinerd@mail.nih.gov or bruntem@slu.edu).
- Assemble these items:
  - Your institution's H&E and Masson's trichrome slides
  - Partially completed HS form (complete section A)
  - Partially completed HT form (complete section A)
  - Envelope marked with the patient's ID no. and code and "Additional reading for NAS=4, PIVENS"
  - Form IP completed with your contact information
- Send all items to Dr. Kleiner or Dr. Brunt by overnight delivery (you may use Federal Express account #2991625081):

David Kleiner, MD
Laboratory of Pathology
National Cancer Institute
Bldg 10, Room 2N212, MSC 1516
Bethesda, MD 20817
301-594-2942

Elizabeth Brunt, MD
4th floor FDT
3635 Vista Boulevard
St. Louis, MO 63110
314-577-8782

Dr. Kleiner will fax the completed HS form to the Clinical Coordinator at the number specified on the IP form and will seal the completed HS form in the envelope provided. He will send the sealed envelope, HT form, and H&E and trichrome slides to Dr. Brunt.

4.2.9. NASH Activity Score (NAS)

Dr. Brunt will complete her evaluation for steatohepatitis and will fax the completed HT form to the Clinical Coordinator at the number specified on the IP form. She will return all materials (sealed envelope from the 1st reader, HT form, and H&E and trichorme slides) to the Clinical Coordinator. Upon receipt, the Clinical Coordinator will complete section C on the HS and HT forms and key the forms.

Plastic boxes capable of holding up to 5 slides may be purchased for these mailings. Specifications are:

5-slide boxes

Distributor:	Fisher Scientific
Catalog No.:	HS15986
Size:	3" x 1" plastic case with flip top
Estimated cost:	$15.32/pack of 25 boxes; $698.80/48 packs (25/boxes/pack)
Tele:	1-800-766-7000

When mailing, wrap the slide box with bubble wrap and place in a padded jiffy bag. Then place inside a Federal Express box. Stuff the box with newspaper or other material to prevent the jiffy bag from sliding around in the box.

4.2.10. 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 96 weeks after randomization) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Form HF will not be completed for unscheduled liver biopsies. 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 3 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 2 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 PIVENS 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
- Other features (Mallory's hyaline, perisinusoidal fibrosis away from septa, hepatocyte ballooning, megamitochondria)

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. Length of biopsy

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

5.3.2. Microvesicular steatosis, contiguous 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 doesn't completely fill the cell. Microsteatosis typically needs ORO for confirmation in routine practice.

5.3.3. Microgranulomas seen (yes/no)

5.3.4. Large lipogranulomas seen (yes/no)

5.3.5. Portal chronic inflammation

0: None
1a: Mild
1b: More than mild

5.3.6. Acidophil bodies

0: Rare/absent
1: Many

5.3.7. Pigmented macrophages (Kupffer cells)

0: Rare/absent
1: Many

5.3.8. Megamitochondria

0: Rare/absent
1: Many

5.3.9. Mallory's hyaline

0: Rare/absent
1: Many

5.3.10. Glyogen nuclei

0: Rare/absent
1: Many

5.3.11. 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

5.3.12. Iron: hepatocellular distribution

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

5.3.13. Nonhepatocellular iron grade (0-2)

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

5.3.14. 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

5.3.15. Steatohepatitis diagnosis

0: No
1a: Suspicions/borderline/indeterminate: Zone 3 pattern
1b: Suspicions/borderline/indeterminate: Zone 1, periportal pattern
2: Yes, definite

5.3.16. 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 (#2991625081)
  - 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 library 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 library 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 1 6. Form and revision

__ 7. Study: **1**=Database; **2**=PIVENS; **3**=TONIC

__ __ / __ __ __ / __ __ 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

__ __ . . . d. Other slide

_____ . . . . . Specify type of stain for other slide

C. Administrative information

__ __ __ 10. CC Initials

_____ 11. CC Signature

__ __ / __ __ __ / __ __ 12. Date form reviewed

__ 13. Tissue adequate: **0**=No ➜ Request original slides from submitting clinic; **1**=Yes

_____ 14. Followup with clinic (*Specify*):

_____ *Patient ID* **D. Histology**

_____ 15. Biopsy length (mm)

H & E stain

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

17. 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. Microgranulomas 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

18. Liver cell injury

_____ . . . a. Ballooning: **0**=None; **1**=Few; **2**=Many

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

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

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

_____ 19. Mallory's hyaline: **0**=Rare/absent; **1**=Many

_____ 20. Glycogen nuclei: **0**=Rare/absent; **1**=Many

Masson's trichrome stain

_____ 21. Fibrosis stage: **0**=None; **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

22. Iron stain

_____ . . . a. Hepatocellular iron grade: **0**=Absent or barely discernible, 40x ➜ **GOTO item 22c**;
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 23**; **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

_____ 23. Is this steatohepatitis? **0**=No; **1a**=Suspicious/borderline/indeterminate: Zone 3 pattern;
1b=Suspicious/borderline/indeterminate: Zone 1, periportal pattern; **2**=Yes, definite

_____ 24. Is cirrhosis present? **0**=No ➜ **GOTO item 27**; **1**=Yes

_____ 25. Is this cryptogenic cirrhosis: **0**=No ➜ **GOTO item 27**; **1**=Yes

26. Features suggestive of steatohepatitis etiology for cryptogenic cirrhosis:

_____ . . . a. Mallory's hyaline (*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: _____

27. Other comments: _____

PIVENS

HF - Liver Biopsy Histology Findings

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

When: Visit s1.

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. Upon completion of this form, the Study Pathologist should give the form to the Clinical Coordinator. If fewer than 2 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 2 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 the patient's NASH Activity Score equals 4, central pathology review is required. Complete form HS - Steatohepatitis Determination and send it with the institution's H & E slide to David Kleiner (instrutions for shipping are on the HS form). If Elig is checked for any item, the patient is not eligible for PIVENS 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 PIVENS and the form should not be keyed.

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: s 1 ____ ____

6. Form & revision: h f 1

7. Study: PIVENS_2

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

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

a. Grade:

< 5% Elig (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) F,Hg

< 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)

13. Hepatocellular ballooning:

None (0) F,Hg

Few (1)

Many (2)

14. Is steatohepatitis present:

No (1) F,Hg

Suspicious/borderline/indeterminate (2)

Yes, definite (3)

D. Exclusion of other liver disease

15. Is there evidence of primary biliary cirrhosis:

Yes (* 1) C No (2)

** Caution: Primary biliary cirrhosis is exclusionary*

16. Is there evidence of Wilson's disease:

Yes (* 1) C No (2)

** Caution: Wilson's disease is exclusionary*

17. 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*

18. 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

** Caution: Autoimmune liver disease and HCV are exclusionary*

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

** Caution: Hemochromatosis or iron overload as defined by 3+ or 4+ stainable iron is exclusionary*

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

** Caution: Alpha-1 antitrypsin deficiency is exclusionary*

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

** Caution: HBV is exclusionary*

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

** Caution: Primary biliary cirrhosis is exclusionary*

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

_____

h. None: (1)

19. Is there evidence of cirrhosis:

Yes (1) No (2)

E. Other features

20. 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. NASH Activity Score

21. NASH activity score (NAS)
(sum of items 10a, 12a, and 13)

___
3-8

(Note: each subscore must be 1 or more)

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

23. Is item 21 (NAS) equal to 4: Yes (* 1) No (2)

** Central pathology review is required. If there are no ineligibility conditions checked on this form (i.e., the patient is deemed eligible pending central pathology determination of steatohepatitis), complete form HS - Steatohepatitis Determination and arrange for central review.*

G. Other comments

24. Other comments:

_____

_____

_____

_____

_____

H. Administrative information

25. Study Pathologist PIN: ___ ___ ___

26. Study Pathologist signature:

_____

27. Clinical Coordinator PIN: ___ ___ ___

28. Clinical Coordinator signature:

_____

29. Date form reviewed:

___ ___–___ ___ ___–___ ___
day mon year

PIVENS

HS - Steatohepatitis Determination - 1st Reading

Purpose: To record results of steatohepatitis determination by 1st Pathologist after the local Pathologist scores an entry biopsy with NAS=4 and checks Suspicious/borderline/indeterminate or Definite steatohepatitis on the HF form.

When: Visit s1.

By whom: Clinical Coordinator and 1st Pathologist.

Instructions: See instruction sheet.

A. Center, patient, and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of visit:

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

5. Visit code: s 1 ____ ____

6. Form & revision: h s 1

7. Study PIVENS_2

B. Steatohepatitis determination

8. Is steatohepatitis present:

No (1)
Suspicious/borderline/indeterminate (2)
Yes, definite (3)

9. 1st Pathologist

a. 1st Pathologist PIN *(use initials if no PIN is available):*

____ ____ ____

b. 1st Pathologist signature:

_____

C. Administrative information

10. Clinical Coordinator PIN: ____ ____ ____

11. Clinical Coordinator signature:

_____

12. Date form reviewed:

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

PIVENS

HT - Steatohepatitis Determination - 2nd Reading

Purpose: To record results of steatohepatitis determination by 2nd Pathologist after the local Pathologist scores an entry biopsy with NAS=4 and checks Suspicious/borderline/indeterminate or Definite steatohepatitis on the HF form.

When: Visit s1.

By whom: Clinical Coordinator and 2nd Pathologist.

Instructions for Clinical Coordinator: See instruction sheet.

A. Center, patient, and visit identification

1. Center ID: ____ ____ ____ ____

2. Patient ID: ____ ____ ____ ____

3. Patient code: ____ ____ ____

4. Date of visit:

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

5. Visit code: s 1 ____ ____

6. Form & revision: h t 1

7. Study: PIVENS 2

B. Steatohepatitis determination

8. Is steatohepatitis present:

No (1)
Suspicious/borderline/indeterminate (2)
Yes, definite (3)

9. 2nd Pathologist

a. 2nd Pathologist PIN *(use initials if no PIN is available):*

____ ____ ____

b. 2nd Pathologist signature:

_____

C. Administrative information

10. Clinical Coordinator PIN: ____ ____ ____

11. Clinical Coordinator signature:

_____

12. Date form reviewed:

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

IP - Instructions for Obtaining Two Additional Pathology Readings when the Local NASH CRN Pathologist Finds NAS=4 for a Patient Screening for PIVENS

Clinical Coordinator (part 1):

If the patient's NAS is equal to 4 (item 23 on the HF form), two of the three pathologists must agree that steatohepatitis is definitely present for the patient to be eligible for PIVENS. The steps for obtaining these readings are:

- Complete section A of the Steatohepatitis Determination - 1st Reading (HS) form
- Complete section A of the Steatohepatitis Determination - 2nd Reading (HT) form
- Complete your address and identification information below:

Name: _____ Clinic address: _____

Email: _____ _____

Telephone no: _____ _____

Fax no: _____ _____

- Alert Dr Kleiner and Dr Brunt to the need for readings for NAS=4 and verify that one of them is available to do the 1st reading (email kleinerd@mail.nih.gov or bruntem@slu.edu).
- Assemble these items:
  - Your institution's H&E and Masson's trichrome slides
  - Partially completed HS form (complete section A)
  - Partially completed HT form (complete section A)
  - Envelope marked with the patient's ID no. and code and "Additional reading for NAS=4, PIVENS"
  - This completed cover sheet
- Send all items to Dr Kleiner or Dr Brunt by overnight delivery (you may use Federal Express account #2991625081):

David Kleiner, MD
Laboratory of Pathology
National Cancer Institute
Bldg 10, Room 2N212, MSC 1516
Bethesda, MD 20817
301-594-2942

Elizabeth Brunt, MD
4th floor FDT
3635 Vista Boulevard
St Louis, MO 63110
314-577-8782

1st Pathologist:

- Complete your evaluation for steatohepatitis and complete section B of Form HS
- Fax the completed HS form to the Clinical Coordinator at the above number
- Seal your completed HS form in the envelope provided
- Send the sealed envelope, HT form and H&E and trichrome slides to the 2nd Pathologist

2nd Pathologist:

- Complete your evaluation for steatohepatitis and complete section B of Form HT
- Fax the completed HT form to the Clinical Coordinator at the above number
- Return all materials (sealed envelope from the 1st reader, HT form, and H&E and trichrome slides) to the clinical coordinator

Clinical Coordinator (on receipt of HS and HT forms):

- Complete section C on the HS and HT forms and key the forms

PIVENS

LT - Liver Tissue Banking

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

When: Visits s1 and f096 and as needed for non-protocol biopsies, 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.

Instructions: Liver biopsy tissue should be obtained by a needle core biopsy (as opposed to a wedge biopsy) using a 16 or greater gauge needle. Whenever more than 2 cm of tissue are obtained during biopsy, place a 1-2 mm segment of liver tissue into a 2.0 mL polypropylene cryovial with preprinted label attached. Flash freeze liver tissue immediately (within 5 minutes following biopsy) by placing labeled cryovial containing liver tissue into a portable liquid nitrogen container. Store the cryovial locally in -70° C (or colder) freezer temporarily and batch ship cryovials on dry ice monthly to the NIDDK Biosample Repository located at McKesson 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 *(s1 or f096):* ____ ____ ____ ____

6. Form & revision: 1 t 1

7. Study: PIVENS 2

B. Liver biopsy

8. Date of biopsy:

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

9. Was the liver tissue obtained using a 16-gauge or greater needle:

Yes (1) No (2)

10. Was liver tissue obtained via a second pass:

Yes (1) No (2)

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

Yes (1) No (2)

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. Flash freeze procedures

13. Was tissue flash frozen within 5 minutes of biopsy by placing in portable liquid nitrogen container:

Yes (1) No (2)

15. ____

14. Explain what was done and why protocol was not followed:

_____

_____

_____

15. Was tissue shipped on dry ice to the Biosample Repository on same day as biopsy:

Yes (1) No (2)

17. ____

16. Describe conditions of local storage prior to shipment to the Biosample Repository *(e.g., temperature, date and time placed in freezer):*

_____

_____

_____

E. Administrative information

17. Clinical Coordinator PIN: ____ ____ ____

18. Clinical Coordinator signature:

_____

19. Date form reviewed:

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

PIVENS

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 that must be returned to the clinical center are recorded.

When: Visits s1, f096, 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: PIVENS_2

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. Date of biopsy specified on the surgical pathology report:

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

C. Requirements for screening biopsy

10. Is this visit s1:

Yes (1) No (2) → **13.**

11. Is the date in item 9 within 6 months (183 days) of the anticipated date of randomization:

Yes (1) No (*2) Elig

** Biopsy date must be within 6 months of randomization.*

12. Were any proscribed medications (antiNASH medications or supplements, antidiabetic medications, antiobesity medications, or nonstable dose of fibrates or statins) used within 3 months of the date of the biopsy:

Yes (*1) Elig No (+2)

** Biopsy must be done when the patient has been free of proscribed medications (antiNASH medications or supplements, antidiabetic medications, and antiobesity medications) for at least 3 months 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 2 unstained slides will be sent to the DCC)

18. Is the institution's H & E stained slide to be sent to the DCC

Yes (1) No (2)

21.

19. Slide sequence number for this slide *(from the NASH CRN label on the slide - use removable overlabels, sequence numbers 81 - 90):*

____ ____ (81-90)

20. Is the H & E stained slide to be returned to the clinical center:

Yes (1) No (2)

21. Is the institution's Masson's trichrome stained slide to be sent to the DCC:

Yes (1) No (2)

24.

22. Slide sequence number for slide *(from the NASH CRN label on the slide - use removable overlabels, sequence numbers 81 - 90):*

____ ____ (81-90)

23. Is the Masson's trichrome slide to be returned to the clinical center:

Yes (1) No (2)

24. Is the institution's iron stained slide to be sent to the DCC:

Yes (1) No (2)

27.

25. Slide sequence number for the iron stained slide *(from the NASH CRN label on the slide - use removable overlabels, sequence numbers 81 - 90):*

____ ____ (81-90)

26. Is the iron stained slide to be returned to the clinical center:

Yes (1) No (2)

27. Is at least one of the stained slides to be returned to the clinical center *(i.e., either item 20 = yes, item 23 = yes, or item 26 = yes):*

Yes (1) No (2)

30. ____

28. 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)

29. Which pathology department did these slides come from:

NASH CRN clinical center's pathology department (1)

30. ____

Other, *(specify):* (2)

_____
name

_____
address

_____
address

_____
address

_____
phone

Note: this is the PIVENS 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

30. Clinical Coordinator PIN: ____ ____ ____

31. Clinical Coordinator signature:

_____

32. Date form reviewed:

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

PIVENS

SS - Specimen Shipment Log

Purpose: To record information about contents of specimen shipment and receipt of specimens at NIDDK Biosample Repository, McKesson 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 responsible for shipping.

Instructions to shipper: Complete one Specimen Shipment Log for every shipment of specimens sent to NIDDK Biosample Repository (McKesson). Clinical Coordinator/laboratory personnel should record Federal Express airbill number at top of page 2, and record date and number of specimens shipped. Place a copy of the Specimen Shipment Log 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 in files. All shipments should be sent via Federal Express priority service (next day, AM delivery). Notify the Biosample Repository of the shipment via fax (301-515-4049) or email (niddkrepository@mckessonbio.com) on the day package is picked up by Federal Express. Include the tracking number in the notification.

Packing instructions:

Check that 1 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 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 envelop 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 a completed Specimen Shipment Log on top of the cooler lid.
Secure fiberboard box with packing tape.

Labeling Shipper:

Place Dry Ice label on side of the box in upper left corner. Record the weight of Dry Ice as 8 kg. Place address label on side of box in lower left corner. Do not cover the printed words, "Diagnostic Specimen."
Use the preprinted Federal Express air bill to ship specimens to McKesson/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 side of box. 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)
McKesson BioServices Corporation
20301 Century Blvd, Bldg 6, Suite 400
Germantown, MD 20874
Telephone: (240) 686-4703

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

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: PIVENS _2_

B. Clinic administrative information

8. Print information of person preparing shipment:

_____
name

_____
telephone number

_____
Email address

C. Specimen shipment information

Record specified information about plasma, serum and liver tissue cryovials shipped in items 9 thru 16. Indicate number of cryogenic vials containing plasma, serum, and liver tissue. McKesson will fill in the Comments section (column h).

	a. Site ID - Patient ID	b. Patient code	c. Visit code	d. Date specimens separated (day-month-year)	e. # of plasma aliquots	f. # of serum aliquots	g. # of liver tissue cryovials	h. Comments (McKesson use only)
9.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
10.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
11.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
12.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
13.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
14.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
15.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				
16.	__ __ __ - __ __ __ __	__ __ __	__ __ __ __	__ __ - __ __ __ - __ __				

D. Comments (to be completed by clinical center)

E. Comments (to be completed by Biosample Repository)

PIVENS

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) on Monday through Wednesday only.
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
- 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: PIVENS 2

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)*:

_____

_____

_____

Center ID: __ __ __ __

B. Slide shipment information

Record specified information about slides shipped in items 9 through 25. Indicate number of slides that are stained or unstained. Personnel receiving the shipment will fill in the Receipt code (column h) 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.** Visit code	**d.** Date of biopsy (day-month-year)	**e.** surgical pathology report (y/n)	**f.** # of stained slides	**g.** # of unstained slides	**h.** 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

Pioglitazone versus Vitamin E versus Placebo
for the Treatment of Nondiabetic Patients with
Nonalcoholic Steatohepatitis: a Multicenter,
Randomized Double Masked, Placebo-Controlled Trial

(PIVENS)

Standard Operating Procedures

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

5 November 2004

Contents

Standards of Care for Adult Patients with Fatty Liver Disorders

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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 Standards 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. 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.

Standards of care are based on the best available information at the time they are developed. Because the understanding of fatty liver disease is rapidly evolving, the Committee anticipates the need to review and revise the guidelines on an annual basis. In July and August each year, the Standards of Care Committee chairperson shall solicit recommendations for revisions from the Committee members and from the Steering Committee members. A revised document shall be presented to the Steering Committee for approval before October 1 of each year. Following approval, the necessary materials for implementation will be prepared by the Data Coordinating Center (DCC) and the new document will go into effect on January 1.

2. Specific recomendations

2.1 Dietary intate

a. Patients without diabetes will be instructed to follow NCEP Step 1 recommendations (Appendix 1). These recommendations will include specific discussions on total caloric intake, the amount and type of fat consumed, the amount of carbohydrate consumed.
b. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the ADA (Appendix 2).
c. Recommendations regarding the use of specific nutritional supplements are addressed below.

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. Type 2 diabetes
    i. Some patients will not have a pre-existing diagnosis of type 2 diabetes but meet diagnostic criteria as a result of testing performed for NASH CRN studies. These patients will be referred to their primary physicians for appropriate management. The use of an insulin-sensitizing agent will be suggested as initial therapy instead of a sulfonylurea or insulin if the primary physician elects to begin pharmacologic therapy.
    ii. Patients with controlled diabetes (Hgb $A_{1C} < 7\%$) will be continued on their current treatment regimens.
    iii. Patients with suboptimally controlled diabetes (Hgb $A_{1C} \geq 7\%$) will receive a recommendation for followup with their primary physician for improved glycemic control.

b. Hypertriglyceridemia
    i. 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
    i. 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
    i. 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)
    i. 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
    i. 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 OSHA regulations.

2.7 Possibly helpful concomitant medication use

a. 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 NAFL such as ALT or imaging studies.

b. Metformin
   i. Patient receiving metformin as a treatment for diabetes may remain on the drug.
   ii. Patients treated with metformin for a diagnosis of NAFL 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.

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

d. Statins
   i. Statins used to treat hypercholestereolemia may be continued with dose escalations as clinically indicated.

e. 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) should be discouraged, but their use will be at the discretion of physicians.

2.8 Possibly harmful concomitant medication use

a. Acetaminophen
   i. Acetaminophen should be restricted to < 3 grams in any given day.
   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
   i. 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 (OCP, HRT)
   i. Estrogen use as oral contraception and hormone replacement therapy will not be discouraged.

d. Amiodarone
   i. Amiodarone can be continued for life-threatening arrythmias.

ii. The continued use of amiodarone for non-lifethreatening 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 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. Vitamin E. Patients will be allowed vitamin E in doses not to exceed 800 IU daily.
b. 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.)
c. Betaine use will neither be recommended nor discouraged.
d. S-adenosylmethionine use will neither be recommended nor discouraged.
e. Herbal supplements
   i. Milk thistle use will neither be recommended nor discouraged.

2.10 Posibily harmful concomitant dietary supplement use

a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5000 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 implement these standards of care immediately in the patients followed at all study sites. For new patients without a biopsy-established diagnosis of NAFL, the standards may be implemented at the discretion of the hepatologist depending on the index of suspicion

of NASH. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. Once a liver biopsy establishes a diagnosis of NASH, the standards should be implemented. 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 develop 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 twice yearly 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. Perhaps a pocket card and a small poster for patient care areas are needed

4.2 Patient brochures

a. What brochures are needed
    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. MVI daily

5. Warnings about herbal remedies
6. Symptoms to report
    a. Angina
    b. Sleep apnea
    c. Irregular menstruation, facial hair

b. Brochure development
    i. Content: Standards of Care Committee
    ii. Design: Need a professional, aesthetically pleasing design
    iii. Printing: Local centers to arrange for printing, distribution, cost recovery
c. Updates to the brochures
    i. Content to be reviewed annually and discussed at Steering Committee meetings
    ii. Revised content and design to be prepared within 4 weeks of review at Steering Committee. Revisions to be distributed to the Steering Committee members for final approval.

4.3 Referring physician information

a. 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.

4.4 NASH CRN website

A public website will be considered in the future, but will not be implemented during the first year of the trial.

5. Appendices

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 (NHLBI, 2001). 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: ADA diet (for patients with type 2 diabetes)

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

Nutritional Principles & Recommendations in Diabetes
American Diabetes Association
Diabetes Care 2004; 27:S36-S46
(Summary of A-level evidence for NASH CRN Appendix)

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

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

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.
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 FDA is currently considering ways to educate the public on the dangers of acetaminophen in combination medications. See their talking points for a meeting held September 19, 2002:
http://www.fda.gov/ohrms/dockets/ac/02/questions/3882Q1_Discussion%20Points%20Final.doc

The best approach to this issue will be to inquire about over-the-counter medication use as part of the medical history. If the FDA or other agencies develop written teaching materials regarding the use of acetaminophen containing products, these will be reviewed by the Standards of Care committee for possible inclusion in subsequent versions.