Baseline Parameters in Clinical Trials for Nonalcoholic Steatohepatitis: Recommendations From the Liver Forum

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease in the world, affecting an estimated 25% of the global adult population.[1] Liver-related morbidity and mortality attributed to NAFLD are substantial, and fibrosis seems to be the strongest independent predictor of outcome.[2] Fibrosis develops among patients with the nonalcoholic steatohepatitis (NASH) phenotype, making it the biologically relevant focus of drug development.[3] Currently, there are no approved therapies to treat NASH, although many drugs are in development. Multiple challenges exist in drug development for NASH, including the inconsistent measurement of baseline parameters, which makes interpretation and comparison of trial data difficult. As drug development proceeds, there is a need to standardize data collected as well as aspects of study design (eg, stratification factors) to make datasets comparable and assist the regulatory agencies' efforts to determine efficacy and safety.

To support efforts in NASH drug development, the Liver Forum first convened after the 2013 American Association for the Study of Liver Diseases and US Food and Drug Administration co-sponsored conference on clinical trial designs and endpoints in NASH.[4] The Liver Forum is an independent collaborative drug development and regulatory science project focused on diagnostics and treatments for NASH based on the established model of the Forum for Collaborative HIV Research.[5] For this particular effort, the Liver Forum invited experts from stakeholder groups in academic medicine, regulatory agencies, the pharmaceutical and medical diagnostics industries, and patient advocacy organizations to develop consensus recommendations for standardized baseline parameters for NASH-related clinical trials.

Methods

A working group of the Liver Forum assessed the state of the science in clinical trials for NASH and made specific recommendations for the categories of data to include in eligibility determinations and baseline assessments. As a first step, we reviewed recent and current placebo-controlled randomized clinical studies registered at clinicaltrials.gov for general patterns of study entry criteria and baseline data collection. For the purposes of this report, we defined broad categories of parameters to recommend for baseline data collection, including demographics and genetics; diet and activity including alcohol, tobacco, and substance use; concomitant medications; laboratory tests; and histology. Further recommendations regarding comorbidities and surgical history, anthropometrics, specialized biomarkers, imaging and other noninvasive diagnostics, and quality of life are available in the Supplementary Materials. For each category, specific variables were assessed for their relevancy with regard to therapeutic goal (ie, whether the drug target is liver fibrosis versus steatohepatitis), phase of trial, and whether the measure is essential, ideal, or should be considered. We further developed consensus strategy for stratified randomization for use in NASH-related trials (Supplementary Materials).

Results

Demographics and Genetics

Age, sex, and ethnicity are known risk modifiers for NAFLD and NASH (Supplementary Table 1). These factors are essential to capture as baseline parameters regardless of trial phase or mechanism of action. Epidemiologic studies suggest that NAFLD is more prevalent in males compared with females, which may be owing to different factors including insulin resistance, visceral adiposity, lifestyle, and sex hormones.[6] Age is a risk factor for NAFLD fibrosis progression and of comorbid conditions such as cardiovascular disease.[7] Aging effects on the liver include decreased volume, blood flow, and mitochondrial dysfunction. Race and ethnicity, often surrogates for unknown genetic polymorphisms, are considered important parameters to capture for proof-of-concept (POC) and phase II trials, and are recommended as essential components for phase III trials. These are typically self-reported given the current lack of better tools to characterize the underlying genetic factors that might contribute to NASH pathogenesis. The prevalence of NAFLD has been shown to vary by race and ethnicity, which is not fully explained by lifestyle or metabolic risk factors.[8]

Genetic polymorphisms in genes including *PNPLA3, TM6SF2*, and *GCKR* have been robustly associated with liver disease severity and/or cardiovascular risk in NAFLD.[9] These variants have specific ethnic distributions, with *PNPLA3* accounting for $\leq$72% of interethnic variation in hepatic triglyceride content in the Dallas Heart Study.[10] DNA (venous blood or extracted from tissue) should be collected and the informed consent process should include the ability to genotype these candidate genes as well as "genome-wide" analyses for future analysis. This is particularly important for phase III trials given their larger size. Genetic testing would also be useful for identifying genes that may predict risk of drug-induced liver injury for study drugs. Furthermore, determining the presence of genetic polymorphisms associated with NASH in trial patients will be essential for evaluating the impact of these polymorphisms on treatment response. Current candidates include *PNPLA3* I148M and *TM6SF2* E167K.[11]

Diet and Lifestyle

The appropriate standard of care for dietary and activity counseling should be provided to NAFLD patients before enrollment in clinical trials, not only because research ethics require that all patients receive standard-of-care treatment, but also to normalize

the potential impact of lower caloric intake and increased activity on weight and NAFLD histologic severity (Supplementary Table 2). We recommend counseling regarding diet and activity take place 4 to 6 weeks before the baseline visit before initiation of the treatment phase. For practical purposes, the time period between the screening visit and baseline visit would be suitable as a "run-in period" to provide healthy lifestyle recommendations and minimize the possible confounding influence of dietary and activity changes. Standardization will be of particular importance in POC trials with changes in liver fat and eventually changes in alanine aminotransferase levels as primary or key secondary endpoints. Specific dietary recommendations exist; for example, the NASH Clinical Research Network (CRN) has recommended the National Cholesterol Education Program Step-1 diet for nondiabetics and the American Diabetes Association diet for diabetics for standard of care in NASH subjects. Similar healthy diet and increased exercise counseling are available in different countries and should be provided to participants in trials outside of the United States. Reinforcement of diet and healthy lifestyle habits should occur at visits throughout studies. Attempts should also be made to capture changes in diet and physical activity habits.

The Alcohol Use Disorders Identification Test (AUDIT-C) is a screening tool developed by the World Health Organization and validated in 6 countries that can be used to exclude potential participants with heavy alcohol consumption and monitor the effect of modest alcohol intake on NAFLD during the course of a trial, as done previously by the NASH CRN.[12] This tool should be used at baseline and throughout the trial. To supplement the AUDIT-C, biochemistry markers such as phosphatidylethanol should be considered for confirming alcohol exposure. Further, the Lifetime Drinking History questionnaire or a similar interview protocol should be considered to assess lifetime alcohol consumption.[13]

Tobacco use, such as smoking, should be considered to collect as a baseline parameter. Although clinical data are limited, tobacco has been associated with accelerated NAFLD pathogenesis.[14] Further, marijuana use should also be assessed given evidence of cannabinoid modulation of liver steatosis and evidence suggesting acceleration of fibrosis and steatosis in chronic liver disease, such as hepatitis C.[15] In that same light, over-the-counter medications and herbal supplements that may interfere with drug metabolism or impact liver function should also be captured.

Concomitant Medications

Concomitant medications such as statins, vitamin E, as well as medications used to treat diabetes or hypertension are essential to capture regardless of phase of trial or medication mechanism owing to potential confounding influence on outcomes (Supplementary Table 3). Such medications may have anti-NASH effects directly or indirectly by targeting elements of the metabolic syndrome. Therefore, it is important to have stable doses of such medications for $\geq$3 months before enrollment. Dose of medication and any dose changes during the study should be captured. Further, medications that may induce steatosis such as corticosteroids, amiodarone, and methotrexate should also be captured regardless of trial phase. Most of these medications lead to exclusion or may need to be considered for stratification.

Laboratory Tests

To the extent possible, absolute values should be used in reporting the results of laboratory tests instead of the use of threshold indicators or values relative to laboratory reference ranges (Tables 1 and 2). The metabolic panel should include fasting glucose, fasting insulin, and hemoglobin A1c regardless of trial phase or drug mechanism of action. Given the increased risk of the NASH phenotype among NAFLD patients with the metabolic syndrome, capturing these component elements in clinical trials is crucial, because their dynamics may be tied to drug action. The Homeostasis Model Assessment can be considered as a marker of insulin resistance in NAFLD. The glucose clamp technique to quantify insulin secretion and resistance is impractical when considering implementation across larger trials, but could be considered in early POC trials. Further, the oral glucose tolerance test minimal model may be considered in early POC trials because it provides a quantitative description of β-cell function and insulin sensitivity.[16]

Table 1. Metabolic Panel

	Early Phase Trials	Late Phase Trials
Fasting glucose	E	E
Fasting insulin	E	E
Hemoglobin A1c	E	E
HOMA	C	C
Glucose clamp	C	NR
OGTT	C	C

C, consider; E, essential; HOMA, homeostasis model assessment; NR, not recommended; OGTT, oral glucose tolerance test.

Table 2. Laboratory Tests

	Early Phase Trials	Late Phase Trials
ALT	E	E
AST	E	E
ALP	E	E
Total bilirubin	E	E
Direct bilirubin	E	E
GGT	E	E
Albumin	E	E
Prothrombin time or INR	E	E
Total cholesterol	E	E
LDL-C	E	E
HDL-C	E	E
TSH	E	E
Triglycerides	E	E
Hemoglobin	E	E
Hematocrit	E	E
Platelets	E	E
BUN	E	E
Creatinine	E	E
Urine microalbumin	C	C
Ferritin	I	I

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C, consider; E, essential; GGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; I, ideal; INR, International Normalized Ratio; LDL-C, low-density lipoprotein cholesterol; TSH, thyroid-stimulating hormone.

Liver-related laboratory tests such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin, γ-glutamyl transpeptidase, albumin, and prothrombin time or International Normalized Ratio should be captured regardless of phase of trial or drug mechanism. At least 2 sets of laboratory tests should be captured several weeks apart to ensure stability before trial entry. If laboratory results show evidence of change, then a third set should be obtained to ensure suitability for trial entry. This is further important to track drug tolerability after a trial begins. Furthermore, dynamic changes to these values may occur owing to activity of the study drug or metabolites, and relative change may be used in the analysis of endpoints. Fasting lipids should also be measured at baseline, because these values may change owing to effect of a study drug. Hematologic tests are also important to capture to track potential drug-related adverse effects and to ensure suitability for inclusion. Further chemistries such as blood urea nitrogen and creatinine should also be obtained given potential influence of drug clearance. For trials enrolling cirrhotic patients, Model for End-stage Liver Disease-Na score and Child-Pugh score should be obtained.

Histology

Liver histology is ideally captured for POC trials and is essential for later phase trials as the standard benchmark (Supplementary Table 4). We recommend all liver biopsy specimens be reviewed and scored according to the NASH CRN system with the overall histopathologic interpretation additionally documented.[17] This scoring system has demonstrated good interrater reproducibility and specifies the degree of steatosis, lobular inflammation, and hepatocellular ballooning needed to calculate the NAFLD activity score and separately evaluates fibrosis stage. Major landmark trials in NASH have used this scoring system for histologic assessment, which allows for comparative analyses. A more recent evolution of this scoring system, the steatosis, activity, and fibrosis score, is semiquantitative.[18] This scoring system also has good interrater reproducibility and separates steatosis from necroinflammation ('activity'), 2 features that potentially have distinct prognostic implications. This distinction may assist in detection of therapeutic benefit where a treatment does not alter hepatic triglyceride accumulation. Because the 2 systems share much in common, it is advised that changes in the steatosis, activity, and fibrosis 'activity' domain be included as a secondary endpoint in future trials.

Further recommendations regarding comorbidities and surgical history, anthropometrics, specialized biomarkers, and quality of life are available in the Supplement Materials.

Discussion

NASH is recognized as a major cause of chronic liver disease leading to cirrhosis, liver transplantation, and hepatocellular carcinoma. Evaluating potential therapies that lead to NASH resolution or prevent its progression is now the focus of multiple, ongoing clinical trials. The ability to compare the results of clinical trials for new therapeutics for NASH depends on the collection of a standardized set of essential parameters at baseline. This paper recommends an essential set of parameters that would allow patient cohorts recruited into different trials to be compared. We fully anticipate that these baseline parameters will continue to evolve over time as new knowledge accumulates with the completion of trials and the identification of new genetic and environment factors that contribute to the pathogenesis of NASH, liver fibrosis, and hepatocellular carcinoma.

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2017.07.024.

References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of non-alcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence and outcomes. Hepatology 2016;64:73–84.
2. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015;149:389–397.e10.
3. McPherson S, Hardy T, Henderson E, et al. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015;62:1148–1155.
4. Sanyal AJ, Friedman SL, McCullough AJ, et al. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–1405.
5. Miller V. The forum for collaborative HIV research: a model for an integrated and inclusive approach to clinical research and drug development. Clin Pharmacol Ther 2009;86:332–335.
6. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Epidemiol 2013;178:38–45.
7. Frith J, Day CP, Henderson E, et al. Non-alcoholic fatty liver disease in older people. Gerontology 2009;55:607–613.
8. Schneider AL, Lazo M, Selvin E, et al. Racial differences in nonalcoholic fatty liver disease in the U.S. population. Obesity (Silver Spring) 2014;22:292–299.
9. Anstee QM, Seth D, Day CP. Genetic factors that affect risk of alcoholic and nonalcoholic fatty liver disease. Gastroenterology 2016;150:1728–1744.e7.
10. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–1465.
11. Brunt EM, Wong VW, Nobili V, et al. Nonalcoholic fatty liver disease. Nat Rev Dis Primers 2015;1:15080.
12. Dunn W, Sanyal AJ, Brunt EM, et al. Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). J Hepatol 2012;57:384–391.
13. Friesema IH, Veenstra MY, Zwietering PJ, et al. Measurement of lifetime alcohol intake: utility of a self-administered questionnaire. Am J Epidemiol 2004;159:809–817.
14. Liu Y, Dai M, Bi Y, et al. Active smoking, passive smoking, and risk of nonalcoholic fatty liver disease (NAFLD): a population-based study in China. J Epidemiol 2013;23:115–121.
15. Purohit V, Rapaka R, Shurtleff D. Role of cannabinoids in the development of fatty liver (steatosis). AAPS j 2010;12:233–237.
16. Breda E, Cavaghan MK, Toffolo G, et al. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. Diabetes 2001;50:150–158.
17. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
18. Bedossa P. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575.
19. Fromenty B. Drug-induced liver injury in obesity. J Hepatol 2013;58:824–886.
20. Blonsky JJ, Harrison SA. Review article: nonalcoholic fatty liver disease and hepatitis C virus–partners in crime. Aliment Pharmacol Ther 2008;27:855–865.
21. Bower G, Toma T, Harling L, et al. Bariatric surgery and non-alcoholic fatty liver disease: a systematic review of liver biochemistry and histology. Obes Surg 2015;25:2280–2289.
22. Obesity. preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:i–xii. 1–253.
23. Siddiqui SHA, Abdelmalek MF, Anstee Q, et al. Case definitions for inclusion and analysis of endpoints in clinical trials for NASH through the lens of regulatory science. Hepatology (Under Review).
24. David K, Kowdley KV, Unalp A, et al. Quality of life in adults with nonalcoholic fatty liver disease: baseline data from the nonalcoholic steatohepatitis clinical research network. Hepatology 2009;49:1904–1912.
25. Cella D, Riley W, Stone A, et al. The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks:

2005-2008. J Clin Epidemiol 2010; 63:1179–1194.

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Supplementary Material

Comorbidities and Surgical History

The following comorbidities are essential to capture across all phases of trials and drug mechanisms: impaired glucose tolerance, type 2 diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, cardiovascular disease, chronic renal disease, obstructive sleep apnea, hypothyroidism, and autoimmune disease (Supplementary Table 5). These comorbidities are often found in nonalcoholic fatty liver disease (NAFLD) patients and are important to consider with regard to drug clearance, tolerability, and safety. Hypertension has been associated with fibrosis progression. Similarly, hypothyroidism is common in NAFLD, and treated and untreated status should be captured because the effects of thyroid hormone on metabolism and thermoregulation may impact disease severity and treatment response. Patients with untreated or uncontrolled hypothyroidism should not be eligible for trials because initiating thyroid replacement may confound results. In addition, chronic kidney disease is essential to capture owing to renal function effects on drug clearance and metabolism. Furthermore, some drugs may be more useful in certain subpopulations of patients with nonalcoholic steatohepatitis (NASH), such as those with type 2 diabetes mellitus or comorbid cardiovascular disease. Some proposed drugs may improve or eliminate types of comorbid disease, such as weight loss potentially benefiting patients with abdominal obesity and obstructive sleep apnea.

Given the influence of other liver conditions on NAFLD-related outcomes, these parameters should be recorded at the onset. Capturing history of drug-induced liver injury and gastrointestinal disease and surgery is essential because these conditions may influence the ongoing pathophysiology of NAFLD. Drug-induced liver injury may aggravate preexisting NAFLD and conversely NAFLD patients are at increased risk to develop drug-induced liver injury.[19] Hepatitis C virus (HCV) infection has been associated with increased disease progression in NAFLD.[20] NAFLD patients with a history of HCV exposure and spontaneously cleared HCV infection may be considered for eligibility. Although active HCV infection would be an exclusion criterion for NAFLD studies, patients with NAFLD diagnosed after successfully treated infection may be considered as a subgroup for exploration based on trial criteria specifying an adequate time duration after documented clearance. Other types of ongoing chronic liver disease would result in exclusion from NAFLD clinical trials and would be identified by history obtained during the screening process or may be uncovered at baseline biopsy. Gastrointestinal disease and surgical history such as cholelithiasis, cholecystectomy, and bariatric surgery would also be important to document because they may impact outcomes. For example, bariatric surgery not only results in rapid sustained weight loss, but has also been associated with significant improvement in NAFLD histologic severity.[21] As such, bariatric surgery should be an exclusion criterion for trial enrollment, although failed gastric banding patients may be considered eligible. Patients that have failed endoscopic bariatric treatment, such as with an intragastric balloon, may also be considered eligible.

Anthropometrics

Anthropometric factors such as weight,* height, body mass index, waist circumference,† and blood pressure are essential to capture for all trials (all phases and all interventions; Supplementary Table 6). Given the increased risk of the NASH phenotype among NAFLD patients with the metabolic syndrome, capturing these component elements in clinical trials is crucial, because their dynamics may be tied to drug action. For example, a particular drug may work by improving insulin resistance, and therefore these parameters may identify a particular type of comorbid NAFLD patient more likely to benefit. The relationship between weight trends, recency, and duration of obesity and NAFLD fibrosis severity and treatment response is not known. An attempt to gather information may help to establish the importance of history of obesity in treatment outcome. Other circumferential measurements to consider include neck, chest, hip, and midthigh.

Specialized Biomarkers

Further validation is required before specific biomarkers can be recommended for use in standardized baseline assessment in NASH clinical trials. At this time, the choice of biomarkers for a given trial should be considered primarily by the mechanism of action of the investigational drug and to optimize companion diagnostics development. A schematic representation of NAFLD disease processes with associated biomarkers is noted in Supplementary Figure 1. It will be incumbent on sponsors to collect further biomarker data to confirm mechanisms of action and/or for pharmacokinetic and pharmacodynamic modeling purposes. Measuring these biomarkers at baseline would allow for evaluation of anti-inflammatory or antifibrotic activity for the given drug by examining response. Furthermore, such biomarkers may show promise for clinical usefulness in measuring drug effect for future studies.

Imaging and Other Noninvasive Diagnostics

The application of imaging technologies to NAFLD is a rapidly evolving field. Some modalities are informative for fat content, some for fibrosis; as of now, none are validated for assessing the degree of inflammation (Supplementary Table 7). Supplementary Table 8 lists these modalities with their potential applications. Evaluation of their strengths and weaknesses are included in a separate manuscript.[23]

The Liver Forum will continue to follow the evolution of all these tests in the evaluation of NASH and its treatment response, and develop recommendations as the science evolves. Inclusion of novel testing modalities in

future trials may help to facilitate the evaluation of these tests in a systematic manner.

Health-Related Quality of Life

No disease-specific quality-of-life instruments have been validated for regulatory purposes for NAFLD (Supplementary Table 8). Existing instruments such as the Short Form 36 and Patient-reported Outcomes Measurement Information System are available and recommended for use in phase III trials, and should be considered for earlier phases. The Short Form 36, which is a well-validated 36-item self-report measure, has been used by the NASH CRN to evaluate quality of life in NAFLD.[24] The Patient-reported Outcomes Measurement Information System instrument accurately measures physical, mental, and social parameters and is rigorously validated across a wide range of chronic illnesses.[25] Given the prevalence of fatigue in NAFLD patients and impact on quality of life, this instrument may be well-suited for use in this chronic disease population. Standardization of NAFLD-specific quality-of-life measures through the use of these instruments would allow for assessment of change in quality of life through the course of a trial and allow for comparability across trials. Further development is needed to develop valid disease-specific instruments.

Stratified Randomization

A stratified randomization strategy in NASH clinical trials would help to reduce the impact of key confounders in assessing outcomes of trial drugs. Such a strategy may protect against type I error, improve power in small trials, and facilitate subgroup and interim analyses. A guiding principle is to have balanced distribution of any stratification factor in each study arm, which becomes cumbersome as stratification schemes become more complex. Therefore, we advocate for a limited stratification strategy aided by standardization of baseline parameter data. Furthermore, the number of strata should consider the total number of patients enrolled. For marketing authorization trials typically enrolling >2000 patients, 3 dichotomized stratification variables should be considered: liver fibrosis stage (stage 2 vs stage 3 or stage 1 vs stage 2 and 3 for those trials enrolling stage 1), vitamin E supplementation ≥400 IU (yes or no), and diabetes mellitus (ye or no). Baseline body mass index would importantly be a necessary covariate. Weight loss is also important to evaluate as a covariate given potential confounding effects on outcomes. As such, we recommend subgroup analyses evaluating change in NAFLD histologic outcomes, namely, NASH resolution and/or improvement in fibrosis, by quantified weight loss.

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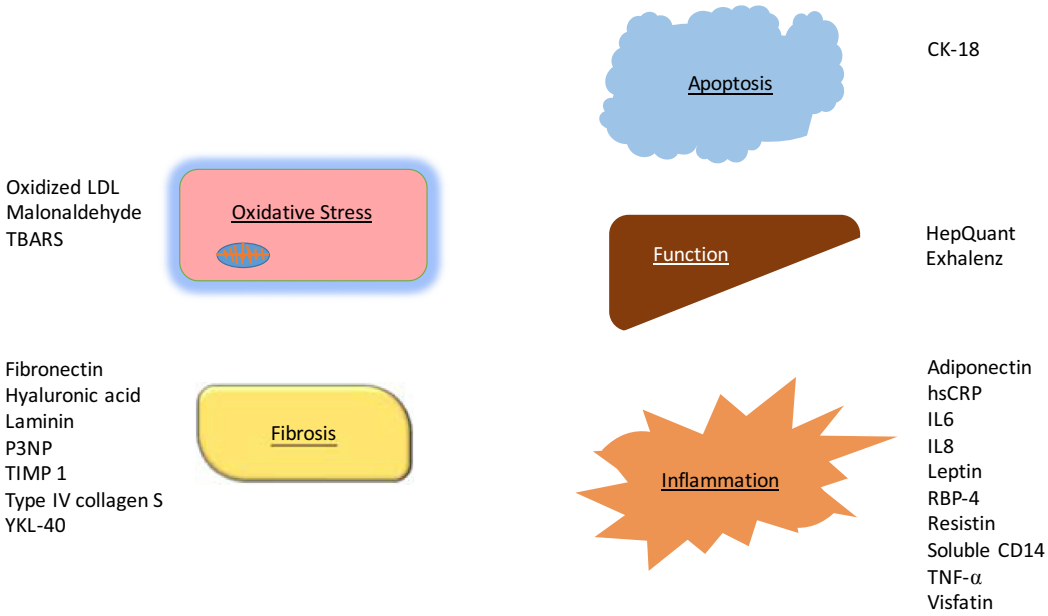
* **Weight should be measured in a standardized fashion on a calibrated scale with bare feet close together and a single layer of clothing.**

† **Waist circumference should be standardized as a measurement midway between the uppermost border of the iliac crest and the lower border of the costal margin. The tape should be placed around the abdomen at the level of this midway point and a reading taken when the tape is snug but does not compress the skin.[22]**



Supplementary Figure 1. Specialized biomarkers grouped by nonalcoholic fatty liver disease activity domain. hsCRP, high-sensitivity C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α.

Supplementary Table 1. Demographics and Genetics

	Early Phase Trials	Late Phase Trials
Age	E	E
Sex	E	E
Menopausal status (women)	E	E
Race	I	E
Ethnicity	I	E
Consent and DNA banking for future analysis	I	E
Candidate gene genotyping (eg, PNPLA3)	I	E
Genome-wide genetic analysis	C	I

C, consider; E, essential; I, ideal.

Supplementary Table 2. Diet and Lifestyle

	Early Phase Trials	Late Phase Trials
Dietary and activity counseling	E	E
Short-term alcohol consumption (AUDIT-C)	E	E
Lifetime alcohol consumption (LDH-q)	C	C
Alcohol exposure (PEth)	C	C
Caffeine intake	E	E
Tobacco use	C	C
Marijuana use	E	E
Over-the-counter medications and herbal supplements	E	E

AUDIT-C, Alcohol Use Disorders Identification Test; C, consider; E, essential; I, ideal; LDH-q, lactate dehydrogenase release; PEth, phosphatidylethanol.

Supplementary Table 3. Concomitant Medications[a]

	Early Phase Trials	Late Phase Trials
Cholesterol medications (statins, fibrates, niacin, fish oil)	E	E
Vitamin E	E	E
Diabetes medications (insulin, metformin, DPP-4 inhibitors, GLP-1 agonists, meglitinides, SGLT2 inhibitors, sulfonylureas, thiazolidinediones)	E	E
Hypertension medications (ACEIs, ARBs, CCBs, beta blockers, alpha blockers, diuretics)	E	E
Corticosteroids	E	E
Amiodarone	E	E
Tamoxifen	E	E
Methotrexate	E	E
Ursodeoxycholic acid	E	E
Anti-depressants (MAOIs, SSRIs, SNRIs, TCAs)	E	E
Oral contraceptives	E	E
Thyroid supplements	E	E

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; DPP-4, dipeptidyl peptidase-4; E, essential; GLP-1, glucagon-like peptide-1; MAOI, monoamine oxidase inhibitor; SGLT2, sodium glucose cotransporter-2; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.
[a]Some of these comedications are exclusionary, and require stability and/or stratification.

Supplementary Table 4. Liver Histology

	Early Phase Trials/Proof Of Concept	Early Phase Trials/Dose Ranging Studies	Late Phase Trials
Liver Histology	I	E	E

E, essential; I, ideal.

Supplementary Table 5. Comorbidities and Surgical History[a]

	Early Phase Trials	Late Phase Trials
IFG or IGT	E	E
Type 2 diabetes mellitus	E	E
Hypothyroidism	E	E
Depression	E	E
Hypertension	E	E
Hypercholesterolemia	E	E
Hypertriglyceridemia	E	E
Cardiovascular disease	E	E
Obstructive sleep apnea	E	E
Chronic renal disease	E	E
Autoimmune disease	E	E
History of drug-induced liver injury	E	E
History of bariatric surgery	E	E
History of gastrointestinal disease and surgery	E	E

E, essential; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.
[a]Some of these comorbidities are exclusionary.

Supplementary Table 6. Anthropometrics and Blood Pressure

	Early Phase Trials	Late Phase Trials
Weight	E	E
Height	E	E
BMI	E	E
Waist circumference	E	E
Neck, chest, hips, mid-thigh circumference	C	C
Blood pressure	E	E

BMI, body mass index; C, consider; E, essential.

Supplementary Table 7. Imaging and Other Noninvasive Diagnostics

	Application	Early Phase Trials	Late Phase Trials
MRI/MRS	Steatosis	E in POC trials with changes in liver fat as primary endpoint	NA
Multiparametric MRI	Steatosis	C (to enrich a population in a POC when biopsy confirmation is not available)	Need further validation
VCE/fibroscan/MRE[a]	Fibrosis	C (to enrich a population in a POC when biopsy confirmation is not available)	I (early identification of patients progressing to cirrhosis)

C, consider; E, essential; I, ideal; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NA, not applicable; POC, proof of concept; VCE, video capsule endoscopy.
[a]Can provide information about liver stiffness and liver fat, although cost and generalized use is limited at present.

Supplementary Table 8. Health Related Quality of Life

	Early Phase Trials	Late Phase Trials
Short Form-36	C	E
Patient-reported outcomes measurement information system	C	E

C, consider; E, essential.