

**CHALLENGES AND OPPORTUNITIES IN DRUG AND BIOMARKER  
DEVELOPMENT FOR NONALCOHOLIC STEATOHEPATITIS: FINDINGS AND  
RECOMMENDATIONS FROM AN AMERICAN ASSOCIATION FOR THE STUDY OF  
LIVER DISEASES (AASLD) - FOOD AND DRUG ADMINISTRATION (FDA) JOINT  
WORKSHOP**

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**List of Abbreviations:**

NAFLD:	nonalcoholic fatty liver disease
NASH:	nonalcoholic steatohepatitis
NAFL:	nonalcoholic fatty liver
HVPG:	hepatic venous pressure gradient
MELD:	model for end stage liver disease
T2DM:	type 2 diabetes mellitus
METS:	metabolic syndrome
FDA:	Food and Drug Administration
AASLD:	American Association for Study of Liver Disease

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**Abstract**

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in North America. It is a growing contributor to the burden of chronic liver disease requiring liver transplantation. Cirrhosis is also associated with an increased risk of hepatocellular cancer which may occur even in the absence of cirrhosis in subjects with nonalcoholic steatohepatitis (NASH) the histological form of NAFLD associated with increased liver-related mortality. The diagnosis of NASH currently requires a liver biopsy. There are also no FDA-approved therapies for NASH. There is therefore a need to develop better diagnostic and therapeutic strategies for patients with NASH targeting both those with early stage disease as well as those with advanced liver fibrosis. There are unique challenges in the design of studies for these target populations.

The long relatively asymptomatic time interval in the progression of NAFLD and NASH to cirrhosis and ultimately liver failure, along with gaps in knowledge regarding disease modifiers combine to present significant challenges in trial design. There is therefore an urgent need to develop methods to identify the populations at particular risk of disease progression and to validate endpoints that reflect meaningful changes in health status in this population.

This manuscript summarizes the discussion at a joint workshop held September 5<sup>th</sup> and 6<sup>th</sup>, 2013, in Silver Spring, Maryland, sponsored by the FDA and the AASLD to develop guidance on diagnostic and therapeutic modalities for NASH.

## 1. Introduction:

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and liver-related morbidity and mortality (1, 2). It has two principal clinical-histological phenotypes: (a) nonalcoholic fatty liver (NAFL) and (b) nonalcoholic steatohepatitis (NASH) (3). Approximately 30% of adults and 10% of children in Western countries have NAFLD (4, 5). NASH is associated with an increased mortality with excess cardiovascular-, liver- and cancer-related deaths (6, 7). There is no approved therapy for NASH. These data underscore the urgent need to develop preventive and therapeutic strategies against NASH.

The Food and Drug Administration (FDA) and the American Association for Study of Liver Diseases (AASLD) jointly sponsored a workshop in September 2013 to tackle this challenge. This workshop assembled major stakeholders to discuss specific challenges and opportunities to facilitate development of therapeutics for NASH. While general consensus was obtained in several areas, there also remain areas where there is a need for ongoing dialogue. This manuscript summarizes the discussion at the workshop.

Individual groups engaged in developing new diagnostics or treatments for NASH are encouraged to communicate directly with the FDA through the pre-IND consultation program for early advice on their development programs. The FDA also has established mechanisms for interactions with outside stakeholders to discuss regulatory science in a non-binding setting e.g. the Voluntary Exploratory Data Submissions (VXDSs) and Critical Path Innovation Meeting (CPIM)<sup>1</sup> pathways.

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<sup>1</sup> Send inquiries about the CPIM to [CPIMInquiries@fda.hhs.gov](mailto:CPIMInquiries@fda.hhs.gov).

## 2. Background:

NAFLD is a clinical-histological syndrome characterized histopathologically by predominantly macrovesicular steatosis with varying amounts of inflammation, cytological ballooning and fibrosis (8). By definition, it occurs despite minimal (< 2-3 units/day) or no alcohol intake (9). The histological and clinical spectrum of the disease is briefly summarized below. Interested readers are referred to several excellent review articles for more details (10-15).

The histological spectrum of NAFLD extends from fatty liver to steatohepatitis (3, 16). Nonalcoholic steatohepatitis (NASH) is characterized by steatosis, inflammation and cytological ballooning with varying amounts of pericellular fibrosis (17, 18). These lesions are classically most pronounced in zone 3 but there may be other patterns of distribution as well. In children, for example, zone 1 inflammation and periportal fibrosis are typically more pronounced than in adults (19). Disease progression is characterized by increasing fibrosis and cirrhosis in a subset of patients.

The principal risk factors for NAFLD include excess body weight, insulin resistance, type II diabetes, hypertension, low high-density lipoproteins and hypertriglyceridemia (10, 20). Subjects, even those with significant histological changes, are frequently either asymptomatic or have nonspecific symptoms (e.g. fatigue). The lack of specific symptoms or signs in such individuals is a significant obstacle to diagnosis before development of cirrhosis when treatment of the underlying disease can potentially avoid cirrhosis the principal cause of liver-related mortality in this population. In addition, there are very little data available to allow differentiation of subjects who will progress from subjects who will not progress to clinically relevant hepatic disease. Therefore, the development of new diagnostics that can differentiate

those with NAFLD who will progress to clinically significant disease represent a major research priority. Liver-related symptoms such as jaundice, pruritus, anasarca, ascites, hepatic encephalopathy, and gastrointestinal bleeding are late manifestations of NAFLD and reflect the presence of end-stage liver disease (i.e., cirrhosis). Occasionally, NAFLD may present with subacute liver failure, with or without a clear cut precipitating factor (21).

### **3. Food and Drug Administration (FDA) Regulatory Pathways**

The FDA has two main pathways for drug development. The first pathway is the “regular” or “traditional” pathway for drug approval, in which a drug is approved based on either a clinical benefit endpoint or a surrogate endpoint that is known to predict clinical benefit on irreversible morbidity or mortality. A clinical endpoint is one that affects how a patient feels, functions or survives.

The second option is the Accelerated Approval Pathway (21 CFR 314.510 and 601.41, Subpart H and E). Accelerated approval applies to drugs and biologics that are intended to treat serious and life threatening illness AND that provide meaningful therapeutic benefit over existing treatments.

Accelerated approval is based on effects of a surrogate endpoint that is “reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict benefit” on irreversible morbidity or mortality. The Agency may grant accelerated approval to a product for a serious or life-threatening condition upon a determination that the product 1) has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit or, 2) an effect on a clinical endpoint that can be measured earlier than an effect on irreversible morbidity or mortality and is reasonably likely to predict an effect on irreversible morbidity or mortality or

other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments.

The term “reasonable likely” implies that some uncertainty remains about the relationship of the surrogate to the clinical benefit to the patient. Therefore, accelerated approval is usually contingent on a sponsor’s agreement to conduct additional post-approval studies to verify and describe the drug’s clinical benefit. This regulatory pathway requires that when marketing approval is granted based on the surrogate, or a clinical endpoint other than survival or irreversible morbidity, clinical trials must be carried out after marketing approval to verify and describe the drug’s clinical benefit.

The preamble to the Accelerated Approval Rule, (Federal Register/Vol.57, No.239/Friday, December 11, 1992/Rules and Regulations), acknowledged that surrogate endpoints can be used for regular or traditional approval when these surrogates are validated by definitive studies. It states that “Ordinarily, products used to treat serious or life-threatening illness, for which approval is based on a surrogate endpoint that is recognized as validated by definitive studies, will be considered for approval under the traditional process rather than under accelerated approval.”

The Agency’s draft Guidance for Industry - Expedited Programs for Serious Conditions—Drugs and Biologics (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358301.pdf>) states: “For purposes of accelerated approval, a surrogate endpoint is a marker, such as a laboratory measurement, radiographic image, physical sign, or other measure that is thought to predict clinical benefit, but is not itself a measure of clinical benefit. Depending on the strength of the evidence supporting the ability of a marker to predict clinical benefit, the marker may be a surrogate endpoint that is known to predict clinical benefit (a validated surrogate endpoint, which

could be used for traditional approval), a surrogate endpoint that is reasonably likely to predict a drug's intended clinical benefit (which could be used for accelerated approval), or a marker for which there is insufficient evidence to support reliance on the marker as either kind of surrogate endpoint (and thus cannot be used to support traditional or accelerated approval of a marketing application). Change in the rate of decline in renal function as measured by creatinine clearance, and changes in blood pressure and Hg A1C have been accepted as validated surrogates to support traditional drug approvals.

However, the preamble does not specifically define the criteria for “validated by definitive studies” that allows a surrogate to be used for traditional approval. Therefore the level of evidence necessary to determine if a surrogate is validated (i.e., acceptable for traditional or regular approval pathways), or if a surrogate is reasonably likely to predict clinical benefit is made on a case-by-case basis by the Agency. Whether an endpoint is reasonably likely to predict clinical benefit is a function of the biological plausibility of the relationship between the disease, endpoint, and the desired effect, and the empirical evidence to support that relationship. The empirical evidence may include “epidemiological, pathophysiological, therapeutic, pharmacologic, or other evidence developed using biomarkers, for example, or other scientific methods or tools.” Clinical data should be provided to support the assertion that a relationship of the surrogate or intermediate clinical endpoint to the outcome is reasonably likely, and should be relevant to the relationship between the specific endpoint to be used and the specific intended clinical benefit of the drug.

What is clear is that there are pitfalls with using surrogates, i.e., examples of where a plausible surrogate that showed improvement with treatment resulted in an overall poor outcome for the patient. Some of these unexpected outcomes may be from off-target effects of a drug.

Several things should be taken into consideration when evaluating a surrogate for use in the regular approval pathway. A surrogate that directly measures tissue loss or organ function e.g. liver failure is particularly plausible for use as an endpoint. Also helpful are the results of controlled trials that show the relationship of the effect on outcomes in trials of other drugs, especially trials involving drugs with different mechanisms, as is the case for example with antihypertensives.

See Guidance “Expedited Programs for Serious Conditions—Drugs and Biologics”:<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358301.pdf> for further details.

#### **4. Clinical Endpoints (Table 1):**

##### **A: Endpoints based on symptoms and functional status:**

Measurements of a change in clinically meaningful symptoms of a disease (how the patient feels and functions) are acceptable as primary endpoints for the regular approval pathway. NASH significantly impacts many aspects of patients’ quality of life. Both adults and children with NASH have poorer health related quality of life scores than healthy controls (22-26).

Most subjects with NAFLD or NASH, are able to function, maintain a job and manage day-to-day activities. There is a need for additional research to identify the frequency and prevalence of physical and mental disability in those with NAFLD, while correcting for confounding variables such as obesity, diabetes and its complications, among others.

Consideration of the risk/benefit balance in this population is important as the majority of patients will not progress; therefore, potential interventions should have a low risk profile. However, as a subset of patients with NASH will progress to cirrhosis, it is important that efforts

continue in developing tools to identify high risk populations and target them for appropriate and safe interventions.

Once cirrhosis develops, there is a progressive impairment in liver function, which eventually leads to multiple symptoms that negatively impact patients' ability to function (27). With progression of disease, measurement of clinical endpoints that assess the patients' symptoms and ability to function becomes feasible. In studies targeting advanced stage disease, it will be valuable to capture the functional status of individual subjects.

In order to assess treatment benefit in clinical trials, well-defined and reliable clinical outcome assessments (COA) may be selected or developed to assess how patients with cirrhosis feel or function in their daily lives. Symptoms can be known only to the patients themselves, and should be assessed using well-defined and reliable patient-reported outcome (PRO) assessments (FDA PRO Guidance for Industry:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM193282.pdf> )

For patients unable to report for themselves (e.g., young children, those with cognitive impairments), other COAs (e.g., clinician-reported outcome (ClinRO) or other observer-reported outcome (ObsRO) assessments) may be used to assess patients' observable behaviors. All COAs require appropriate development and testing within the target patient population in order to ensure they are adequately defined and reliable to detect meaningful and interpretable changes within a clinical trial. Given the high prevalence of symptoms and impaired mental and physical health in patients with cirrhosis, it is important to evaluate outcomes from the patient perspective in the context of long-term clinical trials using appropriately selected or newly-developed instruments.

Potential targets of treatment were discussed and some options that were considered included prevention of progression of fibrosis, prevention of progression to cirrhosis, and prevention or treatment of symptoms. A measurable and objective composite liver-related outcome endpoint could be used to assess events of decompensation in compensated cirrhotic patients. The rates of development of events of decompensation in patients with compensated cirrhosis are well known (28, 29), and specific data for the rate of development of cirrhosis in NASH patients is also known (30). However, even in the cirrhotic population, in view of the rates at which these outcomes develop, large sample sizes or long trials will be required to demonstrate statistically significant differences in rates of development of the events of interest in a reasonable timeframe. These timeframes may be potentially reduced if the treatment effect is large or by enriching the eligible trial population. Enriching the cirrhosis population could be potentially achieved by using patients with a hepatic venous pressure gradient (HVPG) > 10 mm Hg or a MELD score > 10 (31).

Other clinical endpoints are likely to be meaningful to NASH patients with cirrhosis. These include the rates of hospitalization, unscheduled clinic and emergency room visits, tests performed, and lost work days (32). Together with an endpoint measuring a clinically meaningful change in health status, these might provide a more comprehensive picture of an intervention's potential benefit.

## **B. Endpoints based on “hard clinical outcomes”:**

### **B1: Survival as a Clinical Endpoint in Trials for NASH or Cirrhosis:**

All-cause mortality has long been held as the most important outcome and thus a key endpoint in clinical trials evaluating therapies for many chronic diseases that can lead to death, including NAFLD and NASH (7, 15, 33). Liver-related mortality is a component of all-cause

mortality. It is closely linked to the development of hepatocellular cancer, ascites and related complications, variceal hemorrhage, hepatic encephalopathy and eventually acute or chronic liver failure, usually precipitated by sepsis (30, 33). It is well established that the development of any one of these complications heralds an immediate deterioration in health status (i.e., clinical decompensation) and an increase in mortality risk (28, 29). These complications are virtually all linked to the presence of cirrhosis. Hepatocellular cancer is an exception to this rule and can develop in the absence of cirrhosis. Recent studies suggest that almost 50% of hepatocellular cancers related to NAFLD occur in the absence of cirrhosis (34).

NASH progresses slowly to cirrhosis; 15-20% of subjects develop cirrhosis over many years (15, 35). Cirrhosis, the principal cause of liver-related deaths, is associated with a decompensation and mortality rate of approximately 4% annually (30, 36). Therefore, to demonstrate an improvement in mortality rates (all-cause or liver-related) a large number of subjects with early stage NASH would need to be followed for longer than 10 to 15 years. The costs and logistics of such an endeavor are prohibitive, making all-cause or liver-related mortality endpoints difficult to use for drug development and approval. Therefore, all-cause mortality may be impractical as a primary endpoint for most trials that will enroll patients who have NASH without cirrhosis. In subjects with NASH who have established cirrhosis, where decompensation, death or cancer is more imminent, all-cause or liver-related mortality are operationally feasible clinical endpoints but are still likely to require long time frames to measure.

## **B2: Surrogate Endpoints (Table 2):**

As noted above, surrogate endpoints can be used as part of the regular approval pathway if these endpoints have been validated by definitive trials to predict outcomes on clinically

meaningful endpoints. Surrogates may be used to support accelerated approval when there is not an established link of the surrogate to the clinical outcome.

The long duration over which NASH evolves before patients develop clinical outcomes, and the inability to identify the population that will progress, creates a major challenge in designing and conducting clinical trials for those with early stage NASH. This challenge, however, does not diminish the need to develop therapeutics in these patients, and identify who is at risk of progression, given the growing contribution of progressive NASH to the burden of chronic liver disease. It is also important to identify endpoints that can be achieved within a reasonable time interval and that are reliable surrogates for meaningful outcomes. Moreover, these endpoints should both reflect changes in the disease process and be 'biologically plausible', in other words, linked mechanistically to the disease's pathogenesis.

### **B2.1 Histology-based Endpoints in NASH Trials:**

Liver histology currently offers the best short-term method for tracking the progression of NASH. Certain features on histopathology provide some prognostic information regarding risk for progression. Steatohepatitis, not isolated fatty liver, is associated with a substantial increase in the long-term risk of developing cirrhosis and liver-related outcomes (15, 35). This is believed to be related to the underlying inflammation and activation of pro-fibrogenic pathways in NASH. Based on this current understanding of the pathogenesis of NASH, one would expect that reversal of steatohepatitis would reduce the risk of developing cirrhosis. However, steatosis and inflammation can decrease as fibrosis advances (37). Therefore, the reversal of steatohepatitis with no evidence of progression to advanced fibrosis (stage 3 or 4), may be an acceptable surrogate endpoint suitable both for phase 2b and 3 trials that enroll patients with NASH and evidence of early fibrosis. In a recent clinical trial of vitamin E for NASH, patients

who resolved their steatohepatitis also were observed to have improvement in fibrosis (38). Note that due to the uncertainty about the ability of reversal of steatohepatitis to predict a true clinical benefit, to establish the clinical benefit of a drug, outcome trials will be eventually needed to demonstrate that reversal of steatohepatitis does predict clinical outcomes.

A decrease in the NAFLD activity score (NAS) has also been used as an endpoint in clinical trials (39), however, its value is limited by a lack of data linking changes in NAS and its individual component scores, to either progression to advanced fibrosis or clinical outcomes. The advantage of NAS is that it is quantifiable and relatively more reproducible than the diagnosis of steatohepatitis. Additionally, there is ongoing discussion about the need to generate data to either validate the use of the NAS as a valid determinant of NASH clinical outcomes and/or the response to therapy. This would best be accomplished by incorporating a change in NAS as a secondary endpoint in randomized clinical trials.

## **B2.2 Development of Cirrhosis as Potential Surrogate Endpoint for NASH Clinical**

### **Trials:**

There is a body of data that supports that the development of histologically identified cirrhosis predicts a significant worsening of health status. Differences in progression to cirrhosis between treatment and control groups were discussed at the workshop as a potential clinically meaningful outcome measure for clinical trials (27, 28, 30, 33, 36, 40-43). However, there are known weaknesses that are well documented and discussed in the literature with using histopathology assessments in clinical trials and limitations to their use in clinical practice. However, because there is a substantive body of evidence to support that cirrhosis on histology is predictive of clinical outcomes, it was proposed by some at the workshop as a acceptable

surrogate endpoint for “regular” approval under FDA guidelines or as the endpoint for required post-approval studies for treatments approved via the Accelerated Approval pathway.

Development of cirrhosis can be measured with by transient elastography methods; the potential therefore exists that an approval based on non-invasive measures may be acceptable in some cases. There are some data to support the correlation of liver stiffness with clinical outcomes of events of decompensation, HCC and death (44). This would avoid the need for repeated liver biopsies in trials that utilize the difference in progression to histologically defined cirrhosis as an endpoint. However, the use of elastography or other non-invasive methods for the diagnosis of cirrhosis in subjects with NASH has not been explored in great detail and more data will be required to support this endpoint for use in clinical trials. Including such measures as secondary or exploratory endpoints in current trials will provide the necessary data to establish the validity of these potential noninvasive markers.

### **B2.3: Surrogate Endpoints for Clinical Trials for patients with Cirrhosis (Table 3):**

There are also several surrogates that were put forward for consideration at the workshop that may be acceptable as surrogates “likely to predict” mortality or morbidity risk in subjects with compensated cirrhosis, for example the Child Pugh Turcotte score (CPT) and the Model for End Stage Liver Disease (MELD) score, and the hepatic venous pressure gradient (HVPG).

#### **i. Child-Pugh-Turcotte Score:**

The Child-Pugh-Turcotte score was originally developed as a tool to evaluate mortality risk following portacaval shunt surgery in those with cirrhosis (45). It has been shown to be associated with mortality over 1-5 years. Progression from Child class A to class B accurately measures worsening of a given patient’s health status and an increase in mortality risk (46). The CPT score suffers from several limitations however; for example, a bilirubin of 5 mg/dl and 15 mg/dl would both be scored identically while they clearly represent different health states. CPT

also has a subjective element, which may introduce bias in assessment of outcomes. Despite these limitations, it remains a time-tested way to assess 1-5 year mortality risk in subjects with advanced liver disease. Progression from Child class A to B or a 2-point worsening of the CPT score were discussed as potential surrogate endpoints, but the precise change in score to be used as a responder criterion in clinical trials remains uncertain.

**ii. Model for End Stage Liver Disease (MELD) Score:**

The MELD score is among the best predictors of short-term (3 month) mortality risk in those with decompensated cirrhosis. MELD has a more sensitive dynamic range than the CPT score and is used to determine organ allocation for liver transplantation (47, 48). MELD is objective, easy to measure, widely available and supported by a very large body of evidence as a predictor for mortality risk over a 3 month period (47, 49-54).

Patients with NASH and compensated cirrhosis generally have low MELD scores, and the ability of low MELD scores to predict changes in mortality are not as robust as high MELD scores. Subjects with a MELD score > 10 are however more likely to experience decompensation and a liver-related clinical outcome than those with lower scores (31, 55). The benefits of liver transplantation only emerge once the MELD exceeds 14 (56), and therefore a MELD score > 14 is typically the minimal listing threshold for transplantation. While many other factors also determine whether a patient is listed for transplant, a MELD of 14 or higher is an endpoint that meets the criteria for a surrogate that may be acceptable for clinical trials, in that it has a strong relationship to mortality and outcomes, is objective, easy to measure, sensitive and widely available. There was discussion of the potential of MELD as a surrogate endpoint based on sufficient evidence to establish a link to “need for transplant” and “mortality”; specifically, a MELD of 15 or higher with a minimal 2 point change from baseline would establish these links

in a population with cirrhosis. Because “need for transplant” is an irreversible morbidity, if MELD score is the criterion used to make a decision to transplant, achieving the transplantation-qualifying MELD score was discussed as a potentially reasonable clinical trial endpoint for clinical trials intended to support drug approval.

**iii: Hepatic venous pressure gradient (HVPG):**

The HVPG was discussed as a potential surrogate endpoint that might be considered reasonably likely to predict clinical outcome to support accelerated approval. HVPG measures the pressure differential from the portal to the hepatic vein and thus provides a physiological readout that integrates the hemodynamic consequences of increased sinusoidal resistance to flow due to hepatic fibrosis and/or increased portal inflow. Its methodology is well established and it is reproducible (57, 58), with a large body of literature that demonstrates its concordance with liver related outcomes (57, 59-63). It is limited by being an invasive procedure and requiring standardization of the procedures used to obtain the pressure measurement. Cirrhosis related complications heralding clinical decompensation largely occur above a threshold HVPG of 10 mm Hg, which has been established as the cutoff for “clinically significant portal hypertension” (31, 55). Reduction of the proportion of subjects that progress to developing a HVPG > 10 mm Hg was posed as a treatment objective and surrogate primary endpoint for consideration, in the setting of accelerated approval in trials of therapeutics for patients with NASH with advanced fibrosis who have baseline HVPG values below 10 mm Hg at study entry. Lowering HVPG from values > 10 mm Hg to less than this threshold value was also posed for consideration as a surrogate endpoint to support accelerated approval in patients with NASH with bridging fibrosis or advanced cirrhosis and elevated HVPG.

**C. Other Surrogate Endpoints for disease progression**

### **C1: Quantitative liver function tests:**

There has been considerable interest in the development of quantitative liver tests as markers of overall hepatic integrity and functional reserve, with several such tests in various phases of development. In order to be acceptable as surrogate endpoints for clinical trials intended to support Subpart H or E approval, all functional tests must have adequate data to support their use as a surrogate that is “reasonably likely” based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict benefit” on irreversible morbidity or mortality.

### **5. Other Endpoints in Trials for NASH:**

The objectives of early phase trials are to assess safety and to obtain a signal of efficacy that will guide decision-making about further developing a specific drug. The endpoints for such trials should include traditional endpoints for evaluating safety, including potential hepatotoxicity (FDA Guidance - Drug-Induced Liver Injury: Premarketing Clinical Evaluation; <http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf>).

Other endpoints were discussed that may, with additional validation, be useful for supporting efficacy, and have the potential to be used to evaluate efficacy in early phase trials such as proof-of-concept and dose-ranging trials. In NASH, a combination of hepatic triglyceride quantification, liver enzymes and CK 18 are biologically plausible markers of improvement that are also objective, measurable and sensitive to change. Resolution of steatohepatitis almost never occurs without a decrease in hepatic steatosis. Moreover, serum CK18 levels, which are reflective of apoptotic activity, corresponded well to an improvement in liver histology in two phase 2b clinical trials of NASH in adults and in children respectively but need additional validation (64, 65). This additional validation can best be achieved by incorporating these biomarkers as secondary endpoints in early phase clinical trials.

## 6. Identification of the population at risk in early stage disease (Table 4):

In studies of early stage NASH, it is important to study a relatively homogeneous population on the one hand, but the data must also be generalizable to the larger histological spectrum of NASH on the other. As noted above, more research is needed to be able to identify a high risk population among those with early stage disease.

The NASH Clinical Research Network classification distinguishes between definite and borderline NASH but it is not clear if these phenotypes imply different natural courses and outcomes to therapy (16). Data were presented at the workshop suggesting that a diagnosis of steatohepatitis based on presence of steatosis, inflammation and either ballooning or pericellular fibrosis has been shown to correlate with outcomes.(66). Also, if both patients with borderline and definite NASH are included in trials, appropriate statistical methods, for example, stratification of randomization could be used to account for these histological variants of steatohepatitis as variables that may impact outcomes with a given therapy.

Several small studies of subjects with multiple biopsies indicate that the disease progression is bidirectional, with some subjects regressing and others progressing (14, 15). Moreover, in view of the sampling variability associated with liver biopsies (67), there is confusion about whether it is feasible to identify and quantify disease progression within a 3-4 year time frame.

While the clinical implications of progression from stage 1 to 2 disease are unclear, there is general agreement in the literature that progression to advanced fibrosis or cirrhosis represents a clear worsening of the health status of the patient (30). Recently, the NIDDK NASH CRN reported the risk of progression to advanced fibrosis in 239 subjects within a 3-4 year time frame for subjects with early stage disease (68) These data demonstrated that the metabolic syndrome (but not type 2 diabetes alone), persistently elevated ALT, higher baseline inflammation scores,

Mallory bodies and portal/periportal fibrosis were independent predictors of the risk of progression. Of note, over 20% of subjects with stage 1b or stage 2 disease progressed to advanced fibrosis. These data provide guidance on enrichment strategies for trial populations with early stage NASH.

### **7. Pathways for treatment development for advanced stage (stage 3-4) disease:**

There are two potential targets for therapy in those with advanced stage disease. These include therapies directed against the steatohepatitis (the abnormality that drives disease progression) or fibrosis, which is the consequence of disease pathogenesis that leads to clinical events.

Arguably, those with NASH and advanced stage disease are at greatest risk of adverse liver-related outcomes within 3-4 years. Trials for therapeutics directed against fibrosis would generally need to include patients with NASH and bridging fibrosis or cirrhosis (**Table 4**).

Presentations at the workshop supported the contention that the NAFLD fibrosis score and FIB4 (based on age, AST, ALT and platelet count) score have been shown to predict mortality and liver-related outcomes in subjects with NAFLD (**69, 70**), which makes these markers of interest for potential enrichment strategies. A MELD score has been clinically considered to reflect overall liver health, with a score > 10 associated with increased risk of liver-related outcomes in those with otherwise compensated cirrhosis (**31**). In trials in patients with advanced fibrosis or cirrhosis, inclusion of those with advanced fibrosis and a HVPG > 10 mm Hg might also serve as an approach to enrich the population for higher rates of outcome events, thereby maximizing the ability to show a difference between those on treatment and placebo.

### **8. Other Considerations in Trial Design and Endpoints:**

Early phase clinical trials that are attempting to provide evidence of proof-of-concept, or to identify appropriate doses, often utilize endpoints such as transaminases or other noninvasive biomarkers of disease. Toxicity merits particular attention in these trials.

In phase 2b and 3 trials, surrogate measures closely linked to liver-related outcomes may be considered. One such potential surrogate is the proportion of subjects with an increase in HVPG to values  $> 10$  mm Hg. This would necessitate inclusion of subjects with advanced fibrosis and an HVPG between 6-10 mm Hg. In studies that will measure improvement in clinical liver-related outcomes, the ability to demonstrate a benefit may be maximized by including those with compensated cirrhosis and an HVPG  $> 10$  mm Hg and/or a MELD score  $> 10$ . Both FIB4 and the NAFLD fibrosis score may also facilitate enrichment for those populations who are more likely to have outcomes. They have the added advantages of being easy to compute, and are widely available.

It is important to remember that purely anti-fibrotic drugs used to treat advanced stage NASH do not affect steatohepatitis, which is thought to be the driver of disease progression. Therefore even if an anti-fibrotic is effective, efforts to directly attenuate underlying disease pathogenesis must be considered. The nature of such 'maintenance therapy' is not yet defined and could include combinations of agents directed against steatohepatitis, in addition to, or instead of anti-fibrotics once some fibrosis regression has been achieved. Disease progression or regression is not simply reflective of fibrosis alone, however, and long-term studies will be necessary to demonstrate sustained improvement in outcomes, including a beneficial impact of fibrosis regression in reducing the risks of decompensation or hepatocellular carcinoma.

## **9. Biomarker and Diagnostics Development for NAFLD:**

At present, a liver biopsy is required for both the clinical diagnosis of NASH and assessment of the treatment response. Liver biopsies are invasive, painful, subject to sampling variability and occasionally associated with serious complications. Therefore, there is an urgent unmet need to develop biomarkers that facilitate the diagnosis, identification of populations at risk, assessment of disease progression or regression, and/or response to treatment.

There are multiple FDA Guidances related to biomarker development, biomarker implications for clinical trial designs, qualification of drug development tools, and considerations for using a diagnostic as part of a drug development program. Please refer to the FDA guidance webpage<sup>2</sup> for the following biomarker-related Guidances:

- Qualification Process for Drug Development Tools
- Clinical Pharmacogenomics: Premarketing Evaluation in Early Phase Clinical Studies
- *In vitro* Companion Diagnostic Devices
- Standards for Clinical Trial Imaging Endpoints
- Clinical Trial Designs Employing Enrichment Strategies to Support Approval of Human Drugs and Biological Products

In addition, FDA has a web navigational tool informing investigators about IND procedures<sup>3</sup>. Studies assessing biomarkers should have clearly defined objectives, particularly with respect to how a biomarker is to be used to aid a drug development program. The most successful programs are those where the disease pathogenesis and the natural history of the disease are well

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<sup>2</sup> [www.fda.gov/Drugs/Guidance/ComplianceRegulatoryInformation/Guidances/default](http://www.fda.gov/Drugs/Guidance/ComplianceRegulatoryInformation/Guidances/default)

<sup>3</sup> <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm343349.htm>

understood. Biomarkers used as endpoints in clinical trials supporting drug approval should be validated to predict clinically meaningful outcomes. Furthermore, biomarkers for treatment response must be validated for individual drug classes (e.g., antifibrotics) used to treat NASH. Since biomarker development can be difficult for individual companies to pursue, especially in the setting of rare diseases, the creation of consortia of relevant stakeholders (e.g., Pharma, academia, NIH and other government entities, professional societies, and patient advocacy groups) can be most beneficial to share resources, clinical trial data, and patient samples. As a biomarker development program progresses, there are several pathways to enable continued dialogue with FDA and we encourage such exchanges to promote efficient development of effective biomarkers.

#### **10. Pediatric populations with NAFLD and development of therapeutics for such populations:**

There is a substantial burden of disease due to NAFLD in the pediatric population (5, 71-73) but there are also special challenges in drug development for children. Children with NAFLD often have hepatic histology that is different from that seen in adults (19). However, given the long natural history of the disease before clinical outcomes occur, many children are no longer under the care of a pediatrician when such outcomes develop. The impacts of the physiological and behavioral changes associated with growth and sexual maturation on either the disease phenotype or progression have not been defined. Children also represent a vulnerable population who mandate greater attention to potential toxicity, and specific studies may be required to determine pharmacokinetics and to optimize dosing for drug development in this population. Trials in children must comply with the standards as set out in 21 CFR 50.52, which state that “investigations involving greater than minimal risk to pediatric patients must present the prospect

of direct benefit to individual subjects”. Initial pediatric studies should focus on the optimal dosing strategy before clinical trials for efficacy are planned. There is no clear consensus on the optimal pathway for drug development in children, which highlights the need for continued dialogue and discussion to clarify and accelerate drug development pathways for NASH in children.

### **11. Safety related issues in drug development for NASH (Table 5):**

It is imperative to be vigilant about the potential for drug-induced liver injury in all stages of drug/biological development in NAFLD. NASH is associated with type II diabetes, increased cardiovascular risk and cancer-related mortality (74-76). For this reason, it seems important to monitor LDL- and HDL-cholesterol, triglycerides, and diabetes control (e.g. hemoglobin A<sub>1C</sub>) in phase 2b and 3 NASH trials. Those engaged in drug development for NASH are also encouraged to follow FDA guidance related to assessment of safety such as those listed below:

Investigator Responsibilities — Protecting the Rights, Safety, and Welfare of Study Subjects at

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM187772.pdf>

AND

Establishment and Operation of Clinical Trial Data Monitoring Committees at

<http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm127073.pdf>

### **SUMMARY**

The AASLD-FDA workshop discussed several elements in the development of diagnostics and therapeutics for NASH and will hopefully accelerate drug development for the disease. While there were discussions on several important issues, a full consensus was not reached, for example, with respect to specific pathways for validation of biomarkers. These

uncertainties underscore the need for continuing dialogue between all the stakeholders in this arena to identify gaps in knowledge and unmet needs, and to address areas of ambiguity in development of diagnostics and therapies against NASH.

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**Table 1:** *Clinically meaningful outcomes in patients with nonalcoholic steatohepatitis*

Outcome
<b><u>Hard clinical outcomes</u></b>
Mortality: <ul style="list-style-type: none"> <li>- All-cause</li> <li>- Liver-related</li> </ul>
Liver-related outcomes that drive mortality: <ul style="list-style-type: none"> <li>- Ascites and its complications</li> <li>- Variceal hemorrhage</li> <li>- Hepatic encephalopathy</li> <li>- Hepatocellular cancer</li> <li>- Acute on chronic liver failure</li> </ul>
<b><u>How a patient functions?</u></b>
<ul style="list-style-type: none"> <li>- Functional status (disability)</li> <li>- Days of work missed</li> <li>- Ability to manage activities of daily living</li> </ul>
<b><u>How a patient feels?</u></b>
<ul style="list-style-type: none"> <li>- Symptoms</li> <li>- Physical health</li> <li>- Mental health</li> </ul>

**Table 2: Endpoints (markers of outcomes) for early stage disease**

<b>Endpoint</b>	<b>Comment</b>
Development of cirrhosis	<ul style="list-style-type: none"> <li>• Clearly defines a worsening of health status and adversely affects how a patient feels, functions or survives</li> <li>• May serve as a primary endpoint for full approval</li> <li>• May be measured by biopsy or other modalities for the diagnosis of advanced fibrosis/cirrhosis (e.g., liver stiffness measurements)</li> </ul>
Reversal of steatohepatitis without progression to advanced fibrosis (stage 3-4)	<ul style="list-style-type: none"> <li>• Can be demonstrated within a 12-24 month time-frame</li> <li>• Is a surrogate for prevention of cirrhosis</li> <li>• May be suitable for subpart H approval process but will require long-term post-approval follow up to demonstrate that treatment prevents cirrhosis</li> <li>• Advanced fibrosis/cirrhosis may be measured by biopsy or other modalities for the diagnosis of advanced fibrosis/cirrhosis (e.g., liver stiffness measurements)</li> </ul>
NAFLD Activity Score (NAS)	<ul style="list-style-type: none"> <li>• Needs validation to demonstrate that a decrease reflects reduced risk of advanced fibrosis/cirrhosis.</li> <li>• Relative impact of improvement of steatosis vs. inflammation vs. ballooning is not clear</li> </ul>
Reduction of hepatic steatosis by imaging studies along with decrease in ALT and/or other markers of cell injury/death e.g. CK18	<ul style="list-style-type: none"> <li>• MR quantification of steatosis is now a community-accepted validated tool: requires FDA acceptance of the tool for validation and use in clinical trials used for registration*</li> <li>• ALT usually, but not always, improves with improvement in histology</li> <li>• Decreased CK18 has been shown to correlate with histological improvement in trials of subjects with stage 0-3 disease</li> <li>• Best suited for early phase proof of concept (phase 1/2a) studies</li> </ul>

\*It is recommended that sponsors planning to use this tool as an endpoint discuss their plans with FDA in accordance with FDA mechanisms for pre-trial consultation.

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**Table 3: Surrogate markers of the risk of mortality**

<b>Endpoint</b>	<b>Comment</b>	<b>Utility</b>
Death	Strongest endpoint- but sample size and study duration will need to be very large	impractical
MELD score	Score of 14 identifies a point above which in absence of transplant, survival declines	Objective measure Validated
2 point CTP	Transition from Child A to B is clearly associated with poorer survival	Objective-subjective Validated Suffer from ceiling and floor effects
HVPG	Tracks risks of complications and progression	Objective measure Validated
Composite: Ascites Variceal bleed Encephalopathy HCC	<ol style="list-style-type: none"> <li>1. Strongly associated with mortality</li> <li>2. It is quantifiable</li> <li>3. Rates of development in controls are known</li> </ol>	Objective-subjective Validated
Quantitative liver function tests	Quantitative – e.g. cholate clearance test and methacetin breath test	Needs more validation
<b>Endpoint</b>	<b>Comment</b>	<b>Utility</b>
Death	Strongest endpoint- but sample size and study duration will need to be very large	impractical
MELD score	Score of 14 identifies a point above which in absence of transplant, survival declines	Objective measure Validated
2 point CTP	Transition from Child A to B is clearly associated with poorer survival	Objective-subjective Validated Suffer from ceiling and floor effects
HVPG	Tracks risks of complications and progression	Objective measure Validated
Composite: Ascites Variceal bleed Encephalopathy HCC	<ol style="list-style-type: none"> <li>1. Strongly associated with mortality</li> <li>2. It is quantifiable</li> <li>3. Rates of development in controls are known</li> </ol>	Objective-subjective Validated
Quantitative liver function tests	Quantitative – e.g. cholate clearance test and methacetin breath test	Needs more validation

**Table 4: Populations to study in clinical trials of NASH**

Population	Comment
<p><b>For advanced (stage 3-4) disease (mainly anti-fibrotic therapies)</b></p> <p>Biopsy-based populations</p> <ul style="list-style-type: none"> <li>- NASH</li> <li>- Bridging fibrosis or cirrhosis</li> </ul> <p>Non-biopsy based populations:</p> <ul style="list-style-type: none"> <li>- Steatosis (by MR spectroscopy or diffusion-weighted MR)</li> <li>- Elevated ALT (&gt; 40 IU/L) or CK 18&gt; (270 u/l)</li> <li>- Advanced fibrosis/cirrhosis by FDA-approved non-invasive method (e.g., elastography)</li> <li>- Absence of alternate cause of liver disease</li> </ul> <p>Populations may be enriched for risk of clinical outcomes by inclusion of subjects with:</p> <ul style="list-style-type: none"> <li>- MELD &gt; 10</li> <li>- HVPG &gt; 10 mm Hg</li> <li>- High NAFLD fibrosis score or FIB4</li> </ul> <p>Additional considerations:</p> <ul style="list-style-type: none"> <li>- fibrosis-related SNPs</li> <li>- type 2 diabetes</li> </ul>	<p>Biopsy-based populations:</p> <ul style="list-style-type: none"> <li>• Biopsy remains gold standard despite several limitations</li> </ul> <p>Non-biopsy based populations:</p> <ul style="list-style-type: none"> <li>• These represent alternate approaches to identification of NASH with advanced fibrosis.</li> <li>• It is recommended that sponsors discuss with FDA during pre-IND process about these entry criteria</li> </ul> <p>Enrichment of populations:</p> <ul style="list-style-type: none"> <li>• Most useful when a liver-related clinical outcome or meeting minimal listing criteria for transplant (MELD of 15 or higher) is the primary endpoint</li> <li>• Fibrosis-related SNPs may explain variability in treatment response and could be used as an ancillary entry criteria</li> <li>• Type 2 diabetes affects clinical outcomes and should be accounted for by stratification or randomization</li> </ul>
<p><b>For early (stage 0-2) stage disease (anti-NASH therapies)</b></p> <ul style="list-style-type: none"> <li>- Nonalcoholic steatohepatitis (biopsy-proven definite or borderline steatohepatitis)</li> <li>- Grade 2 or greater inflammation</li> <li>- Presence of ballooning with Mallory bodies</li> <li>- Stage 1-2 fibrosis</li> <li>- Persistently elevated ALT over last 6 months</li> <li>- Metabolic syndrome</li> <li>-</li> </ul>	<ul style="list-style-type: none"> <li>• This population has up to 20% probability of developing advanced fibrosis/cirrhosis within 4 years.</li> <li>• Should be feasible to demonstrate reversal of steatohepatitis without fibrosis progression to bridging or cirrhosis within 1-2 years.</li> </ul>

**Table 5: Recommendations about safety-related parameters to be measured in clinical trials for NASH**

Parameter	Comment
<b>Cardiovascular:</b> <ul style="list-style-type: none"> <li>• Low density lipoprotein cholesterol (LDL-C)</li> <li>• Small dense LDL (sdLDL) particle concentration, sdLDL cholesterol, % sdLDL</li> <li>• High density cholesterol (HDL-C) and subclass II and III HDL</li> <li>• Triglycerides and very low density lipoprotein particle size (VLDL-P)</li> <li>• ApoB</li> <li>• Lp(a)</li> <li>• Coronary calcification scores</li> </ul>	<ul style="list-style-type: none"> <li>• Main goal in phase 2b/3 trials is to demonstrate that these parameters do not move in a direction suggesting increased risk.</li> <li>• It is imperative that any drug developed for NASH be at least neutral from a cardiovascular risk perspective and ideally also reduce cardiovascular risks</li> </ul>
<b>Metabolic:</b> <ul style="list-style-type: none"> <li>• Hemoglobin A1C</li> <li>• Fasting insulin and glucose</li> <li>• Fasting free fatty acids</li> </ul>	<ul style="list-style-type: none"> <li>• Main goal is to demonstrate stability over the course of phase 2b/3 trials</li> </ul>
<b>Cancer:</b> <ul style="list-style-type: none"> <li>• Enumerate cancers</li> </ul>	<ul style="list-style-type: none"> <li>• Best studied in phase 4 post-marketing studies</li> <li>• Will be valuable to obtain family history and prior history of cancer to better understand impact of treatment on cancer incidence</li> <li>• All subjects should follow established practice guidelines for cancer screening during long-term trials</li> </ul>
<b>Other safety parameters:</b> <ul style="list-style-type: none"> <li>• Drug-induced liver injury</li> <li>• Behavioral adverse events (e.g., depression)</li> <li>• Other off-target unexpected effects</li> </ul>	<ul style="list-style-type: none"> <li>• Refer to FDA guidance on drug toxicity and criteria for stopping therapy in an individual patient versus trial stopping rules for safety concerns (need citation)</li> <li>• Depression scores should be tracked during therapy</li> </ul>

**REFERENCES (Available on-line as supplementary table)**

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