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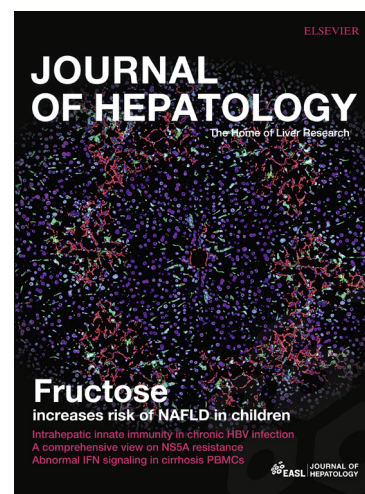
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## **A critical review of end-points for non-cirrhotic NASH therapeutic trials**

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**ABSTRACT**

Nonalcoholic steatohepatitis is a disease without a single, specific, diagnostic marker which means that multiple indicators need to be used to measure therapeutic efficacy. Moreover, drug candidates for nonalcoholic steatohepatitis target many distinct mechanisms that are believed to promote hepatic injury. Therefore, there is a wide range of endpoints that have to be reached, sequentially, as required by the drug development process. Some of these endpoints validate the mechanism of action, others are used to anticipate histological efficacy. Histological endpoints are still considered the best predictors of clinical outcomes but they can only be reliably tested in larger, late phase trials. Here, we will review the rationale and clinical data in favor of the endpoints used at different stages of therapeutic trials. We will also discuss the validity and limitations of current phase 2b histological endpoints, in particular that of a one stage reduction in fibrosis, for their ability to predict progression to cirrhosis which is the ultimate objective of therapeutic trials.

Therapeutic trials need to provide answers to precise questions and these questions are different at the various stages of drug development. Early phase trials (phase 1 and 2a) are designed to inform about pharmacokinetic parameters, early and short-term human safety data and pharmacodynamic aspects. These are common to most therapeutic fields or classes of drugs with little if any specificity for particular diseases such as NASH. In addition, phase 2a trials also demonstrate “proof of principle”, meaning measurable “on target effects” and their biological consequences. An outcome of phase 2a trials that is particularly important is the selection of ideally one or two doses that provide maximum efficacy with acceptable safety and tolerability. This will also allow to gain precious insight into the dose dependency of the therapeutic effect and the safety margin of a particular compound. These doses will be carried over in later stage trials. Typically phase 2b trials will explore whether the biological effects observed in earlier trials translate into hepatic histological improvement. Finally, large scale phase 3 and subsequent outcome trials will provide a comprehensive and statistically robust demonstration of the benefit in terms of histological improvement and clinical long-term outcomes. Since each trial will have to deliver information critical for designing the next step, a careful consideration of how the endpoints should be chosen and if they can be achieved within a given time frame is necessary.

## EARLY PHASE TRIALS

While endpoints in phase 1 studies, as described above, are standard and will not be further developed here, those for proof of principle, phase 2a trials are usually defined based on the mechanism of action of each drug. At least three broad categories can be defined for drugs currently in development for NASH: metabolic, anti-inflammatory and anti-fibrotic effects. The crucial question is, if and how do the metabolic effects of a drug candidate translate into hepatic histological improvement? The same question is to be asked for the potential anti-inflammatory and anti-fibrotic properties since, with few exceptions, most early phase trials do not use direct histological assessments.

### *Metabolic effects*

Since NASH is a disease intimately associated with insulin resistance, adipose tissue dysfunction and the various phenotypic manifestations of the metabolic syndrome, (mainly overweight, visceral adiposity, dyslipidemia, arterial hypertension and glucose abnormalities) there is a wide variety of clinical (weight changes) and biological variables that can be measured to determine how the drug impacts the underlying metabolic abnormalities. Weight reduction is the

most straightforward indicator of a possible benefit for NASH [1]: with diet and lifestyle interventions there is evidence that modest (<5 % from baseline) weight loss can reduce steatosis, higher levels (5-8 %) can improve hepatic inflammation ballooning and even clear NASH, whereas more marked weight loss (>10%) does reduce fibrosis [2]. Although data are currently lacking, it is probable that pharmacologically-induced weight loss of the same magnitude will result in the same histological effects. However, most drugs are either weight neutral and some, such as glitazones, can increase weight while still improving the liver. Therefore, here again, the mechanism of action prioritizes the choice of outcomes and the same information only has value within a particular context. Accurate quantification of insulin action in diabetic subjects and of tissue/pathway specific insulin resistance necessitates the use of cumbersome techniques such as the euglycemic insulin clamp combined with infusion of labelled glucose or free fatty acids [3, 4]. These complex methods are rarely if ever conducted in liver clinics. Instead, improvement in insulin resistance can be accurately demonstrated in non-diabetic subjects by surrogate measures such as HOMA-IR (fasting glucose multiplied by insulin) [5-7] or concentrations of serum adiponectin [8] or the adipose tissue insulin resistance index (the product of free fatty acids and fasting insulin) [9, 10]. Like with all surrogates, the question is, to what extent is an improvement in insulin resistance predictive of a hepatic histological improvement? Trials with glitazones have provided the best evidence so far that improvement in insulin sensitivity can be associated with histological improvement [11-13]. Both pioglitazone and rosiglitazone have a strong antisteatogenic effect [12, 14-16] which is possibly mediated by rises in adiponectin levels and a reduction of adipose tissue-derived lipolysis [10, 17]. Since the action of insulin on SREBP1c and consequently on several of the key enzymes of lipogenesis remains intact in insulin resistance states [18], there is little evidence that an insulin sensitizer drug would change the rate of lipogenesis. Clinical data suggest that an improvement in insulin sensitivity coexists with an improvement in hepatic necro-inflammation: pioglitazone has been shown to resolve NASH more often than placebo [12]. This could be due to a reduction in lipotoxic precursors because of a better control of lipolysis, or to increases in adiponectin which may have anti-inflammatory properties [19]. However much more work is necessary to understand both the clinical reality of this link and its biological determinants. Whether an improvement in insulin resistance results in an improvement in hepatic fibrosis is unknown. Clinical results alone are inconclusive, no matter what meta-analyses are tempted to conclude [20], as no studies designed for fibrosis improvement are available, let alone large scale or longer-term trials. There is of course the possibility of an indirect effect, since improving the conditions that created NASH may subsequently shut-off the fibrogenic process and this could also be facilitated by the silencing of some necro-inflammatory hepatic damage which can then inhibit the triggers for fibrosis. There are also “off-target” effects since, for instance, stellate cells express several nuclear transcription factor receptors such as PPAR $\gamma$ . PPAR $\gamma$  transcriptional

activity is reduced during the activation of stellate cells into a myofibroblastic phenotype [21-23] and activation of PPAR $\gamma$  reduces [21, 22], to some extent [24] hepatic fibrosis. However, more work is needed to understand if there is a more direct effect of insulin sensitization on the myofibroblastic activation of stellate cells that could be mediated through many mediators including adiponectin [19, 25]. As far as the effect of a drug on the phenotypic manifestations of the metabolic syndrome there are multiple standard fasting or dynamic parameters (oral glucose tolerance tests or lipid load tests [26]) of glucose homeostasis and lipid alterations that can be measured. Favorable metabolic effects of a drug are desirable in patients with NASH but not mandatory. Again, it is unknown to what extent the partial correction of these metabolic abnormalities are predictive of hepatic histological improvement. Therefore, these outcomes are mostly useful to validate the pharmacodynamics and biological actions of a drug and less for the prediction of histological improvement.

Metabolic improvement (either through weight reduction or enhanced insulin sensitivity) will result in a reduction in steatosis. Imaging modalities for a precise quantification of steatosis are now available using magnetic resonance spectroscopy [27] and MRI-based proton dense fat fraction (PDFF) [28, 29]. The latter has the advantage of an easier implementation even in multicentric studies on different MRI machines. The physiological amount of liver fat in a healthy population as measured by these imaging methods is around 5.5% [30]. Fat quantification by MRI-PDFF has a strong correlation with the histological semi-quantitative assessment of steatosis as well as changes over time [31]. Paired histology-MRI-PDFF data from a subset of the FLINT trial has shown that a 5-6% absolute change in PDFF correlates with steatosis improvement or aggravation as measured by liver biopsy [32]. Another study has shown the same correlation with histology for a 30% relative change [33]. Several phase 2a proof of principle studies now use steatosis quantification by MRI-PDFF in order to detect short term changes in liver fat upon therapy [34-36].

#### *Anti-inflammatory and anti-fibrotic effects*

There are no good serum or imaging markers of steatohepatitis or of improvement of liver cell injury or cell death or of improvement of the hepatic inflammatory cascade. CK-18 fragments are a rather unreliable marker of steatohepatitis [37] and changes in CK-18 on therapy are at best difficult to interpret, even when using anti-apoptotic molecules [38]. A reduction in soluble markers of systemic inflammation such as IL1 $\beta$ , IL-6, high sensitivity CRP or fibrinogen can result from enhanced insulin sensitivity or reduced adipose tissue insulin resistance, and we lack demonstration of a direct correlation with improvement with hepatic inflammation [39, 40]. It is therefore questionable whether these circulating inflammatory markers are a reliable indicator of changes in

hepatic inflammation and therefore their value as an outcome is simply to validate the metabolic or systemic effects and not necessarily the effects on hepatic injury.

Considering these limitations we are currently left with the measurement of serum aminotransferases as the only non-invasive marker of the hepatic anti-inflammatory effects of a drug. Most, if not all studies that have shown clear hepatic histological improvement, have also documented a robust decrease in ALT levels [12, 13, 40]. A robust decrease is in the range of a 30-40% which is maintained throughout the entire treatment period [11, 15, 38, 41]. ALT values usually do not change in the placebo arm [14, 15, 41] although in some studies/populations there could be an initial decline [12]. However a sustained decline of ALT values in the placebo arm is unusual and should raise suspicion about major diet or lifestyle changes during the trial [42]. Also, some drugs failed to reduce ALT levels which somehow gives credibility to this biochemical signal [43]. Whether a positive signal (ALT reduction) always predicts histological improvement is unknown; despite lingering methodological controversies [44] ursodeoxycholic acid is an interesting example of a strong reduction in ALT values in some NASH studies [41] (and also in chronic hepatitis C [45]) but no histological benefit in other studies [42, 46]. Conversely, most researchers consider a lack of ALT change as indicative of drug inefficacy, and hence a no-go signal for further testing of anti-inflammatory drug candidates. This may be true for steatohepatitis improvement but it is unclear whether it applies to antifibrotic drugs as well. There is at least one recent example where a drug claiming an antifibrotic effect did not change ALT values [39].

Anti-fibrotic effects are even harder to assess without histology, particularly in short-term phase 2a trials. Both fibrosis build-up and fibrosis reversal are slowly evolving processes and it may take years after removal of the primary disease, before a meaningful reduction in fibrosis becomes detectable [47, 48]. One may ask what the rationale for looking for fibrosis changes in 12 week phase 2a trials is, other than trying to understand if there is any chance of a direct antifibrotic effect of a drug. Direct fibrogenic markers could be most useful for this purpose but pro-C3 [49, 50] or matrix remodeling rates [51] are insufficiently validated so far. What is clear is that standard serum fibrosis markers (FibroTest, NFS, ELF) or elastometry can experience a rapid decline that is not correlated with and not explained by a reduction in fibrosis [52, 53]. Inflammation and ALT levels, for instance, influence liver stiffness beyond the amount of fibrotic scar [54] and may confound early changes in elastometry values. The same could hold true for serum fibrosis markers as studies in patients with treated viral hepatitis have shown early changes in serum levels, before changes in fibrosis were documented. Thus, the predictive value of serum markers and elastometry for fibrotic changes lack both specificity and most probably sensitivity in early phase trials. A lot of hope is placed on 2D MRE technology which could be a valuable imaging marker of fibrosis in cross sectional studies [55-57]. It

has been suggested that a 15% relative reduction in MRE stiffness represents a one stage reduction in fibrosis measured by histology [55]. Unfortunately data from clinical trials testing multiple doses of the same drug are not yet entirely demonstrative [58] although more data are needed and will be available in the near future. Recently it was suggested that a T1 mapping technique for fibrosis and inflammation using multiparametric magnetic resonance imaging [59] could be a promising method to grade the severity of steatohepatitis and to predict clinical events [60, 61]. However, these small studies await larger and independent confirmation.

## LATE PHASE TRIALS

### *Overall objectives of phase 2b trials*

Phase 2b trials are typically performed on hundreds of patients, collect safety and tolerability data and test for histological efficacy. These trials are exploratory, meaning that all histological changes need to be documented and studied: changes in steatosis, hepatocyte ballooning and inflammation, fibrosis, NAS, area of fibrosis (i.e. micromorphometry, collagen proportionate area), activation of fibrogenic cells (alpha smooth muscle actin staining) etc. Not all of these lesions necessarily have an association with clinical outcomes and therefore most are not considered approvable outcomes from a regulatory perspective. For instance the NAS has not clearly been shown to predict outcomes and there are conflicting data as to whether baseline values or longitudinal changes are associated with fibrosis progression [62, 63] which may be because NAS is an aggregate score of both disease activity and steatosis. Regardless, what is important in these trials is an exhaustive description of histological changes and particularly of all elementary lesions; this will help understand whether there is potential for histological benefit. However, at this stage, it is also crucial to include the two composite histological outcomes that are reasonably likely surrogates for conditional approval in registrational trials (see below). Moreover, the population of included patients for these trials needs to be very similar to the one agreed upon for inclusion in phase 3, registrational trials. The reason is that these phase 2b trials will inform directly the design of these subsequent trials. It remains to be seen if, in the near future, non-invasive biomarkers will replace histology in phase 2b trials, which is a likely possibility. For this, the demonstration of the diagnostic value of these biomarkers for histological changes will be necessary as well as an approved qualification for use. For the moment, only elastometry measurement by Fibroscan is approved for



detecting stiffness of inner organs, although not yet for changes in liver stiffness induced by therapeutic interventions.

#### *Overall objectives of phase 3 trials*

Phase 3, or registrational trials, are intended for marketing application. Because of the unmet need in NASH therapy, the medicinal agencies have now agreed upon a two-step process designed to accelerate drug approval: an early, conditional approval, based on achieving reasonably likely surrogates and a subsequent, definitive approval, based on achieving a generally accepted surrogate or hard clinical outcomes (for a detailed review see [64]). These are usually combined in a single, long-term trial including an interim analysis after 12 to 18 months of therapy followed by an outcome trial extending for several years. There are two reasonably likely surrogates that could grant conditional approval: 1) resolution of NASH without worsening of fibrosis (i.e. a numerical increase in fibrosis stage); 2) a one or more stage reduction in fibrosis without worsening of NASH (i.e. a numerical increase in the ballooning or inflammatory grade). Whether one or the other of these composite endpoints can be met after a 12 to 18 month treatment period, ultimately depends on the mechanism of action of the drug. A drug with a dominant “antifibrotic” activity will more likely meet the fibrotic endpoint, whereas a metabolic modulator or a drug controlling liver cell injury and inflammation will more likely be successful on the steatohepatitis endpoint, even if fibrosis may improve subsequently. Of course, the ultimate histological benefit cannot always be anticipated based on the mechanism of action in preclinical models or early human studies and some drugs may have pleiomorphic effects. Nevertheless, what ultimately matters is whether the drug candidate inhibits the progression to cirrhosis and results in a reduction in hepatic clinical events (complications of cirrhosis), liver transplantation or death. These are, precisely, the endpoints for the outcome trial; if the benefit over placebo is considered substantial, the drug candidate may then obtain definitive approval.

Hepatocellular carcinoma is a major complication of NASH but current trials do not specifically address a reduction in the incidence of hepatocellular carcinoma as a main outcome. Instead, the number of incident tumors are part of the hepatic clinical events collated together with the other complications of cirrhosis in the outcome trials. The main reason for this is that hepatocellular carcinoma can arise throughout the whole spectrum of NAFLD (steatosis, steatohepatitis with or without fibrosis, with mild or severe activity and cirrhosis). Since phase 3 trials only include patients from part of this spectrum, a reduction in incident HCC would not truly reflect an overall clinical benefit in terms of hepatic carcinogenic complications. For the same reason it is

unknown if resolution of steatohepatitis, through a reduction in ongoing cell injury and inflammation, would result in a reduced rate of hepatocellular carcinoma. Animal models have identified numerous carcinogenic mechanisms that may not be primarily driven by steatohepatitis: a reduction in hepatocyte apoptosis in the steatotic liver of obese rodents, regardless of the presence of fibrosis [65], an increase in circulating levels of IGF-1 [66], increased lipogenesis contributing to liver oncogenesis [67], a tumor-promoting effect of dietary obesity through low grade systemic inflammation [68] or frequent chromosomal alterations in NAFLD HCC [69] to name just a few. Therefore, it is unclear whether the mere reduction in fibrosis stage or the resolution of NASH will have a meaningful effect on the rate of hepatocellular carcinoma.

#### *A review of the histological surrogates and their validity as predictors of cirrhosis occurrence*

##### **Changes in fibrosis stage**

In most chronic liver diseases, patients die of complications of cirrhosis, including primary liver cancer. However, cirrhosis is the result of a protracted fibrogenic process, which is artificially segmented in fibrosis stages, based on pathology-defined landmarks. These stages reflect primarily changes in lobular architecture and not the amount of fibrosis. Regardless, patients need to travel through this fibrogenic process, a journey that is lengthy and to a certain extent reversible. It does make sense then to try to track the progression to cirrhosis by measuring changes in fibrotic stages along the way, both for natural history studies and for drug trials. An efficient drug would either delay or reverse the fibrogenic process, and this could be captured by measuring changes in fibrosis stages. At this point, it is important to keep in mind that the fibrosis stage is therefore only a surrogate for cirrhosis and its complications. Contrary to cirrhosis, stage 1, 2 or 3 fibrosis does not cause a patient's death. The question is, how can we define changes in fibrosis in such a way that it is a robust surrogate for the occurrence of cirrhosis?

Whether one considers that the histological definition of fibrosis stages is arbitrary or not, it clearly has shortcomings. First, the one to four stage division does not reflect a linear increase in the amount of extracellular matrix (mostly collagen). Micromorphometric studies of the fibrosis area have shown that there is relatively little difference in the amount of fibrosis between stages 0, 1 and 2 compared to bridging fibrosis, particularly advanced bridging (METAVIR F3) and a major increase at the cirrhotic stage [70, 71]. This lack of linearity would not be a problem except when trying to measure the potency of an antifibrotic drug by a one stage reduction regardless of which one stage it is. The second shortcoming is that despite their sequential labeling, it is not yet established that

progression to bridging fibrosis for instance, necessarily follows the sequential pattern, meaning stage 1 to 2 than 3. These two shortcomings raise substantial difficulties when using the current fibrosis staging system for the study of the antifibrotic effect of a drug and its ability to prevent fibrosis progression.

However fibrosis stages are defined, and keeping in mind that they are no more than surrogates, natural history studies can bring validity or clinical relevance to their definition. The association with clinical outcomes would increase the value, as a surrogate, of carefully defined changes in fibrosis stage. Accumulating data in several chronic liver diseases, including NASH, has shown an increase in liver-related events (i.e. complications of cirrhosis, hospitalization for decompensated cirrhosis, liver transplantation, liver-related death) in patients with bridging fibrosis compared to the absence of fibrosis [72]. This increase is of course much higher at the cirrhotic stage. For now the data on bridging fibrosis and cirrhosis as a predictor of liver-related events are the most robust we have. This turning point in the natural history of chronic, fibrotic liver diseases have so far provided the rationale for defining the population of patients to be treated or not : before the era of highly potent, well tolerated antivirals, the indication for therapy in HCV patients was rather F2 METAVIR (bridging fibrosis) than F1 (portal fibrosis). In NASH, a few studies with a higher number of patients and a longer follow-up became available and have shown a significant increase in liver related events, even before the bridging fibrosis stage, at NASH CRN stage 2 [73, 74]. It is still controversial whether this also applies to stage 1 [75], as one study has shown [73], but ultimately the demonstration may simply be dependent on following a larger cohort over a longer period of time. All these studies nevertheless come from tertiary referral centers and retrospectively analyze a highly selected population that underwent liver biopsy and survived competing mortality. Even when a large body of data will demonstrate an increase in liver-related events at all fibrosis stages, starting stage 1, this will most certainly provide a rationale for deciding which patients need pharmacotherapy in addition to lifestyle measures. It will not necessarily demonstrate that a one stage reduction is a valid surrogate: if for an early fibrosis stage, the increase in the hazard ratio for liver-related events is only marginal, albeit statistically significant, this may not be sufficient to equate a meaningful reduction of the risk of progression to cirrhosis in patients at that early stage.

#### *Limitations of the current definition of the antifibrotic response*

Currently, the consensus among regulatory bodies and experts, is that a one or more stage reduction in fibrosis without worsening of steatohepatitis is a likely surrogate for progression to cirrhosis and it is actually the basis for conditional approval [64] (although never yet used as such at

the time of this writing). Fibrosis reversal is a legitimate aim when dealing with a progressive fibrotic disease and many studies have shown major fibrosis reversal with therapies that eradicate the cause of liver injury (antivirals in viral hepatitis, immunosuppressants in auto immune hepatitis, etc). However, there is no likely curative treatment for NASH on the horizon, but rather drugs that control or slow down the progression of the disease in a minority of responders, without eradicating the “cause” of NASH. Consequently, only the proportion of patients that worsen the fibrosis stage not the proportion of those that improve it will have a direct impact on the progression to cirrhosis. While in the best case scenario the two can go together, evolving in opposite directions, it is not always the case: a recent therapeutic trial has shown more patients improving fibrosis stage on active drug than on placebo, but the same proportion with worsening of fibrosis in the two arms [39]. Obviously, patients that will progress to cirrhosis are the ones that experience an increase in fibrosis stage not those with a stage reduction. It is therefore doubtful that an improvement in fibrosis is a valid surrogate of progression to cirrhosis, unless an unrealistic 100% response rate is achieved ... Figure 1 shows other theoretical examples where the current definition of the antifibrotic response either cannot predict the progression to cirrhosis or cannot be unambiguously interpreted.

The likely surrogate that best predicts progression to cirrhosis has yet to be defined, but in such a way that is achievable within a 1 to 2 year time-frame. Longer-term exposure may directly capture progression to cirrhosis with a sufficient number of events but this is not compatible with the shorter time-frame of the accelerated conditional approval. A more stringent end-point was proposed, such as a 2 or more stage reduction in fibrosis. This removes some of the uncertainty around the non-linearity of the fibrotic deposition by offering a more robust reduction in fibrosis but still faces the issue of not directly measuring deterioration. The same could be said for complete disappearance of fibrosis, with the added difficulty of low rates of response which makes even statistically significant differences, not convincing, regulatory-wise. Given that bridging fibrosis and cirrhosis are both associated with liver-related mortality in all studies to date [75], a more robust way to define fibrosis improvement or deterioration would be the proportion of patients no longer having bridging fibrosis (if they had it at baseline) or the proportion of patients that progressed to bridging fibrosis (or to cirrhosis if they started at the bridging stage). However, the current NASH-CRN classification which does not distinguish between early (equivalent METAVIR F2) and advanced (equivalent METAVIR F3) bridging, therefore spanning under the term “bridging fibrosis” a very wide range of fibrosis deposition, may limit the sensitivity of this approach.

*Antifibrotic end-points other than histological stage changes*

Micromorphometry correlates with Ishak fibrosis stage and with portal hypertension [76, 77]. Because it is a quantitative variable, it could be more sensitive to change than the histological stage, and therefore could provide additional information on the antifibrotic potency of a drug. Several studies have correlated the area of fibrosis with clinical outcomes, a pre-requisite for it becoming a likely surrogate. Changes in the area of fibrosis predict clinical events of decompensation in patients with HCV recurrence after liver transplantation [78]. In patients with NASH and advanced fibrosis, the area of fibrosis predicts clinical decompensation [79]. The measurement of the area of fibrosis by digital image analysis has been used in several antifibrotic trials [34, 39, 80, 81] but it is not yet an approvable endpoint, only a secondary endpoint intended to support evidence for an anti-fibrotic effect.

### **Resolution of steatohepatitis**

One of the very early findings about the natural history of NAFLD was that steatohepatitis bears a burden of hepatic morbi-mortality that steatosis does not: age and sex-standardized mortality ratios were higher for steatohepatitis than for the general population, while this was not the case for steatosis [82]. Besides, it was believed that steatosis has little or no fibrogenic potential while almost all cases of fibrosis developed in patients with steatohepatitis. It was therefore logical that the disappearance (resolution or reversal) of steatohepatitis would be beneficial as it would place the patient in a very low risk category of disease progression.

Accumulating evidence has strengthened and refined this concept. Patients with steatohepatitis are older [83] and have higher levels of insulin resistance than those with steatosis [84]. They also have a higher prevalence of features of the metabolic syndrome and a more severe phenotype of these complications [83]. Their hepatic disease is more advanced: overall higher levels of fibrosis, more marked biochemical injury (increased ALT), and, by definition, more necroinflammatory activity [85]. Thus, steatohepatitis represents a more advanced form of NAFLD than steatosis and is associated with more advanced metabolic disease. Most importantly, studies with repeat liver biopsy have shown that steatohepatitis, mainly through chronic, uncontrolled necroinflammation, promotes fibrogenesis [86, 87]. Earlier data indicated that necroinflammation is the main risk factor associated with fibrosis progression [88] and this has been recently confirmed when comparing fibrosis progression in patients with steatohepatitis vs. those with steatosis only [89]. Also, on follow-up biopsies, the occurrence of advanced fibrosis coexists with increasing grades of necroinflammation (i.e. disease activity) [63, 86]. Progression from steatosis to NASH increases 7.2 fold the risk of significant fibrosis (stage 2 or higher) on a follow-up liver biopsy [63]. Conversely,

when patients progress from steatosis (without NASH or fibrosis) to significant fibrosis, they almost always develop steatohepatitis in the process [86, 87]. Moreover, there seems to be a quantitative relationship between disease activity and fibrosis deposition: both the baseline necroinflammatory scores and increases in those scores are associated with more fibrosis in studies with serial biopsies [63]. Ballooning grades are correlated with clinical hepatic outcomes in univariate analyses [73]. Finally, interventions modulating disease activity impact on the fate of fibrosis: pharmacological treatment-induced changes in disease activity (the sum of inflammation and ballooning) are positively correlated with changes in fibrosis: a reduction in activity is associated with reduced fibrosis, an increase in activity with increased fibrosis [90].

Data mentioned above is therefore good clinical evidence that steatohepatitis is a main factor behind fibrosis build-up and consequently disease progression in NASH. Current phase 3 trials test the hypothesis that removing this driving force will result in less progression towards cirrhosis. If proven, this will validate its value as a reasonably likely surrogate. It will also, conceptually, promote the idea that fibrosis reversal can be obtained either through a direct antifibrotic action or, indirectly, as a consequence of removing the cause of fibrosis by treating NASH. Of note, the objective is not an improvement in NASH activity but rather its disappearance, a more stringent requirement. The definition of NASH resolution is now consensual among experts (Liver Forum, Manuscript in preparation): it requires disappearance of hepatocyte ballooning and either disappearance or persistence of minimal, residual, lobular inflammation. It is now being used as a regulatory outcome in several large, international, phase 3 studies [91, 92] and has been reported on in at least two recent therapeutic trials [39, 40]. Unfortunately, direct comparison with response rates from older studies is not possible as the definition of resolution of NASH was either different or insufficiently detailed [12, 93].

## CONCLUSION

Many endpoints are currently being measured in NASH trials in an attempt to best predict the biological activity of a drug candidate and its efficacy in improving liver injury in NASH. One of the major breakthroughs in the field was the construction of a regulatory approval framework whereby endpoints that can be achieved within a reasonably short timeframe, compatible with clinical trials, were granted reasonably likely surrogate status. The second major breakthrough was the recognition of progression to cirrhosis as a major therapeutic objective on par with the documentation of liver-related events such as cirrhosis complications, liver transplantation and death. The major challenge of ongoing registrational trials will be to demonstrate that achieving reasonably likely surrogates

such as resolution of NASH and an improvement in fibrosis stage does indeed translate into a reduction in the rate of progression to cirrhosis. While waiting for regulatory acceptable biomarkers of fibrosis, a better histological definition of anti-fibrotic activity taking into account both stage improvement and stage worsening is critical in that regard.

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**Legend to the Figure.**

Three hypothetical scenarios highlighting the limitations of an outcome simply defined by a “one stage or more improvement of fibrosis”. **1a.** Despite a higher rate of fibrosis regression with Drug A then with Drug B, a similar proportion of patients with a 1 or more stage worsening would be expected to result in a similar proportion of progression to cirrhosis; **1b.** Despite a similar proportion of patients with worsening of fibrosis (which is expected to drive progression to cirrhosis) the much higher proportion of patients with fibrosis improvement makes it hard to conclude against an overall antifibrotic benefit; **1c.** A higher proportion of both improvement and worsening of fibrosis with Drug A vs. Drug B makes it uncertain to conclude which of A or B has the best antifibrotic activity.

Figure 1 a.

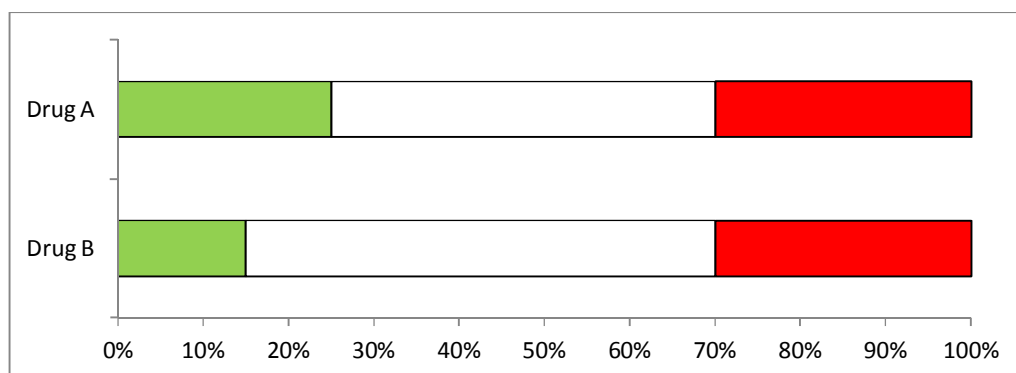


Figure 1b

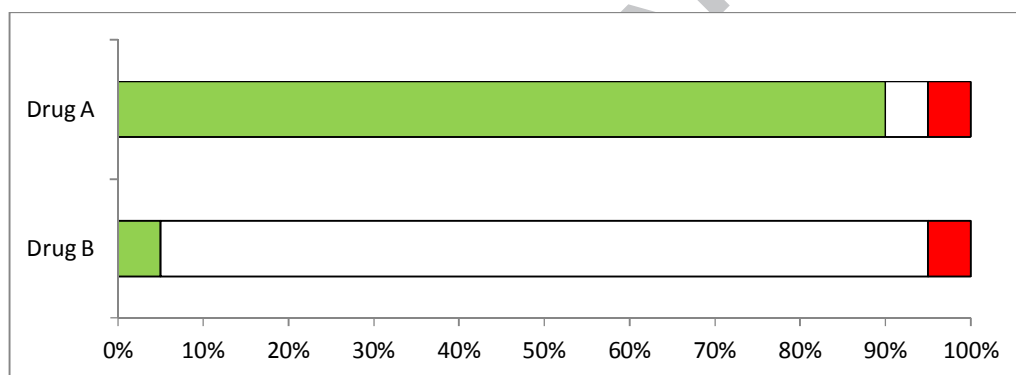


Figure 1c.

