Combatting a Silent Epidemic: Best Practices for Overcoming the Challenges of NAFLD Clinical Trials

As our understanding of Non-Alcoholic Fatty Liver Disease (NAFLD) and diagnostic approaches has evolved, so too have the best practices to be considered when implementing a clinical drug trial. What are the current challenges confronting NAFLD clinical trials, and what are the 'best practices' to be aware of when designing such trials?

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Non-Alcoholic Fatty Liver Disease (NAFLD) is widely deemed the most common form of chronic liver disease in adults in the US, Australia, Asia, and Europe. Globally, it affects about 25% of the population (1).

In the US alone, its prevalence is estimated at approximately 20%-30% of the adult population (2). Children and teenagers commonly experience NAFLD as well (3). Its incidence shows no signs of abatement either. One recent analysis concludes that NAFLD worldwide "is continuing to increase at an alarming rate" – a disturbing trend given that NAFLD is considered the leading cause of liver-related morbidity and mortality (4).

Indeed, NAFLD is a global pandemic – albeit a silent one that is difficult to diagnose and treat. That is because the condition's exact cause remains unknown, it has few symptoms, and it is not a single disorder.

Instead, the NAFLD designation applies to multiple types of the disease marked by excessive amounts of fat in the livers of patients who consume little or no alcohol (5). While some patients accumulate fatty deposits evenly throughout their livers (NAFL), others continue to develop non-alcoholic steatohepatitis (NASH) and subsequent fibrosis.

The more severe NASH involves focal inflammation of the liver (i.e., hepatitis) in addition to fatty deposits.

NAFLD on its own usually presents no symptoms or non-specific symptoms, such as fatigue or weakness. Therefore, it is typically discovered during investigations of other comorbidities. Correlations between NAFLD and obesity, type 2 diabetes, and cardiovascular disease are well known, for example, but are still poorly understood.

The Importance of NAFLD Drug Research

NAFLD involves a dynamic process that includes:

- Fatty deposits >5% evenly spread throughout the liver – i.e., NAFL
- Fatty deposits >5% plus focal inflammation (hepatitis) – i.e., NASH
- Fibrosis (scarring)
- Cirrhosis (severe scarring, permanent liver damage)
    - Hepatocellular carcinoma (liver cancer)
    - End-stage liver disease/chronic liver failure

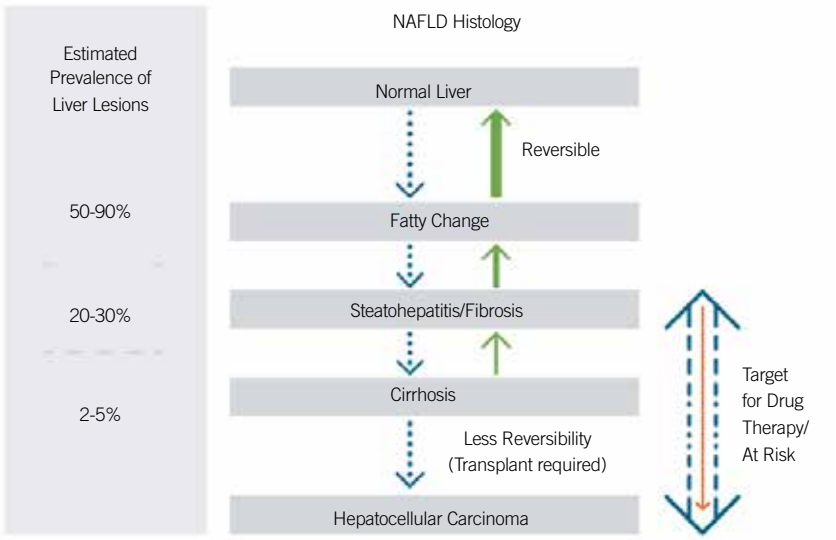

Figure 1: NAFLD pathogenesis (SG Hübscher, 2006)

If NAFLD progresses to cirrhosis, potential complications can become severe and life-threatening.

Patients may experience a decline in liver function (including a reduction in metabolic and digestive processes), a buildup of abdominal fluid (ascites), brain toxicity (hepatic encephalopathy), cancer, and other debilitations.

The further into this dynamic process patient goes, the harder it becomes to improve NAFLD or achieve remission (see **Figure 1**). However, NAFLD can be reversed before it reaches the cirrhosis stage. Thus, clinical research into therapeutic interventions is essential.

At present, lifestyle changes – especially those that lead to weight loss and treatments effectively target comorbidities and risk factors like obesity and diabetes – are the only known ways to alleviate the occurrence of NAFLD or achieve remission (6).

Numerous therapeutic drugs are being researched. Yet to date, no pharmaceutical agents have emerged specifically for treating NAFLD.

The Challenges of NAFLD Drug Research

For researchers, several elements unique to NAFLD exacerbate the typical difficulties associated with clinical trials. These include:

Unknown Etiology

Many different mechanisms, causes, and phenotypes appear to exist under the NAFLD umbrella (7). Genetic factors, environmental factors (such as diet), and metabolic conditions all seem to play a role (see **Figure 2**). This palette of factors means diverse patients may experience different outcomes from the same treatment. It also makes it hard to pinpoint cases of NAFLD within the general patient population. For example, despite its common association with obesity, a significant number of people with NAFLD are of normal weight or lean, so-called 'lean NAFLD' (8).

Disease Complexity

The development and progression of NAFLD is still poorly understood. NAFLD quite often is a staged disease – but not always. Some patients may never progress from NAFL to NASH and beyond; others may do so relatively quickly. In addition, the condition can go into remission on its own or with lifestyle modifications. Consequently, over time, it is nearly impossible to determine whether a patient still has the stage of

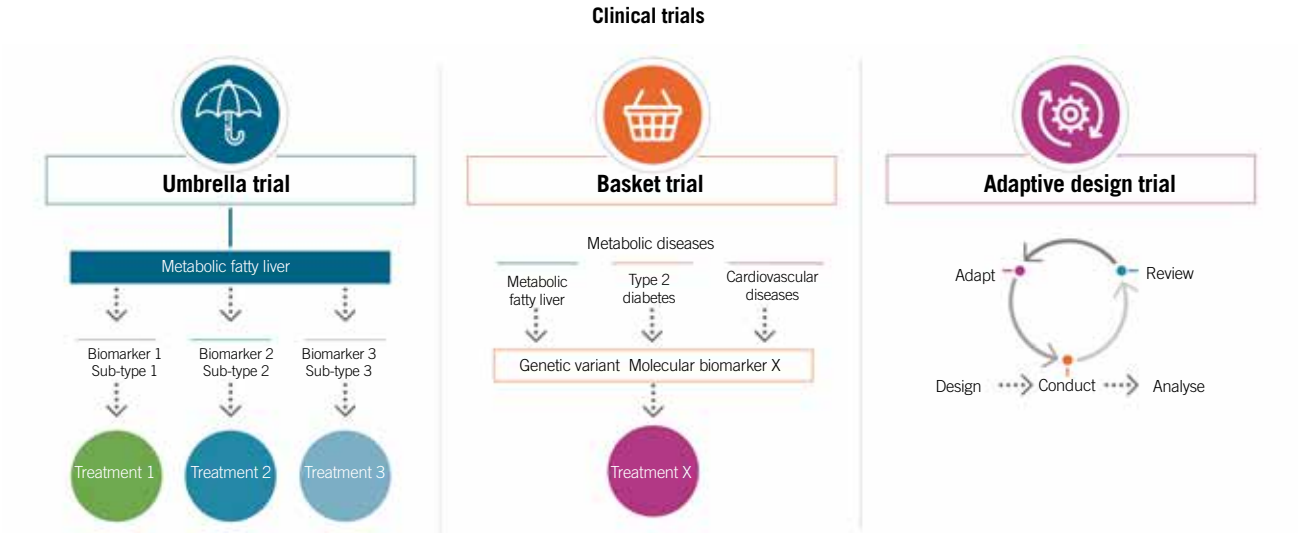

Figure 2: New approaches conducting clinical trials in NASH (Eslam M *et al*, 2020)

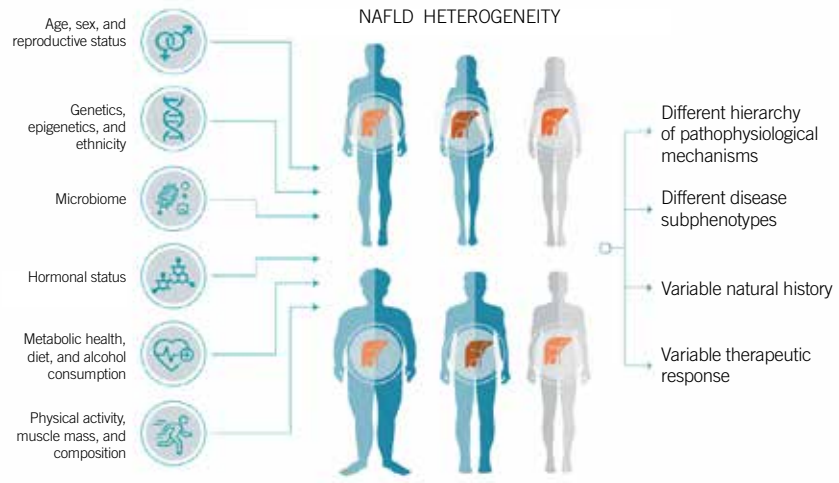

Figure 3: Patient heterogeneity (Arrese M *et al*, 2021)

disease appropriate for the therapeutic agent being studied by a clinical trial. Therefore, the proximate timing of a NAFL, NASH, or fibrosis diagnosis relative to study enrollment is critical for limiting false positive or negative results.

Difficult Diagnosis

Patients seldom present with distinct signs and symptoms of NAFLD. Also, because NASH looks like alcoholic hepatitis at the histological level, alcohol use must be ruled out as a cause before the diagnosis can be made. Likewise, viral hepatitis must be excluded. Currently, an invasive liver biopsy remains the most accurate way to establish a definitive diagnosis of NASH with a degree of ballooning fibrosis, or cirrhosis.

However, it has drawbacks that limit its use in large longitudinal clinical trials. Its invasive nature, risk of complications, patient discomfort, and inherent sampling errors are all reasons that biopsy is difficult to implement on a large scale. Also, due to its local diagnostic capability, a biopsy might not be able to accurately reflect general changes at the organ level. Hence, there is a clear need for minimally invasive methods/alternative biomarkers for assessing liver content (fat, inflammation/fibrosis) in patients during clinical trials. Preferably, these alternatives will be sensitive, reproducible, widely available, and safe. Lately, an increasing number of alternatives have been explored in both clinical and clinical trial settings.

Combined, these considerations make it extremely challenging for researchers to find suitable patients for any given NAFLD study. It also makes it harder to assess whether study outcomes can be attributed to the study drug vs lifestyle changes, or treatments for other associated conditions.

Best Practices in NAFLD Clinical Trial Design

Despite the challenges, many early-phase clinical trials are underway for potential NAFLD therapeutic agents. Quite a few are targeted towards the treatment of NASH. A smaller handful of drugs have reached Phase III trials (9). These include a farnesoid X receptor (FXR) agonist, a thyroid hormone receptor beta agonist, stearoyl-CoA desaturase-1 (SCD1) modulators, lanifibranor (pan-PPAR), and semaglutide (GLP-1RA).

As our understanding of NAFLD and diagnostic approaches have evolved, so too have best practices for clinical trial implementation. These advances demand a more proactive assessment of the reasons why trials fail, as well as more adaptive study designs. Some of the new ways to look at trials include opportunities like adaptive/platform designs, homogeneous cohorts with molecular profiling/genotyping, noninvasive endpoints, and longer duration trials with follow-up to meaningful endpoints (**Figures 2 and 3**).

Alongside such advances come benefits including decreased risks, reduced costs, and minimised bias. To that end, some of the current best practices for NAFLD clinical trial design call on researchers to:

Ensure That Trials Are Of Sufficient Length

Remember that NAFLD often is discovered by fluke during treatment for other, perhaps underlying, conditions. Therefore, patients may have a long, undetected natural history of the disease. Just as it may take a long time to develop NAFL, NASH, or fibrosis, improvements may also take a long time. As both disease development and resolution are connected/codependent, clinical trials that target more advanced disease stages should be of longer duration. Shorter-duration trials of 4-6 months to 12 months might be appropriate for steatosis and NASH, for example, while fibrosis resolution ideally requires longer-duration trials with up to two years of follow-up.

Account For Various Phenotypes By Selecting The Right Patients And More Homogenous Cohorts

Selecting patient populations of the appropriate disease severity is crucial, given NAFLD's spontaneous remission tendency and the effectiveness of lifestyle measures and risk-factor treatments (e.g., obesity or diabetes treatments) (**Figure 3**). A milder patient population may experience a higher placebo rate, jeopardising the efficacy outcomes of a clinical trial. Furthermore, as mentioned earlier, the unknown etiology of NAFLD remains a significant challenge. Race, ethnicity, age, gender, and myriad other genetic, clinical, and environmental factors may play a role in NAFLD outcomes.

Thus, a study will likely increase its odds of demonstrating definitive causal

effects by limiting patient heterogeneity. Grouping patients by, for example, three major sets of determinants – environmental, genetic/epigenetic, and poor metabolic health – could be beneficial. To various degrees, a combination of these factors would help in the selection of a more homogenous cohort/patient population, which in turn could improve targeted treatment efficacy with a lower number of enrolled patients. Otherwise, a larger sample size may be required – to the detriment of costs, risks, bias, invasive biomarkers, and new trial designs.

Choose The Proper Patient Screening Methods Synchronised To The Outcomes Assessment

No patient likes the idea of undergoing invasive biopsy procedures, which also have certain risks including bleeding, pain, pneumothorax, hemothorax, viscous organ perforation, bile peritonitis, infection (bacteraemia. abscess, sepsis), haemobilia, intrahepatic arteriovenous fistula, neuralgia, and rare complications such ventricular arrhythmias (with transvenous biopsy). These risks can deter patient participation, as well as increase the number of adverse events to be reported. Moreover, biopsy-driven endpoints are not considered feasible in a short, proof-of-concept trial. Patients may be reluctant to have multiple biopsies in a relatively short period, and histological changes may not be apparent over this timeframe. So, think about whether histologic endpoints are necessary. If the study drug doesn't target hepatitis, is a biopsy needed? Consider using biomarkers adapted to the disease stage and in line with the underlying mechanisms of the investigational medicinal product (IMP).

Choose More Achievable Endpoints

Researchers can ease patient recruitment and retention by using surrogate endpoints that reduce the need for invasive and costly liver biopsies. Examples include:

- Quantifying NAFL using magnetic resonance imaging to estimate proton density fat fraction (MRI-PDFF), where absolute/relative reduction is also associated with improvement in NASH
- Using a FIB4 score to predict long-term outcomes such as the probability of death or the need for a liver transplant
- Measuring ALT reduction to predict NASH resolution in necroinflammation
- Using MRE/Fibroscan (ultrasound-based transient elastography) to measure fibrosis or cirrhosis

Time Biomarker Measurements Carefully

Many times, a targeted liver biopsy is still considered the gold standard for histological analysis of liver inflammation in clinical trials. It can also be used to stage fibrosis. When requiring liver biopsies to support study eligibility criteria, it is recommended that samples be no more than three months old, and that histology be centrally assessed. This will help mitigate the risk of bias, interobserver variability, and potential self-remission of the disease before the study screening. Similarly, the timing of other surrogate biomarkers should reflect the severity of the disease under study, considering the time to stage development and estimated time to remission.

Conclusion

NAFLD clinical trials are essential but ambitious endeavors. Researchers face a multitude of intricate challenges due to the disease's silence, complexity, and many unknowns. However, as the industry continues to compile and apply best practices in NAFLD study design, so does the ongoing hope that we will find selective and effective therapeutic agents to care for this heterogeneous patient population and combat this worldwide epidemic.

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