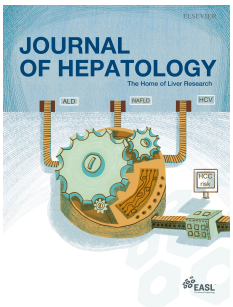

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A proposal from the Liver Forum for the management of comorbidities in nonalcoholic steatohepatitis therapeutic trials

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Abbreviation List:

ADA: American Diabetes Association

ALT: Alanine aminotransferase

ASCVD: atherosclerotic CVD

BMI: Body Mass Index

CV: Cardiovascular

DBP: diastolic blood pressure

DDP4i: dipeptidylpeptidase-4 inhibitors

EASD: European Association for the Study of Diabetes

EMA: European Medicines Agency

FDA: US – Food and Drug Administration

GGT: gamma-glutamyl transpeptidase

GLP1-RAs: glucagon-like protein 1 receptor agonists

HCC: Hepatocellular carcinoma

HDL: high density lipoprotein cholesterol

ICH E9 R1: International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use

LDL: low density lipoprotein cholesterol

MRI-PDFF: Magnetic Resonance Imaging Proton Density Fat Fraction

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

PCSK9 : proprotein convertase subtilisin/kexin type 9

PEth : blood phosphatidylethanol

PPAR: peroxisome proliferator-activated receptor

SGLT2i: sodium-glucose transporter 2 inhibitors

SBP: systolic blood pressure

T2D: Type 2 diabetes

Summary.

The current document has been issued by the Liver Forum who mandated the NAFLD-Associated Comorbidities Working Group - comprised of multi-stakeholder, academic, industry and patient associations to identify aspects of diverse comorbidities frequently associated with NASH that can interfere with the conduct of therapeutic trials and, in particular, impact efficacy and safety results. The objective of this paper is to propose guidance for the management of relevant comorbidities in both candidates and actual participants in NASH therapeutic trials. We relied on specific guidelines from scientific societies, when available, but adapted them to the particulars of NASH trials with the optics of addressing multiple interacting requirements such as maintaining patient safety, reaching holistic therapeutic objectives, minimizing confounding effects on efficacy and safety of investigational agents and allowing trial completion. We divided the field of action in, first, analyzing and stabilizing the patient's condition before inclusion in the trial and, second, managing comorbidities during the trial conduct. For the former, we discussed the concept of acceptable vs optimal control of comorbidities, defined metabolic and ponderal stability prior to randomization and weighed the pros and cons of a run-in period. For the latter, we analyzed non-hepatological co-morbid conditions for changes or acute events possibly occurring during the trial, including changes in alcohol consumption, in order to suggest when specific interventions are necessary and how to manage concomitant drug intake in line with methodological constraints. These recommendations are open to further refinement when additional data will become available and intend to provide a guide for clinical trialists in order to achieve optimal trial objectives while maintaining overall patient health and safety during the trial.

Nonalcoholic steatohepatitis (NASH) is among the most prevalent chronic liver diseases in many parts of the world. Because it is a driver of substantial mortality and health-care costs the search for effective therapies is particularly active with many clinical trials currently ongoing. A characteristic of patients with NASH is the coexistence of numerous comorbidities, most of them related to the metabolic syndrome, either epidemiologically or causally[1]. In clinical trials, particularly those of medium (6-18 months) or longer duration, these comorbidities and their specific therapies can have a significant impact on safety of study participants and also as a confounder of NASH drug efficacy.

The mission of the Liver Forum, which was founded in 2014, is to advance the regulatory science for the treatment of NASH and liver fibrosis by identifying barriers and gaps in the field, and addressing them through a multi-stakeholder consensus building process[2-7]. In NASH clinical trials the Liver Forum recognizes the importance of management of comorbidities as they can become an outcome modifier. It has therefore mandated a Working Group to assess how concurrent comorbidities and their treatments impact on the conduct of NASH trials and what the most reasonable management options can be in this context. The working group is comprised of experts from academic medicine, from the pharmaceutical industry and from patient organizations. Recommendations regarding lifestyle interventions during a clinical trial have been presented in a previous paper released by the Liver Forum[8].

A BRIEF OVERVIEW OF FREQUENT COMORBIDITIES IN PATIENTS WITH NASH RELEVANT TO THERAPEUTIC TRIALS

Most patients with NASH (~80%) are overweight (body mass index (BMI) ≥ 25 kg/m^2) or obese (BMI ≥ 30 kg/m^2) and around ~50% have type 2 diabetes (T2D) or impaired glucose homeostasis[9]. Weight gain, worsening of insulin resistance/glycemic control or incident T2D are associated with fibrosis progression[10, 11] and are independent risk factors for cirrhosis, hepatocellular carcinoma (HCC)[12-14], hospital admission and liver-related mortality[15]. Some other metabolic risk factors, like combined dyslipidemia (characterized by elevated plasma triglycerides and low high density lipoprotein (HDL) cholesterol levels), although linked to the physiopathology of the disease and related to cardiovascular risk in Nonalcoholic Fatty Liver Disease (NAFLD)[16], are less frequent and seem to have minor impact on the

severity and progression of the disease[17-19] although obesity, T2D and atherogenic dyslipidemia have a high degree of collinearity making it difficult to dissect out the contribution of each of them. High blood pressure is found in almost half of the patients with NAFLD and has been shown to be associated with fibrosis progression and disease severity[20]. Conversely, NAFLD is associated with increased risk of incident arterial hypertension[21], atrial fibrillation[22], early atherosclerosis[23] and clinical cardiovascular (CV) events[24]. Overall, the interaction between the components of the metabolic syndrome on the one hand and NAFLD on the other hand are complex and multidirectional (**Table 1**).

Because of the high prevalence of T2D and arterial hypertension, both contributing to renal function impairment, chronic kidney disease is frequently found in patients with NAFLD (20% to 50% of patients)[25] and correlates with the severity of the liver damage[26]. Obstructive sleep apnea affects almost 40% of obese patients and has been frequently described in patients with NAFLD in association with the histological severity[27]. Other comorbidities can be either associated as an underlying cause or have been involved in the pathogenesis of NASH (e.g. hypothyroidism).

RATIONALE FOR DEVELOPING A MANAGEMENT PLAN FOR COMORBIDITIES IN NASH TRIALS

Guidelines developed by scientific societies deal with almost every aspect of the comorbidities frequently coexisting with NASH. When necessary, this document will refer to these guidelines as it is not intended to substitute for them. The purpose of this paper is rather to identify aspects of these comorbid associations (or their therapies) that can interfere with the safety of participants in NASH therapeutic trials or with the interpretation of the results, both on the efficacy and the safety/tolerability side. Two situations will be considered: before randomization and during the trial itself.

Before randomization the presence of comorbid associations may raise several issues. The first one is to ensure a stable condition at baseline for each study participant. This will ensure that during the trial, changes in different parameters in the active and control arms will reflect as much as possible the impact of the study drug rather than that of overall improvement (or worsening) of the general condition that is unrelated to the liver disease *per se* or to the therapeutic intervention. Parameters that are particularly prone to change in the weeks leading

to randomization and that therefore can impact trial outcomes are, for instance, body weight, alanine aminotransferase (ALT) values, glycemic control, blood pressure and liver fat content.

Equally important is another issue, related to safety. Ideally, for patients enrolled in a trial, all conditions at risk other than those associated with the study drug should be minimized. Uncontrolled comorbidities or an unstable overall health condition may favor the emergence of adverse events during the trial or increase their severity. They may also obscure the assessment of the causal relationship of those adverse events with the study drug. Finally, should the patients need specific therapeutic interventions to manage unstable comorbidities, decisions based on heterogeneous local practices without centralized guidance for therapeutic trials, may result in a considerable center effect, particularly in international trials that involve a large number of centers. This center effect could impact the ability to assess drug efficacy.

Guidance on management of comorbidities in therapeutic trials is also justified after patient enrollment. First, associated comorbidities should be adequately controlled and monitored during the trial as some comorbidities may act as significant disease modifiers (obesity, T2D, possibly arterial hypertension, dyslipidemia and hypothyroidism) interfering with study drug effects. The same holds true for some of the drugs used to treat these comorbidities that may impact on the histological condition: observational studies suggest that statin use is associated with less fibrosis and less cirrhotic complications[28, 29] and metformin with less HCC[30, 31]. A major challenge is the use of glucagon-like protein 1 receptor agonists (GLP-1 RAs) that may improve liver histology [32]. Indeed, the choice of an anti-diabetic drug is no longer guided solely by its hypoglycemic efficacy, but also by its efficacy in terms of cardiovascular and/or renal protection. In the new joint recommendations of the EASD and the ADA,[33] which were recently published, GLP-1 RAs are positioned as first-line drugs in the case of established atherosclerotic cardiovascular disease (ASCVD). The same is true for sodium-glucose transporter 2 inhibitors (SGLT2i), which also became the first option in many frequent clinical situations observed in patients with T2D, such as in ASCVD (with the same level of recommendation than GLP-1 RAs); ii) heart failure, and iii) chronic kidney disease[33]. Although the level of evidence for NASH resolution with SGLT2i is currently lower than for GLP-1 RAs, pre-clinical data suggest that this class of anti-diabetic drugs may impact the course of the hepatic disease while improving, in humans, extrahepatic outcomes (cardiac, cardiovascular or renal)[34]. In some trials certain drugs are not allowed at baseline because of a possible confounding effect on liver histology; during the trial, however, if treatment with these drugs becomes the best option for the patient for an indication other than the liver, ethical concerns may justify trial discontinuation. An alternative could be to roll-in the patient in an

open-label trial of the investigational compound. Second, the study drug itself can have side effects on blood pressure, lipid levels or glycemic control[35, 36] that requires specific management[37] in order to control for potential long-term safety issues (mainly cardiovascular events) or, more rarely, for acute risks (such as pancreatitis in case of hypertriglyceridemia). Another concern, for instance when managing increases in low density lipoprotein cholesterol (LDL-C) levels occurring on therapy, is whether aiming at reversing the levels to pre-randomization values is sufficient or whether the aim should be to provide optimal control as recommended per individual cardiovascular risk, necessitating potential trial discontinuation. This is increasingly becoming a real challenge as the LDL-C target is lower in high (<70 mg/dL) and very high (<55 mg/dL) cardiovascular risk populations[38], which are common in NAFLD, especially with concurrent type 2 diabetes. Achieving this LDL-C target often requires intensified therapy with a combination of several treatments (statins, ezetimibe and PCSK9 inhibitors). Finally, controlling for changes in metabolic parameters during the trial will help minimize events that could impact drug efficacy.

BEFORE INCLUSION IN A CLINICAL TRIAL

Before inclusion in a clinical trial careful evaluation of co-morbidities which should be *adequately* and *stably* treated to achieve at least a *moderate control[39, 40]* is recommended. Importantly, patients for which inclusion in therapeutic trials is considered should ideally be in an overall stable condition and their life expectancy should largely exceed the duration of the trial. Therefore, the risk of life-threatening events in the short-term should be minimized by excluding patients with uncontrolled comorbidities (see below) and also those who experienced recent major events. This needs to be decided on a case-by-case basis also taking into account the risk of recurrence in the short-term. Traditionally, a 6-month interval since a first acute cardiovascular event is considered acceptable in most cases (stroke, myocardial infarction, acute coronary syndrome) if secondary prophylaxis is implemented. Such an interval also guarantees a stability of the metabolic parameters (blood pressure, lipid and glucose parameters). In cases of recurrent events or high risk of recurrence despite implementation of appropriate medical measures, inclusion in a trial is not recommended as the priority of medical management should be given to conditions of more immediate concern than NASH. Moreover, early drop-outs for underlying medical reasons (i.e. unrelated to NASH or the study drug) can only be detrimental for the ability of the trial to correctly assess study drug effects. Along the same lines, patients with a history of neoplasm of less than 5 years since complete remission

need to be excluded. Indeed, chances of cancer recurrence during the trial should be minimized and concerns can be raised over the potential of new drugs with insufficiently established safety and carcinogenic profile, to favor neoplastic resurgence in patients with a history of cancer.

Uncontrolled comorbidities

Uncontrolled comorbidities must be avoided as they can impact the clinical or histological course of the liver disease and potentially confound the effect of the tested drug. Uncontrolled comorbidities could also trigger severe adverse events sometimes requiring hospitalization, which even if unrelated to the study drug can mandate permanent or temporary trial product discontinuation. Moreover, introduction of new drugs to manage insufficiently controlled comorbidities may be necessary and this may interfere with the mechanism of action or, ultimately, the efficacy of the tested drug.

Uncontrolled T2D exposes to acute complications such as diabetic ketoacidosis or hyperosmolar hyperglycemic syndrome, potentially leading to coma, or sepsis; as well as a worsening of macro or microvascular complications in the target organs, all of which can deteriorate the health condition and force trial product discontinuation. However, sometimes it is difficult to achieve an "acceptable" control of T2D and many patients are struggling for years to do so. Requiring a strictly "optimal" control of T2D will unnecessarily exclude many patients while increasing the difficulty of trial enrollment and reducing the ability to translate data into real-world settings. Therefore, these patients could be allowed to enter the trial if the T2D is at least moderately controlled (e.g. HbA1c $\leq$ 9.0 %, 75 mmol/mol) with stable doses of antidiabetic medication. In some late-stage trials, the HbA1c threshold could be increased to reflect expected use in the real-world setting. However, this should be carefully considered as accepting too high an HbA1c level presents the risk of increasing the placebo response due to intensified or optimized management during the trial (via improvement in both body weight and glycemic control). In this case baseline stratification on HbA1c level (for instance above 8.0%) could be an acceptable option.

Untreated or treated but uncontrolled blood pressure significantly increase (more than double) the risk of heart disease and cerebrovascular related death[41]. Hypoxemia due to severe obstructive sleep apnea can worsen hepatic inflammation and fibrosis[27] and therefore consideration for assisted breathing device should be given before trial inclusion. Chronic kidney disease is frequent in patients with NASH and is associated with the severity of liver

damage[42]. On the other hand, renal impairment alters protein binding, volume distribution and elimination of drugs cleared by the kidney while changing the bioavailability of drugs eliminated by hepatic and intestinal transport. It is therefore important to set boundaries for unacceptable control of various comorbidities. Patients can be included in trials once these comorbidities are under control within an acceptable (even if not optimal) range.

Acceptable control of comorbidities

Ideally, before inclusion in the trial, all patients should have an *acceptable*, even if not a perfect, control of their main cardio-metabolic comorbidities (obesity, T2D, blood pressure, dyslipidemia and CV risk) and must be as much as possible in a stable condition before inclusion and once the trial starts **(Table 2).**

Dyslipidemia and cardiovascular risk. Scientific societies guidelines recommend lipid-lowering therapy adapted to the individual CV risk[38, 43] in both primary and secondary CV prevention. Individual CV risk is determined based on the presence of CV risk factors – history of atherosclerotic CVD (ASCVD), T2D, high blood pressure, age > 50 years, male sex and tobacco use[44]. National or transnational guidelines[43, 45] may differ, but in large international trials we encourage the implementation of guidelines that apply to the specific place of practice, regardless of possible differences, since during the trial, patients will stay and be managed in their original environment. Clinicians are encouraged to implement the relevant recommendations[43, 45] for achieving the lipid targets as soon as the diagnosis of NASH is made; hence if a clinical trial is considered, there will be no delays in patient recruitment. Optimal management of blood lipid levels including statin treatment when indicated and clinically feasible before trial inclusion is also preferable for the methodological integrity of the trial. This is particularly encouraged for longer (1 year or more) trials and may not be a priority for shorter (1-6 month) trials unless specifically indicated for trial-independent medical reasons. If indicated, statin therapy should be initiated at the appropriate dose (low/moderate or high intensity dose) to reach LDL-C target in patients who are not currently taking statins or up-titrated in patients already taking statins. In some cases, an intensification of lipid-lowering therapy with ezetimibe and/or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors is required to reach LDL-C target, especially in patients at very high CV risk or intolerant to statins. For instance, introducing a moderate or high dose statin lowers the LDL level by 30% to 50%. This will limit excursion in LDL-cholesterol levels induced by some drugs[46] and will provide a clear assessment of the real benefit that some other drugs may have on top of

established lipid lowering medications[47]. Moreover, it may reduce the need for starting therapy during the trial which carries the risk of imbalance between active drug and control arms. This is an important consideration given the claims, based on retrospective databases, that statins, for instance, reduce the risk of fibrosis[28], cirrhosis decompensation[29, 48, 49] or HCC[50-52]. Conversely, as statins are associated with increases in aminotransferases in some patients, their introduction during the trial might trigger adverse events that complicate the interpretation of trial conduct, which is again an argument to start them prior to the inclusion in the trial if they are indicated. Regarding ezetimibe[53, 54] and PCSK9 inhibitors, their action on NASH progression in humans appears quite neutral based on genetic data[55], although some preclinical data suggest they may be involved in the pathogenesis of NASH[56], [57]. Long-term follow-up of hepatic data, especially with PCSK9 inhibitors, is important to provide more certainty about their potential effects on NASH pathogenesis.

Arterial hypertension. There is a continuous relationship between uncontrolled blood pressure and cardiovascular mortality[58, 59], and therefore uncontrolled blood pressure may constitute a competing risk, particularly in phase 3 NASH clinical trials. In patients with uncontrolled blood pressure higher than 160/100 mmHg, the investigator may decide either for dose escalation of an existing antihypertensive drug or introducing a new drug in line with local practice or existing guidelines[60, 61]. Even if the target blood pressure is not achieved before the trial starts, any 10 mmHg reduction in systolic blood pressure (SBP) or a 5 mmHg reduction in diastolic blood pressure (DBP) is associated with significant reductions in all major CV events by 20%, all-cause mortality by 10-15%, stroke by 35%, coronary events by 20%, and heart failure by 40%[62], irrespective of baseline BP within the hypertensive range, the level of CV risk, comorbidities (e.g. T2D and chronic kidney disease), age, sex, and ethnicity[63]. Since there is no available data in humans in favor of a beneficial effect on liver histology[64-67] of the different classes of antihypertensive drugs, including the angiotensin receptor blockers, they can all be used in patients enrolled in NASH clinical trials.

Type 2 diabetes. Untreated patients that exceed the thresholds associated with the diagnosis of T2D (HbA1c ≥6.5% or 48 mmol/mol and/or fasting plasma glucose ≥126 mg/dL or 7.0 mmol/L and/or 2h fasting glucose ≥200 mg/dL or 11.1 mmol/L) should be started on lifestyle changes and eventually anti-diabetic medication before trial inclusion. Although we acknowledge that managing T2D first may delay trial inclusion, we recommend to avoid the inclusion of patients with newly discovered T2D (≤6 months). On one hand uncontrolled T2D is associated with higher aminotransferase levels and more advanced liver damage[68, 69] but

also with a higher rate of diabetes complications, particularly micro-vascular (retinopathy, nephropathy)[70, 71] which are highly dependent on the glycemic control. Also, the decrease of HbA1c may lead to improvement of the liver condition independent of confounders[72]. Additionally, the initiation of antidiabetic therapies during the trial might interfere with the safety of the study drug as well as with the efficacy assessments especially in longer phase 3 trials (see below).

An "acceptable" glycemic control before starting the trial is preferable but as long as patients are below values described above in the uncontrolled comorbidities section (HbA1c ≤ 9.0%, 75 mmol/mol), we recommend that inclusion in trials is possible. According to the recent guidelines, the anti-diabetic treatment should be started once T2D has been diagnosed (HbA1c ≥ 6.5%, 48 mmol/mol) and adjusted if HbA1c level is ≥1.5% above the glycemic target[73]. Instead of establishing one single glycemic target for all patients, the recent joint European Association for the Study of Diabetes (EASD)-American Diabetes Association (ADA) guideline recommends to define individualized optimal glycemic targets depending on the patient's clinical profile: from <6.5% (48 mmol/mol) for patients with short duration diabetes, long life expectancy, no CV comorbidities, to <8.0 % (64 mmol/mol) for patients with multiple comorbid conditions, with long-standing T2D, high risk of hypoglycemia and multiple glucose lowering agents including insulin[74]. As discussed earlier in the article, the management of diabetes is not limited to glycemic control and the management of comorbidities associated with diabetes is a major consideration in the choice of anti-diabetic medication.

The choice of the antidiabetic medication should be left to the discretion of the practicing physician according to the local standard of care and the national guidelines. However, several aspects need to be considered. Some antidiabetic drugs may change the liver fat content and interfere with Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF) assessments in early phase 2 trials, which are typically of short duration. Examples of these drugs are pioglitazone[75, 76], GLP-1 RAs[77, 78] and probably SGLT2i[79], [80]. Some of them (mainly GLP-1 RAs but also SGLT2i) also induce weight-loss[77, 78, 81] which significantly impacts liver histology in NASH. These drugs should be on stable dose for ideally 6 months before the baseline liver biopsy or the baseline MRI-PDFF measures are performed. Other anti-diabetic drugs have no proven efficacy on the intrahepatic triglyceride content (sulfonylureas, dipeptidylpeptidase-4 (DDP4) inhibitors[82], metformine[83], glargine insulin)[84] and hence dose escalation for optimal glycemic control should be allowed before starting the trial. Beyond their role in glycemic control and body weight loss, some anti-diabetic

drugs have also demonstrated some beneficial effects on ASCVD in large randomized controlled trials such as GLP-1 RAs and SGLT2i[85] that might impact hard outcomes and safety profile in NASH trials.

Endocrine deficiencies. Several endocrine deficiencies have been documented in patients with NAFLD. Obese patients have low levels of growth hormone (GH), mainly related to impaired GH secretion which is inversely associated with abdominal fat[86]. Conversely, massive weight loss restores pituitary growth hormone secretion[86]. Several studies suggest that GH and insulin-like growth factor-1 (IGF-1) could be some potential disease modifiers in the development and progression of NAFLD. Indeed, in obese patients steatosis[87] and NASH with advanced fibrosis[88, 89] is associated with more severe GH deficiency. In NASH trials, serum levels of IGF-1 could be tested at screening and, in case of severe deficit, the GH/IGF-1 axis should be further explored to confirm GH deficiency and investigate its etiology. Unless there is a specific primary defect of GH secretion, no replacement therapy appears necessary. Testosterone deficiency is also related to adipose tissue expansion in obese patients[90] and favors weight gain, hepatic steatosis and insulin resistance[91]. In NASH-CRN participants low free testosterone level is associated with NASH and liver fibrosis severity[92]. Because it is a frequent finding in men with NAFLD[90], serum testosterone levels could be tested at screening and end of treatment. To date, only few small studies have investigated the effect of testosterone therapy on hepatic steatosis, with conflicting results. Therefore, additional studies are warranted to determine whether these endocrine deficiencies, given their strong biological link with steatohepatitis and adipose tissue dysfunction, could contribute to the individual heterogeneity in treatment response. However, hormone replacement therapy is not recommended for these two conditions, as long as they are secondary to the weight gain. Finally, low thyroid function is significantly associated with liver fat in diabetic patients[93], with NASH[94] and with advanced fibrosis[94]. The association holds true across the whole range of values including subclinical hypothyroidism and normal-low values[95, 96], with some studies even reporting negative prognostic implications in patients with NAFLD[97]. Measurement of thyroid function with TSH dosage at screening is therefore advisable with thyroid hormone supplementation in case of documented hypothyroidism.

Metabolic and ponderal stability

Weight changes heavily impact most, if not all, histological features of NAFLD, aminotransferase values and some biochemical and metabolic features such as glycemic control or plasma lipids. While weight gain is associated with incident NAFLD, weight loss, depending on its magnitude, can improve steatosis, steatohepatitis, and in some patients, even fibrosis[98]. The impact of weight changes on the histological features has been described both in obese[99] but also in lean patients with NAFLD[100]. Significant weight fluctuations might also impact the baseline assessment: if weight changes occurred after the baseline liver biopsy (most studies allow up to 6 month-old historical biopsies), then the biopsy findings no longer represent the true actual baseline histology.

Since improvement and, possibly, also deterioration of histology with weight changes is both continuous and prone to individual variability, the threshold for defining substantial weight changes is arbitrary. In line with regulatory guidance, we recommend 5% weight change from baseline as a maximum allowed that would minimize the impact on complex or composite histological lesions (such as steatohepatitis and fibrosis) as opposed to steatosis, which can fluctuate for lesser degrees of weight change[98]. The time frame ordinarily accepted for this <5% weight change is 6 months. This assumes that a new histological baseline is reached after a period of 6 months of stable weight, although evidence for this is lacking. It is important to emphasize that the 6 months period be defined prior to screening for the baseline clinical, biochemical or imaging criteria that serve as parameters for efficacy assessment (and hence definition of endpoints) and likewise prior to the date of the baseline liver biopsy for histological eligibility criteria (as well as efficacy endpoints if applicable).

This concept of ponderal stability can be extended to a more general definition of a "*metabolically stable*" condition, defined as no change or minimal changes in comorbidities and their treatment that have no impact on the liver condition or trial outcomes. While some parameters like body weight or glycemic control primarily impact on the severity of liver histology and therefore on the primary study outcomes[10, 11, 101], other comorbidities (e.g. dyslipidemia or arterial hypertension) are more likely to impact on non-hepatic outcomes and safety evaluation[46, 47]. This implies that screening should be delayed in patients with a recent introduction of antidiabetic therapy or statin therapy, given the expected short-term impact of disease control-induced and/or drug-induced alterations of liver enzymes with these medications. The reasonable time period required for metabolic stability is of 3 months before the baseline liver biopsy and/or other baseline measures that serve for efficacy assessment. This requirement will not apply for new drugs introduced for less than 3 months that, to the best of

our knowledge, do not impact liver histology, aminotransferase levels or liver fat content and for drugs with rare, idiosyncratic liver toxicity.

Finally, if bariatric surgery is contemplated in the short-term (i.e. before the end of the trial) it would be preferable to refrain from including the patient in the trial. Apart from massive weight loss, the surgical procedure will improve many associated comorbidities, induce hormonal changes and trigger overall histological improvement. More difficult is to decide when a history of bariatric surgery is no longer an exclusion criteria. Maximum weight loss and metabolic improvement usually occurs in the first two years[102]. In case of massive weight regain and persistence of metabolic comorbidities (such as type 2 diabetes or arterial hypertension) five years after bariatric surgery, in a patient displaying steatohepatitis of required histological severity, trial inclusion can be considered.

Ensuring comparability among treatment arms

"Stratified randomization may be used to ensure balanced distribution of known prognostic factors[103]. Little is known about determinants of patient heterogeneity in NASH and their influence on patient outcomes, although as far as metabolic risk factors, presence of type 2 diabetes has the highest influence on disease course. Select baseline medications for treating associated comorbidities, such as GLP1RAs, may also be confounding some of the histological trial endpoints. Other important factors are fibrosis stage, or, in trials with ALT reduction as an endpoint, a threshold of increased ALT. For drugs that primarily address metabolic dysfunction, stratification on diabetes status is recommended. Other metabolic risk factors may not have a similar impact to justify stratification. There are at least two reasons why stratified randomization is often necessary in NASH trials. One of them is that many small-sized early phase trials include an interim analysis and imbalances between treatment groups for prognostic factors are more likely to occur with small samples. The second is that subgroup analyses are facilitated by stratified randomization, thus allowing a more robust comparison of treatment effect across pre-randomization characteristics[103], mainly type 2 diabetes or obesity. However, the number of strata should be chosen parsimoniously, and one or two strata are usually retained, with type 2 diabetes being the most used one".

Pre-randomization lead-in (run-in) period

The run-in period is a time-lapse prior to randomization when trial participants are given either the active drug or placebo, usually in an attempt to optimize treatment response, tolerability, and adherence or to stabilize disease severity or optimal management. Active drug run-ins expose to multiple problems including overestimation of the drug effect, underestimation of treatment risk and carry-over effects. They can be used for enrichment of adherent participants but at the expense of general applicability of the results. They are not recommended in NASH trials unless the route of administration (parenteral) or the safety profile severely impact compliance thus running the risk of minimizing estimates of treatment effects. An additional potential problem with a run-in period is that, at least theoretically and depending on the duration, drug exposure may induce improvements which can impact on liver histology; in that event, a) patients may no longer meet the criteria for trial inclusion and b) this may impact the overall histological benefit measured at the end of the trial. In trials for T2D or obesity, with non-invasive, easily measurable efficacy parameters, this aspect is less problematic.

More commonly, placebo run-ins are observational periods used in some clinical trials to "stabilize" the pathological condition in the new environment defined by trial requirements, often different from the "real-life", to optimize patient management, to capture and homogenize modifications induced by enrollment in the clinical trial, or to evaluate adherence[104, 105]. The run-in period is required by Food and Drug Administration (FDA) and European Medicines Agency (EMA)[106, 107] in both hypertension and T2D trials that recruit patients insufficiently controlled by standard of care. In obesity trials, the use of the run-in period is subject of debate: it is not recommended by the FDA while is being reevaluated by the EMA[108]. In NASH the relevance of a run-in period should be considered in light of the efficacy endpoints that are being tested and of trial duration. Long trials (one year and more) with histological endpoints will most probably not be impacted by short-term adjustments at trial inception. Short-term studies (3 to 4 months) with metabolic endpoints or liver fat content measurement could benefit from a stabilization period and an optimal pre-trial management that would lead to more conservative, more realistic efficacy estimates of the active drug over placebo. However, applying strict selection criteria that include "metabolic stability" will reduce the relevance of a run-in period. Also, even if improvement in placebo arms can dilute the efficacy of the active drug, therefore pleading in favor of a run-in period, some of the current drugs in development are strongly anti-steatogenic; this could render a run-in period to control for an arguably mild

spontaneous improvement dispensable, at least for parameters such as liver fat content. As far as reductions in aminotransferases values in some[109] but not all[54, 110] short-term trials in patients on placebo, two issues are worth considering. First, serum ALT is known to fluctuate over time in NASH patients as part of the natural history of the disease and a reduction could simply reflect repeated measurements and not exclusively a Hawthorne effect. Second, in cases when a sustained aminotransferase reduction occurs throughout the entire trial duration, the impact of a run-in period may be limited.

For all these reasons, we do not recommend the general use of a placebo lead-in phase even in short term trials with metabolic, imaging or biochemical endpoints. It could be considered on a case-by-case basis, particularly in trials with ALT as the primary endpoint. In this case, however, an acceptable solution could be to measure aminotransferase values on two occasions prior to randomization and to use the mean of these values as the baseline value (see REGENERATE trial NCT02548351). The same may apply to metabolic parameters, when the drug tested acts on metabolic control and if secondary endpoints are metabolically-related.

MANAGEMENT OF COMORBIDITIES DURING THE TRIAL.

During a clinical trial, patients can experience a new event/condition or changes of an already existing condition, either disease or treatment-related. In clinical trial settings, according to the definition of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH E9 R1), such events are referred to as ***intercurrent events*****[111]**. Here we will only discuss events that are related to well-known comorbidities of NASH patients as opposed to randomly occurring health events or unpredictable off-target effects of drugs. The events discussed could require additional medication or changes in the background medication, which may significantly impact the trial's outcomes. Changes in weight, glycemic control, dyslipidemia and high blood pressure control are the most common non-liver related intercurrent events occurring in NASH trials *(Table 3).*

Weight changes

While stable weight is required at randomization and prior to baseline liver biopsy, weight changes are frequently seen during the course of NASH trials. Weight loss during the trial could be specifically induced by the investigational agent [47, 77, 81] or could result from successful dietary and lifestyle changes favored by trial participation[109] Overall, weight loss

is beneficial for NASH patients, hence no other action besides carefully capturing changes during the trial is necessary. On the contrary, weight gain and, in particular, central fat is detrimental hence for medical and ethical reasons, all efforts should be made to reinforce dietary and lifestyle counseling. Aside from exceptional circumstances, weight gain during a trial is not massive enough to warrant approved anti-obesity medication and therefore we consider non pharmacological interventions sufficient for management during the trial. Weight gain can also be an expected side effect of some medications[112]. Weight changes can influence biochemical, metabolic or histological outcomes and therefore extensive documentation is necessary: to a minimum, body weight, waist circumference as a proxy of visceral fat mass and lower limb edemas should be captured.

Glycemic control and diabetes management

Patients without diabetes at baseline that experience rising glycaemia levels during a trial should be managed depending on the trial duration and the magnitude of increase, with the obvious exception of acute, life-threatening events. In short-term trials (6 months or less) small or moderate increases in glycemic levels could be managed through reinforcement of nutritional counseling, non-pharmacological interventions and pharmacological therapy can be deferred until the end of the trial. In longer trials a progressive increase in HbA1c (by 1.5% from baseline or above 7.0%) would require pharmacological intervention. These thresholds are only indicative and, if necessary, can be adapted to personalized glycemic targets according to national or regional guidelines. If possible and depending of the presence of associated comorbidities, it is important not to use drugs that could have an effect on trial endpoints, but instead widely recommended medications such as metformin, as well as sulfonylureas or DPP4 inhibitors, which are neutral on NASH progression[82]. Based on current data on drug efficacy in NASH, drugs to be avoided as first-line therapy include GLP-1 RAs and SGLT2i as these drugs could improve histological or biochemical parameters[32, 80]. However, it may be considered unethical, based on recent ADA/EASD recommendations, not to initiate GLP-1 RAs or SGLT2i therapy as a first option in case of concomitant ASCVD, as well as SGLT2i therapy in case of concomitant heart failure or CKD.[33]

Patients with T2D at baseline with deteriorating glycemic control should be managed by intensifying the antidiabetic medication. Worsening of glycemic control is usually due to progression of preexisting diabetes, independent of the investigational product itself which justifies intervention regardless of the length of the trial. In other cases, the investigational

product could cause worsening of glycemic control of variable intensity. If, in view of the investigator, glycemic control is inadequate to prevent long-term complications of diabetes (this would usually be the case when HbA1c rises above 7.5%) reinforcement of therapy is entirely acceptable from the trial participation point of view. This includes initiation of insulin therapy or even SGLT2i in patients at high cardiovascular risk (at least for trials with histological endpoints, since no data on histological improvement with SGLT2i is yet available). In contrast, introduction of GLP-1 *RAs* for managing glucose control should be a last recourse because of anticipated confounding hepatic effects of this class of drugs[32]. In the absence of a formal indication (for ASCVD management in particular), the initiation of GLP-1 RAs treatment may be reconsidered at the end of the clinical trial, in agreement with the diabetologist in charge of the patient. However, when the patient presents a major cardio-renal event during the trial, the initiation of GLP1 RAs or SGLT2i is justified. In case of the occurrence of ASCVD event, SGLT2i may be preferentially used if the clinical trial tests a GLP-1RA or another incretin-based therapy; conversely, a GLP-1 RA will be used in a clinical trial seeking to evaluate the efficacy of a SGLT2i. In case of heart failure or major renal event (doubling of plasma creatinine, appearance of macroproteinuria), treatment with SGLT2i may be initiated, including in non-diabetic patient.

Lipid changes

Because some of the drugs tested in NASH clinical trials may change lipid levels[46, 47], LDL-cholesterol concentrations should be carefully monitored during the study and each subject has to be evaluated according to: (1) the magnitude of LDL-C change in the context of the individual cardiovascular risk profile, (2) the risk-benefit assessment of statin or alternative lipid lowering therapy (ezetimibe, PCSK9 inhibitors)[43, 45] and (3) the trial's duration. If a NASH drug increases LDL-C levels, the magnitude of increase, the level of reversibility of such increase with statins and the potential increase in long-term cardiovascular risk should be understood. For patients who were not on lipid lowering drugs at baseline, and who experience LDL-C increases during short-term clinical trials (<6 months), lipid lowering therapy could be safely deferred. This will inform on the magnitude of increase to be expected with the specific investigational agent without increasing the cardiovascular risk for the patient. For those on lipid lowering drugs, doses should be up-titrated regardless of the duration of the trial if LDL-C increases exceed 15-20% from baseline. This will inform on the reversibility of LDL-C

changes with statin or other lipid lowering drugs. In case of elevated triglycerides, in long-term clinical trials, action should be taken depending upon triglyceride levels. Thus, in moderate to high hypertriglyceridemia (2.0 to 9.9 mmol/L), the priority should be the prevention of cardiovascular events and the primary goal is to achieve LDL-C or non-HDL-C targets (lifestyle changes and statins as appropriate). In patients with severe hypertriglyceridemia (≥10 mmol/L) occurring during the trial, the priority is to prevent acute pancreatitis and the primary goal is to reduce triglyceride levels (lifestyle measures, control of comorbidities, fibrates, omega 3 fatty acids)[113].

Arterial hypertension

Blood pressure lowering treatment should be adjusted during the clinical trial if repeated measures reveal uncontrolled blood pressure ≥160 mmHg or ideally, if blood pressure is above thresholds according to concomitant comorbid conditions (currently defined as ≥140/90 mmHg in patients without clinical CVD and low CV risk and ≥130/90 mmHg in patients with clinical CVD, high CV risk or associated comorbidities among T2D, chronic kidney disease, heart failure or peripheral artery disease). In patients with newly diagnosed arterial hypertension, the treatment should be initiated following local standard of care and national guidelines. There is insufficient data to believe that any of the antihypertensive medications would have an impact on NASH trial endpoints so the choice of the medication should be adapted to the patient's condition and guided by relevant guidelines.

Renal function

If arterial hypertension and diabetes are adequately controlled and other acute conditions excluded, the most probable causes for deterioration in renal function are nephrotoxicity induced by other drugs, contrast agents for imaging procedures or the investigational agent itself. Documentation of nephrotoxicity should go beyond the measurement of serum creatinine levels (for instance by measuring cystatin-C) as some drugs such as peroxisome proliferator-activated receptor (PPAR) alpha agonists can increase creatinine levels[114, 115] without consequences on renal function[116]. In case of a mild reduction in glomerular filtration rate from baseline but with levels maintained above 60 mL/min, creatinine levels should be monitored closely and investigators should check for concomitant nephrotoxic drugs and ensure the optimal control of comorbidities (especially T2D and high BP*).* These considerations are also relevant as some patients may be at the edge for normal estimated glomerular filtration rate

at study inception only to drop below the lower limit of 60 ml/min soon after study start. In case of severe renal impairment (glomerular filtration rate <30 mL/min), decisions regarding trial discontinuation should be considered on an individual basis, according to the known or suspected study drug nephrotoxicity, baseline renal function status and associated comorbidities and also on alterations in systemic exposure for renally cleared drugs.

Alcohol consumption

Alcohol consumption is a major disease modifier impacting biochemical and histological endpoints and clinical outcomes, which are critical in NASH trials. Investigators cannot assume that the baseline, self-reported consumption of alcohol is accurate and that it will not change during the trial. Moreover, documenting an increase in alcohol consumption during the trial is essential for a better understanding of changes in trial endpoints that could otherwise be attributable to the investigational agent. The objectives therefore are to identify covert alcohol consumption in excess of the allowed daily thresholds in order not to include in a NASH trial, patients with moderate or heavy alcohol consumption. Equally important is to document changes in alcohol consumption during the trial in order to asses the confounding effect of variations in alcohol consumption on the trial endpoints. The consequences of alcohol consumption should be interpreted in relation to biological sex, as women are more susceptible than men to alcohol effects, particularly for small/moderate amounts of alcohol[117]. Binge drinking, defined as episodic heavy alcohol consumption of ≥ 60 g/day in men and ≥ 48 g/day in women but less than 140 g/week has been associated with fibrosis progression[118], and should also be asked about.

Alcohol consumption can be documented through questionnaires and alcohol biomarkers. Validated questionnaires such as the Alcohol Use Disorders Identification Test (AUDIT) or the Timeline Follow-Back (TLFB) can be used, with the AUDIT being a short questionnaire designed to identify at-risk alcohol consumption while the TLFB being a much more detailed assessment of number, volume and type of alcoholic beverages over a defined period of time (typically 1 week to 1 month)[119]. An open and destigmatizing approach by the research team, prior to completing the questionnaire is crucial for collecting reliable data. Currently recommended alcohol biomarkers are[120]: urine ethyglucuronide (which measures alcohol exposure over the past 2-5 days), hair ethylglucuronide (which measures alcohol exposure over the past 3-6 months) and blood phosphatidylethanol (PEth) which documents alcohol exposure over the past 2-4 weeks[121] with very high sensitivity and specificity[122].

Since abstinence is not a prerequisite for inclusion in NASH trials, urine ethylglucuronide measurments are of limited interest. Despite the demonstration of the value of hair ethyglucuronide detection in NAFLD patients[123] and the fact that this test is associated semi-quantitatively with alcohol consumption, there may be issues with patient acceptance, sample availability, complex handling and false positive results[124] which reduces its utility for NASH trials. Ranges of PEth levels are associated with no or minimal drinking (<20 ng/ml), low/moderate drinking (2-3 drinks per day, 20-80 ng/ml) or excessive drinking (4 drinks per day or more, 80-200 ng/ml)[124]. Although not yet validated in this context, we recommend that PEth levels be measured at baseline, at end of treatment and, possibly, every 3-4 months at scheduled study visits thus allowing to document (and quantify) changes in alcohol consumption. This could be a first step towards accounting for alcohol consumption as a potential confounder of biochemical or histological trial endpoints. As far as questionnaires, AUDIT may be sufficient in patients with dully demonstrated abstinence (including repeat PEth levels). In those consuming alcohol at baseline or during the trial, the option of using TLFB should be considered. Finally, in case of significant daily consumption (anywhere above 50 g per day) or binge drinking, if documented more than once, trial discontinuation should be strongly advised. The conditions that led to an increase in alcohol consumption, in particular depression or other psychiatric disorders should be explored. Conversely, mild to moderate (e.g.<50 g/day) consumption of alcohol occurring during the trial should be discouraged but could be tolerated, particularly in long term phase 3 or outcome trials. Indeed, future NASH drugs need to show some benefit even against low/moderate levels of alcohol consumption which can commonly coexist with NASH in real-world settings.

CONCLUSIONS

Randomized trials are designed to test drug performance in tightly controlled settings thus fulfilling methodological requirements for equal distribution of known confounders in all but the studied intervention. Patients with NASH, however, largely differ in terms of associated comorbidities, their severity and level of control by different medications. Moreover, trial participants can also experience changes in the status of these comorbidities during the trial, especially since some of the trials are of year(s)-long duration. These comorbidities and their management introduce a substantial source of heterogeneity between trial participants with potential impact on assessing trial outcomes, adverse events and patient compliance, not to

mention the applicability of trial results to real-life effectiveness. Hence, in this document, we provide guidance in defining acceptable boundaries in the management of these comorbidities. These boundaries should be compatible with the methodological requirement of clinical trials and with the specifics of NASH but also with the medical and ethical standards individual patients are entitled to. We acknowledge the large variations in standard of care around the globe and the fact that precise official recommendations from different regional and national societies exist and which this document is not intended to replace. Therefore, we chose not to issue precise and detailed algorithms but rather to favor a flexible approach based on patterns of practice and already existing recommendations. Our guidance is intended to operate within a framework that is compatible with the specifics of NASH as a multisystem disease but also with concerns about trial feasibility and integrity. This guidance should be revisited as concepts, therapies and standard of care evolve in the foreseeable future.

Table 1. Interplay between NASH and its most frequent comorbidities.

	Epidemiology	Outcomes			Treatment impact		
			Impact on extra-hepatic outcomes		Of comorbidities on NASH		Of NASH on comorbidities[§§]
	Prevalence* among NASH	Impact** on liver related outcomes[***]	Non-liver cancer	CV disease	Hepatic histology	Liver-related outcomes[***]	
Overweight/Obesity	High/very high	Probable	Established	Established	Established, regression	Established[#]	Neutral
Type 2 diabetes	Medium/high	Established	Established	Established	Probable, regression[§]	Uncertain	Neutral
High blood pressure	Medium	Uncertain	Neutral	Established	Neutral	Neutral	Neutral
Dyslipidemia	Medium	Neutral	Neutral	Established	Uncertain[¶]	Uncertain[¶]	Neutral
Obstructive sleep apnea	Low	Uncertain	Neutral	Uncertain	Neutral	Neutral	Neutral

* ***Prevalence***: low: < 40%; medium: 40% – 50%; high: 50% – 75%; very high: 75%;

** ***Level of evidence:*** *Established*: large amount of data, good level of evidence; *Probable*: suggestive but not definitive demonstration; *Uncertain*: conflicting data or low level of evidence; *Neutral*: no data or no impact so far in most/all studies.

*** ***Liver related outcomes***: Progression to cirrhosis, cirrhosis decompensation, hepatocellular carcinoma, liver transplantation

[§] For GLP1-RAs and pioglitazone

[¶] Retrospective data for statins

[#] Established for massive weight loss following bariatric surgery.

[§§] There are no data to support the impact of NASH resolution on the associated metabolic comorbidities as a direct consequence of NASH improvement, (i.e. independent of extrahepatic, direct effects on metabolic dysregulation).

Table2. Abbreviated recommendations for the management of major metabolic comorbidities before inclusion in NASH therapeutic trials.

TYPE 2 DIABETES	HIGH BLOOD PRESSURE	DYSLIPIDEMIA	WEIGHT
Newly diagnosed T2D: initiate life style changes and specific treatment as appropriate according to local or international guidelines. -Delay inclusion/liver biopsy by at least 6 months if initiation of GLP1 R ag or pioglitazone or 3 months if other antidiabetic therapies **Known T2D**- optimize glycemic control: Consider individualized glycemic targets - Patients with non-optimal but "acceptable" diabetes control (e.g. HbA1c ≤ 9.0%, 75 mmol/mol) may be included in therapeutic trials - For antidiabetic drugs that reduce liver fat content (pioglitazones, GLP1 RAs, SGLTi) a stable dose is required for at least 6 month before LB	If **uncontrolled blood pressure** (> 160/90 mmHg) follow local or international guidelines and consider either dose escalation of an existing antihypertensive drug or introducing a new drug. Stable dose of antihypertensive drugs is not required as there are no data to support their beneficial effect on liver histology.	Clinicians should use local or international guidelines to reach optimal **lipid target (LDL-C or non-HDL-C) according to the individual CV risk**, particularly in longer (≥ 1 year) trials. Optimal lipid control is not mandatory for shorter (< 6 months) trials. As appropriate, consider: - starting a statin - statin dose escalation - add ezetimibe and/or PCSK9 inhibitors	Allow a maximum 5% weight change between baseline liver biopsy and randomization (6 month period)

CV: Cardiovascular**; GLP1 RAs:** glucagon-like protein 1 receptor agonists**; HDL-C:** high density lipoprotein cholesterol; HbA1c: glycated hemoglobin; **LB:** liver biopsy**; LDL-C:** low density lipoprotein cholesterol;**SGLT2i:** sodium-glucose transporter 2 inhibitors; **T2D**: Type 2 diabetes

Table 3. Abbreviated recommendations for the management of major metabolic comorbidities after inclusion in NASH therapeutic trials

TYPE 2 DIABETES	HIGH BLOOD PRESSURE	DYSLIPIDEMIA	WEIGHT
In short term trials (< 6 months) favor non-pharmacological measures and nutritional counseling. If possible defer therapeutic intervention until after trial completion. In longer trials (≥ 1 years) consider adapting treatment if HbA1c ≥ 7.0% or if there is > 1.5 % increase in HbA1c from baseline. Consider dose escalation of existing treatment or introducing a new drug according to guidelines. Favor drugs without impact on liver histology (e.g. Metformin, Sulfonlyureas, or DPP4i and if possible avoid GLP1 RAs.	Treat to target blood pressure in accordance with local or international guidelines, concomitant comorbid conditions and CV risk.	In short-term trials (< 6 months), lipid-lowering therapy could be deferred in case of LDL-C increase in those patients that are not already taking statins. In patients already taking statins, for every 15-20% increase in LDL-C, the dose of statin should be up-titrated regardless the duration of the clinical trials. If necessary, new drugs (ezetimibe or PCSK9 inhibitors) can be added. In case of mild hypertriglyceridemia (2 to 9.9 mmol/l) occurring during the trial, statin therapy should be continued and the priority should be given to the prevention of CV events. In case of severe hypertriglyceridemia (≥ 10 mmol) statins should be discontinued and the priority should be given to prevention of acute pancreatitis (start fibrates, omega 3, etc).	Monitor changes in weight and compliance with diet and lifestyle recommendations Aside from exceptional circumstances avoid initiating treatment for weight loss with weight loss agents

CV: cardiovascular; **DPP4 i**: dipeptidylpeptidase-4 inhibitors; **GLP1 RAs**: glucagon-like protein 1 receptor agonists ; **HbA1c**: glycated hemoglobin; **LDL-C**: low density lipoprotein cholesterol; **PCK9**: proprotein convertase subtilisin/kexin type 9.

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Table 1. Interplay between NASH and its most frequent comorbidities.

	Epidemiology	Outcomes			Treatment impact		
			Impact on extra-hepatic outcomes		Of comorbidities on NASH		Of NASH on comorbidities[§§]
	Prevalence* among NASH	Impact** on liver related outcomes[***]	Non-liver cancer	CV disease	Hepatic histology	Liver-related outcomes[***]	
Overweight/Obesity	High/very high	Probable	Established	Established	Established, regression	Established[#]	Neutral
Type 2 diabetes	Medium/high	Established	Established	Established	Probable, regression[§]	Uncertain	Neutral
High blood pressure	Medium	Uncertain	Neutral	Established	Neutral	Neutral	Neutral
Dyslipidemia	Medium	Neutral	Neutral	Established	Uncertain[¶]	Uncertain[¶]	Neutral
Obstructive sleep apnea	Low	Uncertain	Neutral	Uncertain	Neutral	Neutral	Neutral

Prevalence: low: < 40%; medium: 40% – 50%; high: 50% – 75%; very high: 75%;
*** Level of evidence:** *Established*: large amount of data, good level of evidence; *Probable*: suggestive but not definitive demonstration; *Uncertain*: conflicting data or low level of evidence; *Neutral*: no data or no impact so far in most/all studies.
****Liver related outcomes***: Progression to cirrhosis, cirrhosis decompensation, hepatocellular carcinoma, liver transplantation
[§] For GLP1-RAs and pioglitazone
[¶] Retrospective data for statins
[#] Established for massive weight loss following bariatric surgery.
[§§] There are no data to support the impact of NASH resolution on the associated metabolic comorbidities as a direct consequence of NASH improvement, (i.e. independent of extrahepatic, direct effects on metabolic dysregulation).

Table2. Abbreviated recommendations for the management of major metabolic comorbidities before inclusion in NASH therapeutic trials.

TYPE 2 DIABETES	HIGH BLOOD PRESSURE	DYSLIPIDEMIA	WEIGHT
Newly diagnosed T2D: initiate life style changes and specific treatment as appropriate according to local or international guidelines. -Delay inclusion/liver biopsy by at least 6 months if initiation of GLP1 R ag or pioglitazone or 3 months if other antidiabetic therapies **Known T2D**- optimize glycemic control: Consider individualized glycemic targets - Patients with non-optimal but "acceptable" diabetes control (e.g. HbA1c ≤ 9.0%, 75 mmol/mol) may be included in therapeutic trials - For antidiabetic drugs that reduce liver fat content (pioglitazones, GLP1 RAs, SGLTi) a stable dose is required for at least 6 month before LB	If **uncontrolled blood pressure** (> 160/90 mmHg) follow local or international guidelines and consider either dose escalation of an existing antihypertensive drug or introducing a new drug. Stable dose of antihypertensive drugs is not required as there are no data to support their beneficial effect on liver histology.	Clinicians should use local or international guidelines to reach optimal **lipid target (LDL-C or non-HDL-C) according to the individual CV risk**, particularly in longer (≥ 1 year) trials. Optimal lipid control is not mandatory for shorter (< 6 months) trials. As appropriate, consider: - starting a statin - statin dose escalation - add ezetimibe and/or PCSK9 inhibitors	Allow a maximum 5% weight change between baseline liver biopsy and randomization (6 month period)

CV: Cardiovascular**; GLP1 RAs:** glucagon-like protein 1 receptor agonists**; HDL-C:** high density lipoprotein cholesterol; HbA1c: glycated hemoglobin; **LB:** liver biopsy**; LDL-C:** low density lipoprotein cholesterol;**SGLT2i:** sodium-glucose transporter 2 inhibitors; **T2D**: Type 2 diabetes

Table 3. Abbreviated recommendations for the management of major metabolic comorbidities after inclusion in NASH therapeutic trials

TYPE 2 DIABETES	HIGH BLOOD PRESSURE	DYSLIPIDEMIA	WEIGHT
In short term trials (< 6 months) favor non-pharmacological measures and nutritional counseling. If possible defer therapeutic intervention until after trial completion. In longer trials (≥ 1 years) consider adapting treatment if HbA1c ≥ 7.0% or if there is > 1.5 % increase in HbA1c from baseline. Consider dose escalation of existing treatment or introducing a new drug according to guidelines. Favor drugs without impact on liver histology (e.g. Metformin, Sulfonlyureas, or DPP4i and if possible avoid GLP1 RAs.	Treat to target blood pressure in accordance with local or international guidelines, concomitant comorbid conditions and CV risk.	In short-term trials (< 6 months), lipid-lowering therapy could be deferred in case of LDL-C increase in those patients that are not already taking statins. In patients already taking statins, for every 15-20% increase in LDL-C, the dose of statin should be up-titrated regardless the duration of the clinical trials. If necessary, new drugs (ezetimibe or PCSK9 inhibitors) can be added. In case of mild hypertriglyceridemia (2 to 9.9 mmol/l) occurring during the trial, statin therapy should be continued and the priority should be given to the prevention of CV events. In case of severe hypertriglyceridemia (≥ 10 mmol) statins should be discontinued and the priority should be given to prevention of acute pancreatitis (start fibrates, omega 3, etc).	Monitor changes in weight and compliance with diet and lifestyle recommendations Aside from exceptional circumstances avoid initiating treatment for weight loss with weight loss agents

CV: cardiovascular; **DPP4 i**: dipeptidylpeptidase-4 inhibitors; **GLP1 RAs**: glucagon-like protein 1 receptor agonists ; **HbA1c**: glycated hemoglobin; **LDL-C**: low density lipoprotein cholesterol; **PCK9**: proprotein convertase subtilisin/kexin type 9.