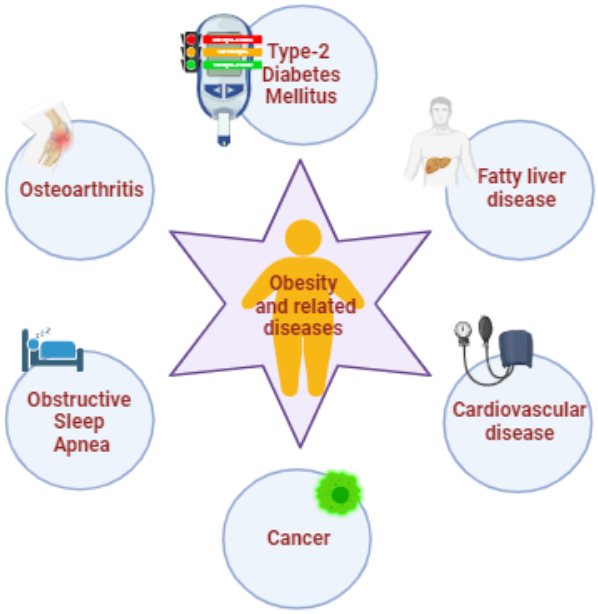
# Therapeutic Concepts for Obesity and Related Diseases

According to the World Health Organisation (2016), more than 1.9 billion adults, 18 years and older, were overweight. Of these, over 650 million were obese. At current, most of the world's population live in countries where overweight and obesity kills more people than underweight. In view of these alarming figures, health programmes and drug developments have been initiated worldwide to promote healthy lifestyles and prevent the development of obesity and related diseases in all ages.




*Created by BioRender.com*

Obesity together with physical inactivity, insulin resistance, and genetic predisposition are the leading causes of the *Metabolic Syndrome* that puts people at higher risk of cardiovascular diseases (coronary heart disease, heart failure, or stroke), Type-2 Diabetes Mellitus (T2DM), and diseases related to fat accumulation in organs such as Non-alcoholic Fat Liver Disease/ Non-Alcoholic Steato-Hepatitis (NAFLD/NASH). Various innovative treatment strategies based on incretin mimetics such as glucagon like agonists on the peptide 1 receptor (GLP1R) or agonists on the glucose dependent insulinotropic peptide receptor (GIPR) are now in development as therapeutic options to treat obesity and its related diseases.

The **Charité Research Organisation GmbH** has longstanding experience in the conduction of early phase projects for new medicines to improve metabolic diseases. We want to share our expertise in this special Whitepaper that comes in three parts.

In **Part 1**, new treatment approaches for *obesity with or without T2DM* are presented and discussed based on current research and applicable guidelines. The management of T2DM patients has much improved since the introduction of GLP1R agonists and sodium-glucose cotransporter 2 inhibitors (SGLT2i). Their use as treatment intensification for T2DM patients with cardiovascular and/or renal disease and/or corresponding risk factor obesity will be described according to national guidelines.

In **Part 2**, the key drivers in the pathophysiology of NAFLD/NASH and the recommended diagnostic tools to be used in clinical trials will be discussed. The recently approved THR-ß agonist Resmetirom and other pharmacological approaches for the treatment of *NAFLD/NASH with or without obesity* will be presented. Most of these are targeting metabolic dysregulation and intrahepatic lipotoxicity or inflammation. Other strategies include modification of genetic risk factors or activation of inflammatory immune cells.

In **Part 3**, the diverse phenotypes of heart failure (HF) will be discussed with a special focus on the *cardiometabolic phenotype HF with preserved ejection fraction (HFpEF)*, in which chronic cardiometabolic stress resulting from T2DM and/or obesity are key drivers of HF pathophysiology. The new treatment options for the cardiometabolic HFpEF population will be described based on the recent focused update of the ESC Guideline in 2023.

## Introduction

Human being is seemingly genius to circumvent its natural limits. Magnificent monuments like the Pyramids of Giza in Egypt or the Colosseum in Rome bear witness to his genius.

Since then the big *Industrial Revolutions*, *Scientific-Technical Revolution* and now the *Digital Evolution* have made even the unbelievable possible. Reaching out to the moon and the stars - Whatever it takes! Any challenge is welcome, no hurdle too high. If not in real - the virtual is fine. Quantum computing, virtual and augmented reality, nanotechnology, driverless technology, artificial intelligence, incredible fast internet, workplace automation, robotics, reusable rockets will soon be part of our everyday lives. Everything around us is already moving faster than we ever could. Every tool we use is becoming smaller, but we are becoming fatter and fatter just moving our brains but almost no muscle.

### What are the numbers we are talking about?

According to the World Health Organisation (WHO), obesity has nearly tripled since 1975. In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these, over 650 million were obese. Most of the world's population lives in countries where overweight and obesity kills more people than underweight [WHO 2024]. In view of these alarming figures, health programmes have been initiated worldwide to promote healthy lifestyles and prevent the development of obesity in all age groups. Education on healthy eating and avoiding a sedentary lifestyle is a top priority. For example, it is important to avoid soft drinks with high-fructose corn syrup as a sweetener as those increase the consumption of dietary fructose and contribute to the manifestation of overweight and/or obesity. In addition, the consumption of high-fat red meat should be reduced.

### But how is overweight/obesity actually defined and what health risks can be derived from it?

Overweight and obesity mean abnormal or excessive fat accumulation in the body that may impair health. The Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters ($kg/m^2$). For adults, overweight is a BMI ≥ 25; and obesity is a BMI ≥ 30 (**Figure 1**).

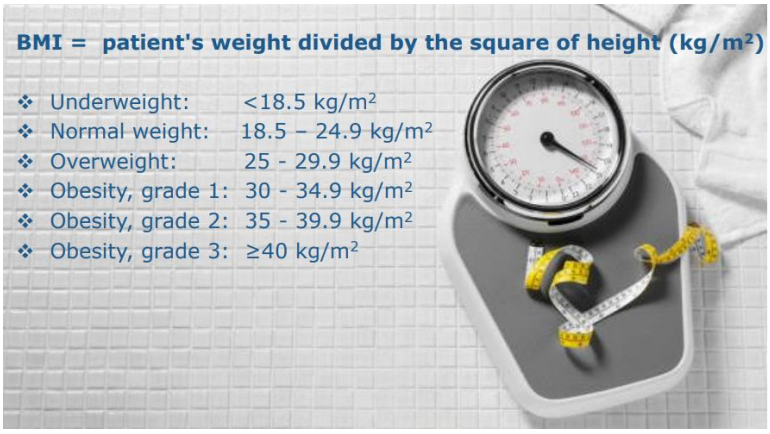



**Figure 1: BMI Categories** [WHO 2000]

Obesity together with physical inactivity, insulin resistance, and genetic predisposition are the leading causes of the *Metabolic Syndrome* [Hanefeld & Leonhardt 1981] [**Figure 2**]. Metabolic syndrome including obesity puts people at higher risk of cardiovascular diseases (coronary heart disease and heart failure, or stroke), type-2 diabetes mellitus (T2DM), and diseases related to fat accumulation in organs such as non-alcoholic fat liver disease (NAFLD). In addition, obesity may provoke musculoskeletal disorders (especially osteoarthritis) and some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon). It is estimated that 4–9% of all cancer diagnoses are attributable to obesity, and that obesity correlates with poorer prognosis for multiple malignant diseases (reviewed in Müller et al. 2022]. Obesity can consequently lead to serious diseases. Thus, it is of utmost importance to implement prevention programs stimulating life-style changes in the population and to develop drugs to treat obesity and related diseases.

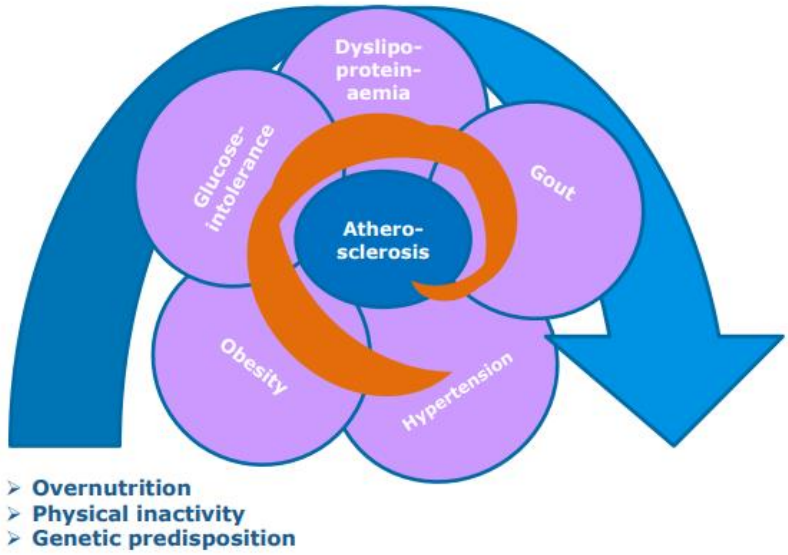



**Figure 2: Metabolic Syndrome**
*Source: Adopted from Hanefeld & Leonhardt 1981*

Various innovative treatment strategies based on incretin mimetics such as glucagon like agonists on the peptide 1 receptor (GLP1R) or agonists on the glucose dependent insulinotropic peptide receptor (GIPR) are now in development as therapeutic options to treat obesity and related diseases. The GLP1R agonists Liraglutide and Semaglutide have been already approved for weight reduction and/or improved control of hyperglycemia in T2DM patients. Dual incretin mimetics are in development and some of them such as Tirzepatide (GIPR/GLP1R agonist), which has been approved by the FDA in 2023, will probably soon be approved in Europe. And, triple incretin agonists are already on the horizon such as Retatrutide (GIPR/GLP1R + glucagon receptor (GCR) agonist).

In the following, we will give an overview of the current management of *Obesity (body weight reduction)* based on applicable guidelines.

## Obesity

In July 2020, obesity was recognized as an independent chronic disease with a high tendency to re-occur. This change in status is inextricably linked to the right to treatment [National Diabetes Strategy-2020] and serves also to destigmatize the common belief that obese people have insufficient self-discipline. The basic therapy for obesity consists of nutrition, exercise and behavioral therapy. The treating physician should skillfully motivate a comprehensive change in lifestyle risk factors including self-management education, nutritional counselling (5:2 diet, Mediterranean diet), increasing physical activity, and psychosocial counselling (if indicated). As smoking is associated with an increased risk of diabetes, possibly through increased insulin resistance [Maddatu et al., 2017], smokers should give up tobacco consumption. The goal is to achieve a decrease of at least 5 % of the body weight for BMI 25-35 kg/m$^2$ and of more than 10 % of the body weight for BMI >35 kg/m$^2$ [German Interdisciplinary S3 Guideline, 2014]. As lifestyle changes are often not sufficient to achieve a significant and long-lasting weight loss, adding pharmacological and/or surgical interventions is often indicated. Bariatric surgery represents the most effective approach to weight loss, leading to decreased mortality from cardiovascular diseases or cancer by 30% and 23%, respectively [Carlsson et al. 2020a].

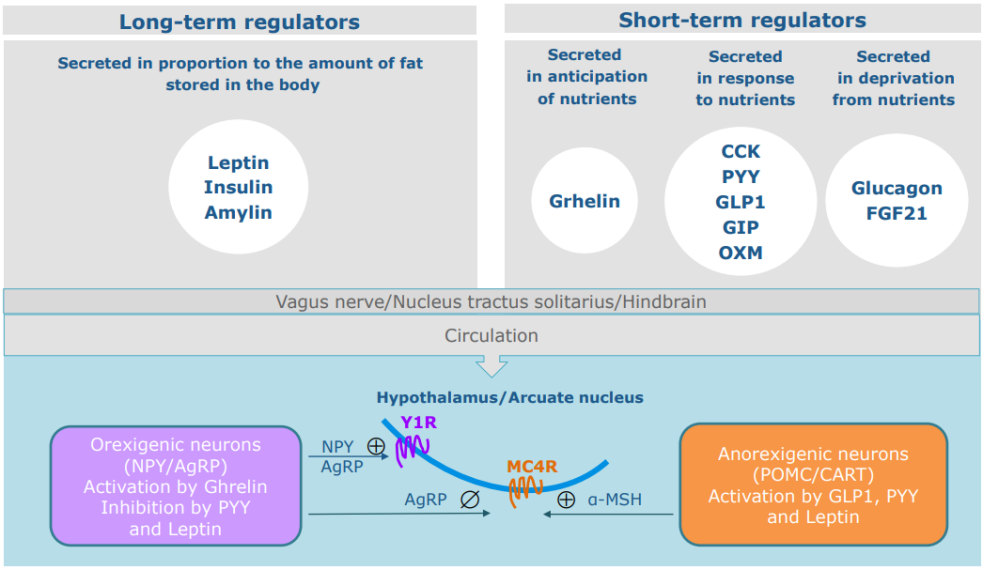



**Figure 3: Regulators of food intake signaling to the brain**
*Figure based on review by Müller et al., 2022*

In contrast, to achieve weight loss with adequate tolerability and safety long-term pharmacotherapy was until recently a seemingly impossible task. Many drugs that were initially authorised had to be withdrawn due to cardiovascular adverse effects (amfepramon), increased suicide risk (rimonabant) or an increased likelihood of dependence and abuse (methamphetamine). In Germany, the lipase inhibitor Orlistat (generic) is still used to reduce triglyceride absorption; however, therapy is often discontinued due to gastrointestinal side effects (fatty stools, flatulence) and impaired absorption of hormone preparations. In 2021, EMA approved the melanocortin-4 receptor analogue Setmelanotide as therapy for genetic obesity (confirmed biallelic proopiomelanocortin (POMC) or leptin receptor (LEPR) deficiency). Over the last two decades, our knowledge about the regulation of food intake has expanded enormously. We learned that various food intake-regulating hormones are involved in the central control of homeostatic and hedonistic eating behaviour [Müller et al., 2022] (**Figure 3**).

The gut-brain communication is driven by complex molecular communication between peripheral organs (gut, liver, and pancreas) and brain to avoid extreme situations such as starvation or overfeeding. The positive effects of the glucagon like peptide 1 (GLP1) on postprandial metabolism (improved glucose-dependent insulin secretion and insulin sensitivity of liver cells) led to the development of GLP1R agonists that are now integrate part of modern antidiabetic therapy. Weight loss however leads to a reactive influence on this complex gut-brain control system. Appetite and its hormonal mediators increase permanently. This is the biological reason for recurrent weight gain after dieting (*yo-yo effect*). If treatment is discontinued or reduced, a relapse inevitably occurs and long-term therapy seems unavoidable. The discovery that GLP1 receptor agonism in the arcuate nucleus (hypothalamus) causes an increased release of α-melanocyte stimulating hormone/activation of the melanocortin 4 receptor (MC4R) and a greater feeling of satiety, leading to weight loss has stimulated the development of new anti-obesity drugs such as Semaglutide (Wegovy®, NovoNordisk) that achieves clinical relevant and sustained weight loss without severe side effects [Müller et al., 2022; Rubino et al., 2021]. Similarly, the glucose dependent insulinotropic peptide (GIP) functions by improving insulin sensitivity in peripheral organs as well as in white adipose tissue and skeletal muscle [Samms et al., 2020]. The functional GIP-receptor (GIPR) is also expressed in human brain regions regulating food intake (such as hypothalamic nuclei and brainstem). As central or peripheral administration of GIPR agonists lowered weight by reducing caloric intake in animals, it is supposed that GIPR positive cells in the brain may enhance GLP1R signaling to anorexigenic neurons [Adriaenssens et al., 2019]. For these reasons, dual GLP1R/GIPR agonists have been developed such as Tirzepatide, which has been approved by the FDA in 2023 (and which will probably approved by EMA in 2024).

Finally, triple incretin mimetics are already on the horizon such as Retatrutide (GIPR/GLP1R and glucagon receptor (GCR) agonist). GCR agonism is known to delay gastric emptying, and reduces body weight by increasing energy expenditure with additional positive effects on lipid metabolism (reduced serum triglycerides and cholesterol). Retatrutide has already shown positive phase 2 results in overweight or obese people which will be discussed in more detail in the next chapter.

The new drug candidates for the treatment of obesity (body weight reduction) are described in detail in Part 1 of this Whitepaper, which can be accessed via this LINK: https://www.charite-research.org/de/white-paper-therapeutic-concepts-obesity-and-related-diseases-inc-type-2-diabetes-mellitus-part-1. In the following we focus on the pathophysiology and potential treatment approaches for obese patients with fat liver diseases including NAFLD/NASH.

## Obesity and NAFLD/NASH

*Non-Alcoholic Fat Liver Disease* (NAFLD) is the growing epidemic of the 21st century as it affects 25% of the global adult population and is the second-leading cause of end-stage liver disease and liver transplantation in Europe and America [Devarbhavi et al., 2023]. The term NAFLD covers a broad range of liver conditions without fibrosis with little to no symptoms affecting people with metabolic syndrome without significant alcohol intake. Overweight individuals with T2DM or a family member with NAFLD are at a higher risk to develop the disease. In approximately 20-30% of NAFLD patients the disease progresses to Non-alcoholic Steatohepatitis (NASH) [Yanai et al., 2023]. A sedentary life-style in combination with a hypercaloric diet, rich in saturated fatty acids, refined carbohydrates and fructose, but low in fibre favours overweight/obesity with a visceral fat distribution pattern. And, approximately 34% of obese people also have NASH [Quek et al., 2023].

*DELPHI Consensus 2023: New nomenclature for steatotic liver subclasses*

In recent years, there is emerging evidence that obesity and associated insulin resistance are pathological drivers of the metabolic dysfunctions observed also in patients with fatty liver disease regardless of the amount and pattern of alcohol intake [Eslam et al., 2020]. There are obviously overlapping biological processes that contribute to both NAFLD and alcohol-associated/related liver disease (ALD). Thus, the term "non-alcoholic" does not accurately capture the aetiology of NAFLD. And last but not least, the term "fatty" has been considered to be stigmatising by some stakeholders. Therefore, a new nomenclature of steatotic liver disease (SLD) subclasses was stipulated in the new DELPHI Consensus 2023 (**Figure 4**) introducing the terms *metabolic dysfunction associated steatotic liver disease* (MASLD) to replace NAFLD and *metabolic dysfunction-associated steatohepatitis* (MASH) to replace NASH. MASLD patients with an increased alcohol consumption are now classified as MetALD. Within MetALD, there is a continuum where, conceptually, the condition can be seen to be MASLD or ALD predominant. MASLD is present if at least one of five cardiometabolic risk factors is present in people with SLD. These are (pre-) diabetes, obesity, high blood pressure, elevated triglycerides and elevated LDL cholesterol. The renaming and categorization as SLD offers the opportunity to increase awareness of liver diseases in the future, to make the diagnosis more precise and to refer patients to appropriate monitoring at an earlier stage.

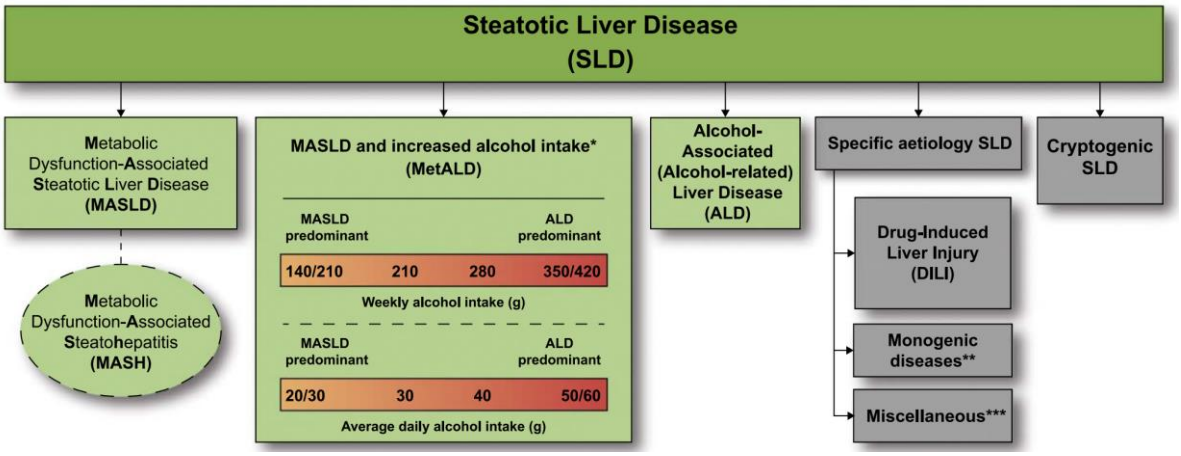



**Figure 4. Steatotic liver disease (SLD) subclassification**

SLD, diagnosed histologically or by imaging, has many potential aetiologies. Metabolic dysfunction associated steatotic liver disease (MASLD), defined as the presence of hepatic steatosis in conjunction with one cardiometabolic risk factor (CMRF) and no other discernible cause, ALD, and an overlap of the 2 (MetALD), comprises the most common causes of SLD. Persons with MASLD and steatohepatitis will be designated as metabolic dysfunction associated steatohepatitis (MASH). Within the MetALD group, there exists a continuum across which the contribution of MASLD and ALD will vary. To align with current literature, limits have been set accordingly for weekly and daily consumption, understanding that the impact of varying levels of alcohol intake varies between individuals. Other causes of SLD need to be considered separately, as is already done in clinical practice, given their distinct pathophysiology. Multiple aetiologies of steatosis can coexist. If there is uncertainty and the clinician strongly suspects metabolic dysfunction despite the absence of CMRF, this may be early MASLD and prompt additional testing (e.g., Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and oral glucose tolerance tests). Those with no identifiable cause (cryptogenic SLD) may be re-categorized in the future pending developments in our understanding of disease pathophysiology. Finally, the ability to provide an affirmative diagnosis allows for the coexistence of other forms of liver disease with MASLD, for example, MASLD + autoimmune hepatitis or viral hepatitis. *Weekly intake 140–350 g female, 210–420 g male (average daily 20–50 g female, 30–60 g male). **eg, Lysosomal acid lipase deficiency (LALD), Wilson disease, hypobetalipoproteinaemia, inborn errors of metabolism. ***eg, HCV, malnutrition, celiac disease, human immunodeficiency virus (HIV).

*Source: DELPHI Consensus 2023*

*Pathways leading to NASH*

Lipid accumulation in liver cells (steatosis) causes lipotoxicity - the key driver in the pathophysiology of NAFLD: Metabolic injury results in pathogenic cascades including endoplasmic reticulum (ER) stress, oxidative stress and mitochondrial dysfunction in hepatocytes. Lipotoxicity, as a consequence of free fatty acid (FFA) overload and increased de novo lipogenesis, leads to release of stress signals and induction of cell death mechanisms of the metabolically stressed hepatocyte, which in turn activate immune responses [Tacke et al., 2023] (**Figure 8**). The required life-style changes are however often ignored, and NAFLD may "silently" progress to a Non-Alcoholic Steato-Hepatitis (NASH) and become symptomatic with aggravating liver fibrosis. The manifestation of NASH and liver fibrosis may lead to serious diseases such as liver cirrhosis, liver cancer or liver failure, which are all associated with an increased mortality. Potential pharmacologic approaches target the metabolic dysregulation and injury of hepatocytes as well as extrahepatic inflammatory signals. Other strategies include modification of genetic risk factors, inflammatory activation of immune cells or reduction in metabolic stress on hepatocytes [Tacke et al., 2023; Wirth et al., 2022] (**Figure 5**).

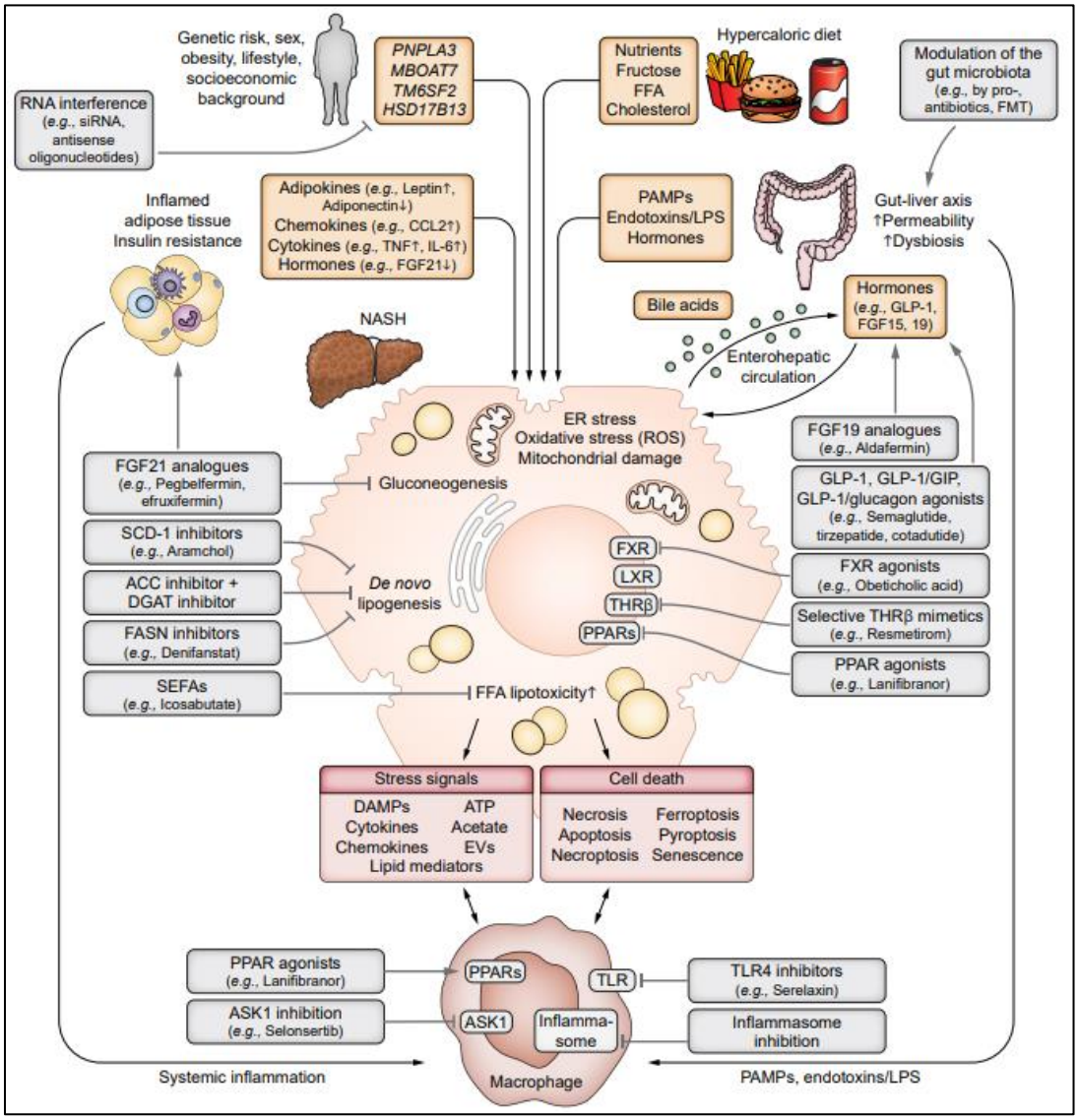



**Figure 5: From metabolic injury to inflammation: Targeting the activation of inflammatory cascades.**
*Source: Figure adopted from Tacke et al., 2023*

There is increasing evidence that a high number of key regulatory genes regarding liver homeostasis are directly mediated via thyroid hormone receptors (THRs). In chronic liver injury, a decrease in DIO1 activity coupled with an increase in DIO3 activity leads to increased conversion of T4 to *inactive* reverse T3. The resulting loss of THR-ß activity leads to accumulation of lipotoxic species, cyclic liver injury and *local intrahepatic hypothyroidism*. THR-ß activity in the liver is also impaired in NASH, leading to a reduction in mitochondrial function and beta-oxidation of fatty acids, which in turn drive inflammation and liver fibrosis. THR-β agonists, such as Resmetirom can selectively restore intrahepatic thyroid hormone function by increasing fatty acid β-oxidation, mitochondrial biogenesis, de novo lipogenesis, cholesterol and bile acid synthesis, and decreasing LDL cholesterol levels. [Karim & Bansal, 2023] (**Figure 6**).

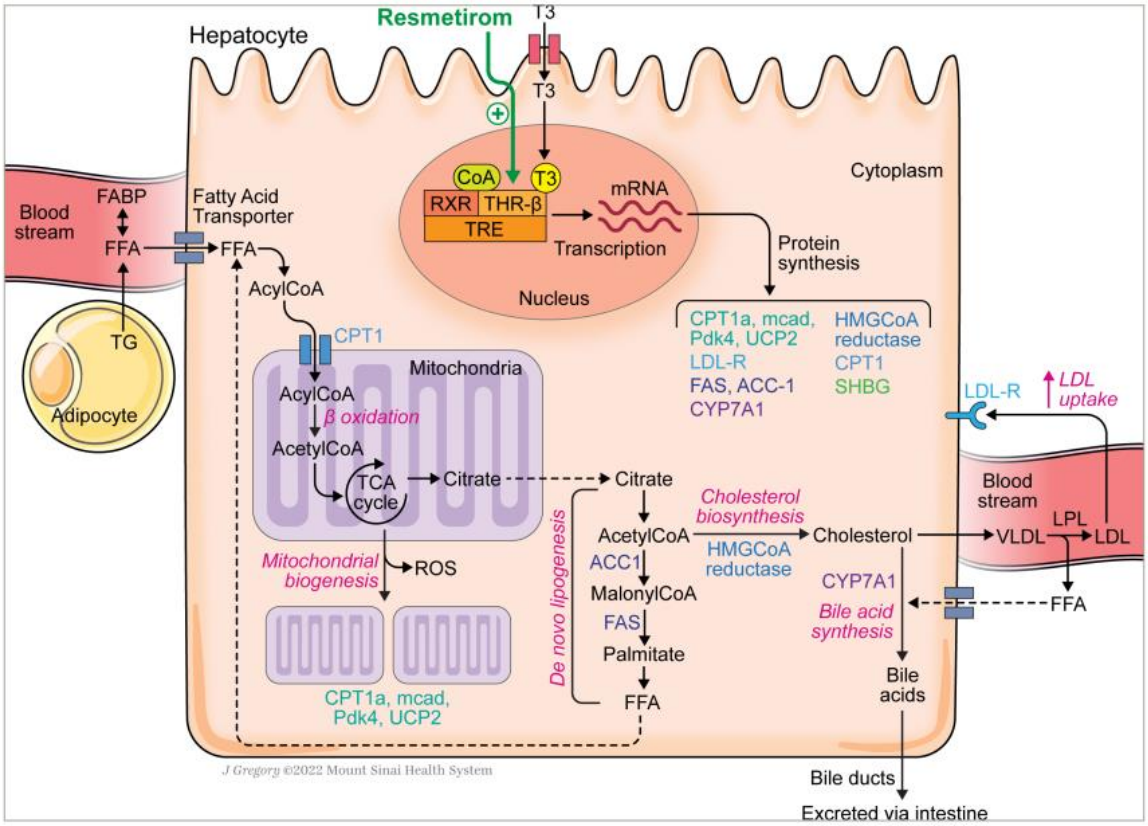



**Figure 6. Proposed hepatocyte pathways activated by thyroid hormone receptor-β agonists in NASH**

THR-β agonists, such as Resmetirom, modulate several genes that promote uptake of free fatty acids from both external sources (CPT1), as well as increased production and uptake of internal free fatty acids from de novo lipogenesis (ACC1, FAS) and lipophagy. Increased mitochondrial biogenesis (CPT1a, mcad, PDk4, UCP2) and mitophagy of unhealthy mitochondria increase capacity to metabolize and burn the increased flow of free fatty acids. While increased cholesterol is produced due to upregulation of HMGCoA reductase, bile acid synthesis increases through increased CYP7A1 and excretion, and LDL uptake by the liver increases by induction of the LDL-receptor. As a result, there is increase of fatty acid β-oxidation, mitochondrial biogenesis, de novo lipogenesis, cholesterol and bile acid synthesis, but decrease LDL cholesterol levels. The sex hormone binding globulin (SHBG), which transports androgens and oestrogens in blood and regulates their access to target tissues, is also a known downstream target of THR-β and may be a biomarker of target engagement

Abbreviations: ACC1 = acetyl coenzyme A carboxylase 1; acetylCoA = acetyl coenzyme A; acylCoA = acyl coenzyme A; CPT1 = carnitine palmitoyltransferase I; CYP7A1 = cholesterol-7-alpha-hydroxylase; CoA = coenzyme A; FABP = fatty acid binding protein; FAS = fatty acid synthase; FFA = free fatty acids; HMGCoA = 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; LDL = low-density lipoprotein; LDL-R = low-density lipoprotein receptor; mcad = medium-chain acyl-coenzyme A dehydrogenase; Pdk4 = pyruvate dehydrogenase kinase 4; ROS = reactive oxygen species; RXR = retinoid X receptor; SHPG = sex hormone binding globulin; T3 = triiodothyronine; TCA = tricarboxylic acid; TG = triglycerides; THR-β = thyroid hormone receptor β; TRE = tetracycline-responsive element; UCP2 = uncoupling protein 2; VLDL = very-low-density lipoprotein.

*Source: Karim & Bansal, 2023*

*Resmetirom - the first approved medication for NASH*

Until recently worldwide there were no approved medications available for the treatment of NAFLD or NASH. In March 2024, the selective agonist on the thyroid hormone receptor-ß (THR-ß) **Resmetirom** (Madrigal Pharmaceuticals) became the first drug which has been received the FDA approval for the treatment of NASH with liver scars due to fibrosis under the trade name Rezdiffra™.

The approval is based on the pivotal phase 3 MAESTRO-NASH with about 1750 patients, where resmetirom achieved both liver histological improvement endpoints: resolution of NASH and reduction of liver fibrosis. In both MAESTRO-NASH (NCT05500222) and the phase 3 safety study MAESTRO-NAFLD-1 (NCT04197479), LDL cholesterol and triglyceride were reduced by resmetirom and patients achieved potentially meaningful improvements in noninvasive measures of liver health.

Rezdiffra's label reflects the clinical safety results. The drug does not carry a boxed warning, and its label notes that the most common side effects are diarrhoea, nausea, pruritus, vomiting, constipation, abdominal pain, and dizziness.

*Incretin analogues as potential treatments for NASH*

The principle pathological drivers of MASH are obesity and associated insulin resistance, rendering them key therapeutic targets for incretin analogues such as the GLP1 receptor agonists and dual/triple agonists [Newsome and Ambery, 2023] **Figure 7**:

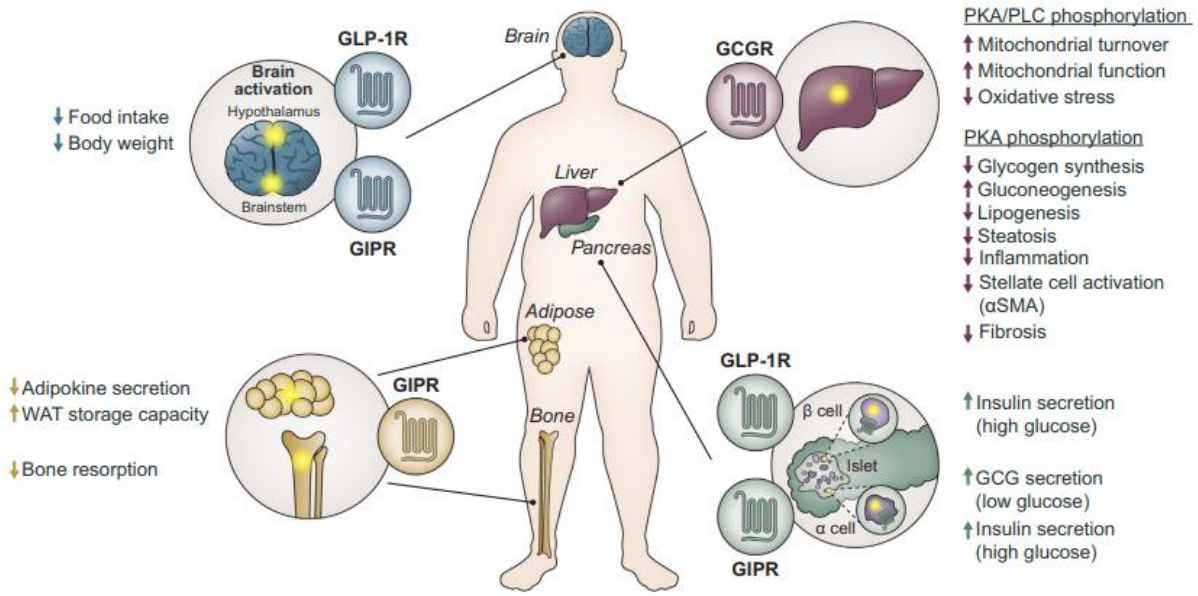



**Figure 7. Major modes and sites of action of the relevant incretin hormones/receptor agonists**

Abbreviations: GCG = glucagon, GCGR = glucagon receptor, GIPR = glucose-dependent insulinotropic peptide receptor, GLP-1R = glucagon-like peptide 1 receptor, WAT = white adipose tissue
*Source: Newsome and Ambery, 2023*

The results of the first phase 2 and phase 3 trials with incretin analogues in patients with NASH let assume the following beneficial effects [Newsome and Ambery, 2023]:

- Increased glucose-dependent insulin release and reduced glucagon levels in response to GLP1 agonist therapy are associated with a reduction in liver fat fraction, improvements in glucose control and a reduction in liver injury in patients with NASH.
- Glucagon agonist administration drives increased gluconeogenesis and glycogenolysis, reduced hepatic lipid accumulation, increased mitochondrial turnover, improved mitochondrial function, and reduced oxidative stress.
- GIP receptors are expressed within the central nervous system and modulation of these receptors is thought to regulate body weight and food intake. However whether GIP antagonism or agonism reflects the appropriate way to target these receptors is the subject of debate.
- Combinations of incretin analogues offer the potential for greater weight loss, as well as acting via synergistic mechanisms to enhance beneficial effects on liver injury.
- The efficacy of combination therapy needs to be considered alongside the increased risk of side effects, drug-drug interactions and costs.

Applying our understanding in the metabolic pathways and probable reasons for NASH, there is a bundle of compounds including incretin analogues which try to proof the efficacy in the indication. Since those mechanisms try to regulate essential metabolic pathways, the tolerability part in long-term treatment deserves great attention.

*NAFLD/NASH diagnostics in clinical trials*

Although NAFLD can be distinguished from NASH solely by histopathology, a liver biopsy does not qualify as routine diagnostics due to the associated potential for serious complications of this invasive method. In addition, the heterogeneous nature of NAFLD is not well covered by the small size of the biopsy sample. In clinical practice, a liver biopsy is justified only in reasonable cases with therapeutic implications and in specialised centres with a high intra- and inter-reader reliability for histopathology. The same holds true for clinical studies in individuals with NASH. In a large study that examined inter- and intra-reader variabilities, agreement among three pathologists on eligibility criteria was found in only 53.7% of patients [Davison et al., 2020]. Therefore, non-invasive biomarkers and /or tests have been established as scores, which are proven as useful for an approximate assessment of the disease stage and an evaluation of the need for a liver biopsy. These include both blood-based markers and imaging procedures [Harrison et al., 2023a] (**Figure 8**).

| NIT | Description/formula | Correlation with MALOs |
|---|---|---|
| **FIB-4** | Score based on age and simple blood tests (AST, ALT and platelets) | Baseline FIB-4 and change in FIB-4 independently associated with occurrence of MALOs |
| **NFS** | Score based on age, body mass index, diabetes status and simple blood tests (AST, ALT, platelets and albumin) | Baseline NFS and change in NFS independently associated with occurrence of MALOs |
| **ELF** | Score based on circulating markers of hepatic matrix turnover consisting of hyaluronic acid, TIMP-1 and propeptide of type III collagen | Cutoff of <9.8: rule out short-term occurrence of MALOs (negative predictive value=90%)<br>Cutoff of ≥11.3: fivefold increase in the risk of developing a MALO |
| **LSM** | FibroScan: measures the velocity of an elastic shear wave propagating through the liver | Baseline LSM and change in LSM independently associated with occurrence of MALOs |
| **MRE** | Imaging technique using a modified phase contrast pulse sequence to visualize rapidly propagating mechanical shear waves | Cutoff of ≥6.48 kPa independently associated with occurrence of MALOs |

MALOs, major adverse liver outcomes.

**Figure 8: Correlation between non-invasive tests and major adverse liver outcomes**

Abbreviations: ELF = enhanced liver fibrosis test, FIB-4 = fibrosis 4 score, LSM = liver-stiffness measurement, MRE = magnetic resonance elastography, NFS = NAFLD fibrosis score

*Source: Harrison et al., 2023a*

In addition, Magnetic Resonance Imaging (MRI)-derived proton density fat fraction (MRI-PDFF) has emerged as an accurate method to quantify liver steatosis for clinical trials. A reduction of more than 30% of fat fraction in MRI-PDFF has been associated with histological NASH improvement and is therefore frequently used as an efficacy endpoint, together with the average relative and absolute change in liver fat content [Loomba et al. 2021]. Taken together, carefully selected non-invasive tests, including blood-based ALT or enhanced liver fibrosis (ELF) tests, and ultrasound-based vibration-controlled transient elastography (VCTE; using the FibroScan device), in combination with MRI-PDFF will aid in interpreting changes in fat liver content in clinical trials that use histology as a primary endpoint. In addition, liver stiffness can accurately be quantified by magnetic resonance elastography (MRE) that recently has become a standard staging tool for liver fibrosis.

## *Pipeline Small Molecule-based drug developments for NAFLD/NASH*

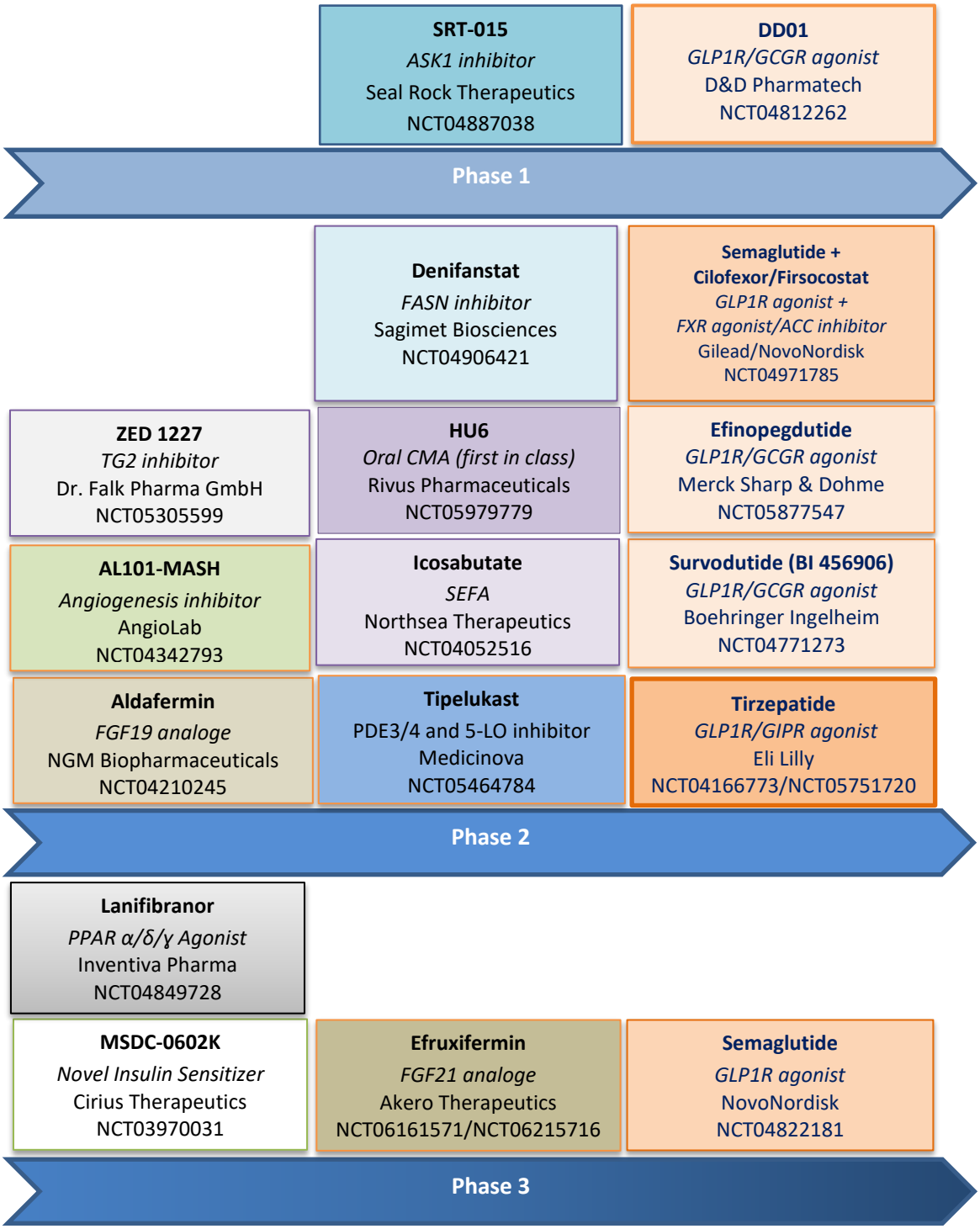



**Figure 9. Current Drug Pipeline for NAFLD/NASH with or without obesity**

ACC=Acetyl-CoA Carboxylase, ASK1 = Apoptosis Signal-regulating Kinase 1, FASN= Fatty Acid Synthase
FGF=Fibroblast Growth Factor, FXR= Farnesoid X Receptor, GCGR =Glucagon receptor
PNPLA3 = patatin-like phospholipase domain containing 3 protein, PPAR=Peroxisome Proliferator-Activator Receptor
SEFA=Structurally Engineered Fatty Acids, THR-ß=Thyroid Hormone Receptor β, TG2 = Transglutaminase 2

In the following, the above listed **new drug candidates** for the treatment of *NASH with or without obesity* are presented and discussed according to their development phase.

### *Phase 1*

**DD01** (D&D Pharmatech) is a *long-acting* dual agonist of GLP-1 and glucagon receptors. DD01 augments the benefits of incretin therapy by acting additionally through the glucagon receptor resulting in enhanced liver lipolysis. Treatment with DD01 caused significant weight loss, reduced liver fat, and improved glucose tolerance in various preclinical animal models of obesity, diabetes, and fatty liver, including non-human primates. The *first-time-in-human* trial (NCT04812262) was completed in December 2022. Eligible participants included T2DM who are overweight/obese for SAD part and obese with T2DM and NAFLD for MAD part with a minimum BMI of 30 kg/m$^2$ and hepatic fat contents of ≥10% determined by MRI-PDFF. Subcutaneous injections of DD01 were generally safe and well tolerated with gastrointestinal adverse events like those reported for selective GLP-1 receptor agonists. Following only 4 weeks of once-weekly treatment, up to 100% of patients achieved >30% liver fat reduction by MRI-PDFF. A mean relative reduction in liver fat content of >50% was observed in a pooled analysis of the two high doses of DD01, whereas the change from baseline in placebo-treated subjects was <5%. Rapid improvements in steatosis were observed at well-tolerated doses and accompanied by increased insulin, decreased HbA1c, and weight loss at higher doses. DD01 had a half-life of 7-8 days in obese/overweight patients with T2D and NAFLD. Based on these phase 1 results, the FDA has granted a Fast Track designation for the investigation of DD01 for the treatment of adults with MASLD or MASH [HTTPS://WWW.BIOSPACE.COM/ARTICLE/RELEASES/D-AND-AMP-D-PHARMATECH-GRANTED-FAST-TRACK-DESIGNATION-FROM-US-FDA-FOR-DD01-FOR-THE-TREATMENT-OF-NASH-MASH/].

**SRT-015** (Seal Rock Therapeutics) is a *second generation* oral inhibitor of the Apoptosis Signal-regulating Kinase 1 (ASK1) with a liver-preferential distribution in development for liver diseases including NASH. SRT-015 has demonstrated direct anti-fibrotic, anti-inflammatory, and anti-apoptotic activities *in vitro* and *in vivo*. SRT-015 has shown efficacy in multiple preclinical models of acute and chronic liver injury. Different stress stimuli induce reactive oxygen species (ROS) that result in the oxidation and dissociation of thioredoxin from ASK1 leading to ASK1 activation. In turn, activated ASK1 induces phosphorylation and activation of the JNK and p38 MAP downstream kinase cascades3 to result in apoptosis, inflammation and fibrosis, all key components of NASH [Elias et al., 2022] (**Figure 10**).

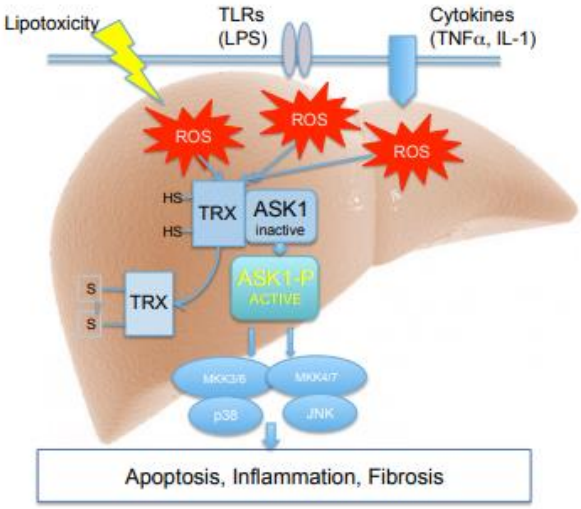



**Figure 10: Mechanisms of action of SRT-015 treatment in a NASH**

*Source: Elias et al., 2022. Poster presentation at The Liver Meeting® of the American Association for the Study of Liver Diseases (AASLD).*

Abbreviations: ASK-1 = Apoptosis Signal-regulating Kinase 1; TRX = Thioredoxin; ROS = Reactive Oxygen Species

SRT-015 has completed the *first-time-in-human* clinical trial (NCT04887038) in healthy volunteers, achieving therapeutically relevant exposure with an *excellent* safety and tolerability profile. SRT-015 significantly (P< 0.05) decreased galectin-3, an inflammatory monocyte marker and total liver lipids [Elias et al., 2022] (**Figure 11**).

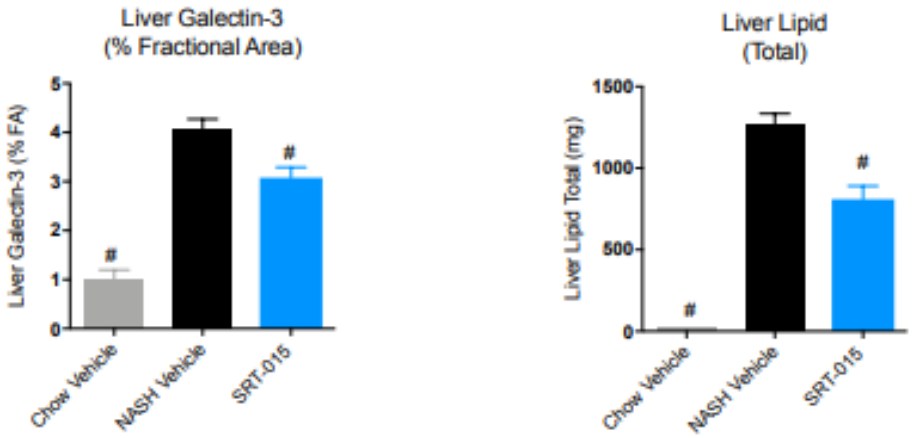



**Figure 11: SRT-015 decreased galectin-3 (an inflammatory monocyte marker) and liver lipids in healthy volunteers using a therapeutic model of NASH**
*Source: Elias et al., 2022. Poster presentation at The Liver Meeting® of the American Association for the Study of Liver Diseases (AASLD).*

### *Phase 2*

**AL101-MASH** (ALS L1023, AngioLab) is a dried extract of ethyl acetate, prepared by activity-guided fractionation from Melissa leaf (lemon balm). AL101-MASH is supposed to suppress liver fat accumulation by inhibiting angiogenesis, to protect liver cells from injury by inhibiting the activation of IL-6 and nitric oxide inflammatory pathways, and to improve fibrosis of liver cells by inhibiting matrix metalloproteinase activity. The potential efficacy of AL101-MASH to reduce steatosis and liver fibrosis was evaluated in a phase 2 trial (NCT04342793) in patients with NASH by MRI-PDFF and Magnetic Resonance Elastography (MRE). The trial was completed in May 2021, but no results have been published so far.

**Aldafermin** (NGM Biopharmaceuticals) is a biological analog of the human gut hormone FGF19 involved in bile acid synthesis and metabolism. Modifications within the amino terminus of Aldafermin eliminate tumorigenicity while retaining potent activity to suppress CYP7A1, which encodes the first and rate-limiting enzyme of bile acid synthesis (biased FGFR4-KLB receptor signaling). Since accumulation of bile acids is thought to be hepatotoxic, leading to progressive injury and dysfunction, Aldafermin may improve outcomes in cirrhotic NASH patients through modulation of bile acid metabolism [Sanyal et al., 2021]. Aldafermin also acts on the FGFR1c-KLB receptor to potentiate insulin sensitivity and fat metabolism [Lan et al., 2017].

The phase 2b results for Aldafermin in Patients with NASH with different fibrosis stages or cirrhosis have been recently published: The ALPINE 2/3 trial (NCT03912532) was conducted in patients with biopsy-confirmed NASH and stage 2 or 3 fibrosis. Patients stratified by fibrosis stage received placebo or Aldafermin once daily for 24 weeks. The primary endpoint (fibrosis improvement of at least one stage with no worsening of NASH) was not met. However, Aldafermin led to a significant, rapid, and dose-dependent reductions in liver fat and improved liver histology at 12 or 24 weeks [Harrison et

al., 2022]. In the ALPINE 4 trial (NCT04210245) patients with compensated NASH cirrhosis received once daily Aldafermin doses or placebo for 48 weeks. Aldafermin 3 mg resulted in a significant reduction in Enhanced Liver Fibrosis (ELF) score in patients with compensated NASH cirrhosis [Rinella et al., 2024] (**Figure 12**).

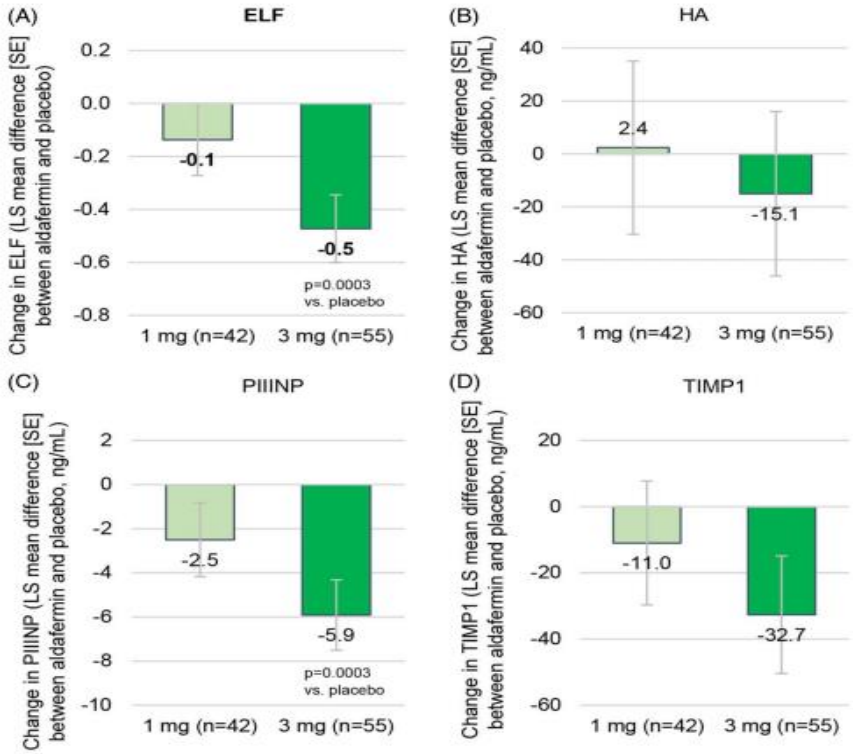



**Figure 12: Change from baseline in ELF score at week 48 (A: Primary Endpoint) and Change from baseline to week 48 in the individual components of ELF: HA (B), PIIINP (C), and TIMP-1 (D)**
Shown are LS mean differences between the aldafermin group (1 or 3 mg) and the placebo group.
Abbreviations: ELF = Enhanced Liver Fibrosis score, HA = hyaluronic acid, LS = least-squares, PIIINP = N-terminal pro-peptide of type III collagen, TMP-1 = Tissue inhibitor of metalloproteinase-1.
*Source: Rinella et al., 2024*

**Cilofexor** (Gilead Sciences) is a farnesoid X receptor (FXR) agonist and has shown positive "*proof of concept*" phase 2a results in 2020 (NCT03987074). Activation of the hepatocyte nuclear FXR by agonists like obeticholic acid or cilofexor, have been shown to improve the histological features of NASH. Consistently, clinical data with obeticholic acid reported an improvement of liver fibrosis in non-cirrhotic NASH, but not in patients with compensated cirrhosis [Younassi et al., 2019]. In 2023, the FDA Gastrointestinal Drug Advisory Committee voted however against obeticholic acid (Ocaliva, Intercept Pharmaceuticals) approval for NASH due to its unfavorable benefit-risk profile.

Cilofexor was tested in an open label, *proof of concept* trial (NCT03987074) in combination with Semaglutide in patients with mild-to-moderate liver fibrosis due to NASH. The combined treatment with Cilofexor and Semaglutide resulted in additional improvements in liver steatosis and biochemistry compared to Semaglutide alone [Alkhouri et al., 2022]. Gilead and NovoNordisk are currently conducting a Phase 2b double-blind, placebo-controlled study (NCT04971785) to investigate the safety and efficacy of the GLP1R agonist Semaglutide, and a fixed-dose combination of Cilofexor and Firsocostat, alone and in combination in people with compensated cirrhosis (F4) due to NASH. The four-arm study in approximately 440 patients will evaluate the treatments' impact on liver fibrosis improvement and NASH resolution. Study completion is expected in December 2024.

**Firsocostat** (GS-0976 from Gilead Sciences) is a liver-directed allosteric inhibitor of acetyl-CoA carboxylase (ACC). De novo lipogenesis (DNL) plays a major role in fatty acid metabolism and contributes significantly to triglyceride accumulation within the hepatocytes in patients with NASH. ACC1 primarily is present in the cytosol and catalyzes the first committed reaction in DNL. ACC2 primarily is present in the mitochondria and catalyzes the formation of malonyl-CoA which functions as a potent allosteric inhibitor of carnitine palmitoyl-transferase 1 (CPT1) thereby inhibiting the transfer of fatty acids into the mitochondria for β-oxidation. The net effect of activating both isoforms is an increase in hepatic TG and complex lipids leading to lipotoxicity. Therefore, inhibiting ACC pharmacologically represents an attractive approach for treating NASH [Alkhouri et al., 2020] (**Figure 13**).

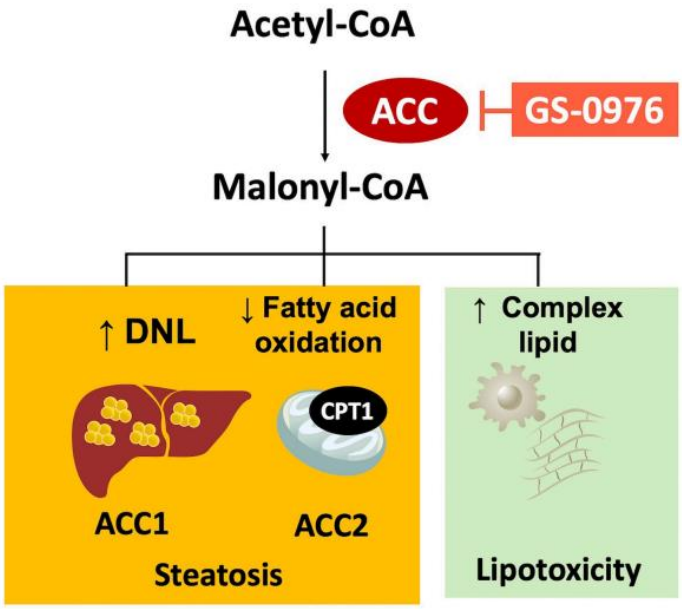



**Figure 13. Mechanism of action of Firsocostat**
Abbreviations: ACC = acetyl-CoA carboxylase; ACC1 = acetyl-CoA carboxylase; ACC2 = acetyl-CoA carboxylase 2; DNL = De novo lipogenesis
*Source: Akhouri et al., 2020*

A phase 2b trial (NCT04971785) is currently testing whether a fixed combination of Firsocostat and Cilofexor alone or in combination with Semaglutide may improve liver fibrosis and/or resolve NASH in patients with compensated cirrhosis (F4) due to NASH. Study completion is expected in Q42024.

**Denifanstat** (TVB-2640, Sagimet Biosciences Inc.) is a *first in class* oral Fatty Acid Synthase inhibitor (FASNi). Denifanstat has been evaluated in a randomized, double-blind, placebo-controlled Phase 2b trial (NCT04906421, FASCINATE-2) in NASH patients with fibrosis. Positive top-line results from this trial were announced in January 2024 demonstrating statistically significant improvements relative to placebo on both of the primary endpoints of NASH resolution without worsening of fibrosis with ≥2-point reduction in NAS, and ≥2-point reduction in NAS without worsening of fibrosis. Denifanstat-treated patients also showed statistically significant fibrosis improvement by ≥ 1 stage with no worsening of NASH, and a greater proportion of MRI-PDFF ≥30% responders relative to placebo. Based on these data, Denifanstat may be clinical effective in NASH patients without cirrhosis as backbone monotherapy or in combination with other drugs. In future, Denifanstat will be tested also in other NASH indications such as cirrhotic (F4) NASH and pediatric NASH.

**Efinopegdutide** (MK-6024, Merck Sharp & Dohme LLC) is a dual GLP1R/GCGR agonist (for mode of action, please refer to **Figure 7**) that is currently studied in a phase 2 trial (NCT05877547) *head to head* with Semaglutide compared to placebo in participants with pre-cirrhotic NASH. Study completion is expected in December 2025.

**Pemvidutide (ALT-801, Altimmun)** is a dual GLP1R/GCGR agonist (for mode of action, please refer to **Figure 7**) that is currently studied in a phase 2 trial (NCT05989711/IMPACT) compared to placebo in non-cirrhotic patients with NASH. Study completion is expected in September 2025.

**Survodutide** (BI 456906, Boehringer Ingelheim) is a dual GLP1R/GCGR agonist (for mode of action, please refer to **Figure 7**). In February 2024, Boehringer Ingelheim announced groundbreaking results of a phase 2 trial (NCT04771273) that evaluated weekly subcutaneous injections of Survodutide in adult patients with MASH and fibrosis (F1-F3) with and without T2DM. The trial met its primary endpoint with Survodutide reaching a biopsy-proven improvement in MASH after 48 weeks, without worsening of fibrosis stages F1, F2 and F3 (mild to moderate or advanced scarring). Up to 83.0% of adults treated with Survodutide achieved a statistically significant improvement of MASH versus placebo (18.2%) [Response difference: 64.8% (CI 51.1% - 78.6%), p<0.0001]. Survodutide also met all secondary endpoints, including a statistically significant improvement in liver fibrosis. [HTTPS://WWW.BOEHRINGER-INGELHEIM.COM/HUMAN-HEALTH/METABOLIC-DISEASES/SURVODUTIDE-TOP-LINE-RESULTS-MASH-FIBROSIS].

**HU6** (Rivus Pharmaceuticals, Inc) is a *first in class* oral controlled metabolic accelerator (CMA) that provides a novel, measured approach to activating proton leak and mitochondrial uncoupling, a natural process in the body that regulates and dissipates energy. HU6 is metabolized to 2,4-dinitrophenol (DNP) and increases proton leakage via adenine nucleotide translocase, thereby increasing fat oxidation and reducing the production of reactive oxygen species [Brand 2000]. DNP was used as a weight loss drug in the early 1930s, but was never approved as drug due to its high peak concentrations which induce potentially dangerous side-effects, such as hyperpyrexia and increased basal metabolic rate. HU6 was therefore designed as a controlled-release formulation that minimizes the rapid absorption and high peak blood concentrations of DNP to provide a wider therapeutic index and improve safety.

HU6 has shown positive results in a phase 2a trial (NCT04874233) in subjects with NASH and high BMI between 28.0 and 45.0 kg/m$^2$ (inclusive), where it met the primary endpoint of reducing liver fat (**Figure 14**) as well as secondary endpoints such as body weight loss, while conserving skeletal muscle mass. In addition, improvements in markers of insulin resistance and inflammation were observed [Noureddin et al., 2023].

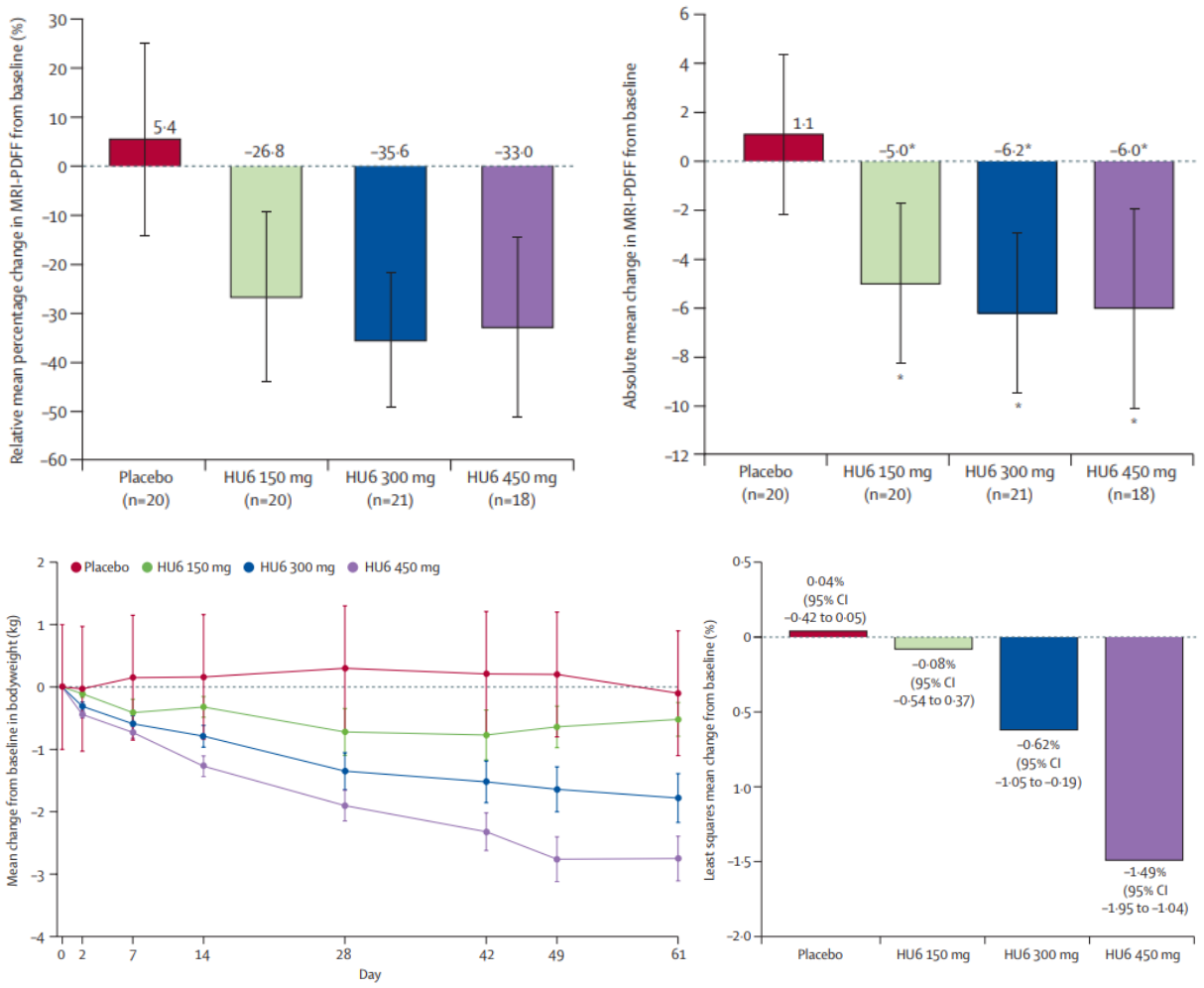



**Figure 14. Effects of HU6 on liver fat, body weight and glycated albumin from baseline to day 61.**

(A) Relative mean percentage change and absolute mean change from baseline to day 61 in liver fat (A). Error bars represent SDs.
(B) Relative mean percentage change in liver fat. Responder cutoff represents participants with at least a 30% reduction from baseline to day 61 in liver fat.
(C) Mean change in bodyweight. Error bars represent SDs.
(D) Percentage change from baseline to day 61 for glycated albumin.

*Source: Noureddin et al., 2023*

HU6 is now tested in a phase 2 trial (NCT05979779) to evaluate the safety and efficacy of three HU6 dose levels versus placebo in obese subjects with T2DM at risk of NASH. HU6 will be administrated daily for 6 months. Study completion is expected in November 2024.

**Icosabutate** (NST-4016, Northsea Therapeutics) is a structurally engineered fatty acid (SEFA*)*, a new class of fatty acids drugs. Icosabutate was studied in a Phase 2b clinical trial (NCT04052516, ICONA) to evaluate its safety and efficacy in participants with NASH. Positive interim results for the ICONA trial were announced in January 2021, showing significant decreases in NASH and fibrosis biomarkers independent of fibrosis stage and disease severity. Icosabutate was also well-tolerated, with no serious safety signals to date, a key attribute for patients with NASH, who require chronic therapy. Top-line 52-week histology results were expected in Q1-2023, but not published yet.

**Tipelukast** (MN-001, MediciNova) is a novel, orally bioavailable small molecule compound which has demonstrated anti-fibrotic and anti-inflammatory activity in preclinical models, including leukotriene (LT) receptor antagonism, inhibition of phosphodiesterases (PDE) (mainly 3 and 4), and inhibition of

5-lipoxygenase (5-LO). The 5-LO/LT pathway is considered to be a novel approach to treat liver fibrosis. MN-001 has been shown to down-regulate expression of genes that promote fibrosis including LOXL2, Collagen Type 1 and TIMP-1. MN-001 also down-regulates expression of genes that promote inflammation including CCR2 and MCP-1. In addition, histopathological data shows that MN-001 reduces fibrosis in multiple animal models.

A phase 2 trial (NCT05464784) is currently evaluating the safety, tolerability and efficacy of MN-001 in patients diagnosed with NAFLD, T2DM, and hypertriglyceridemia. The primary outcome measure is a composite of (i) the mean change in controlled attenuation parameter (CAP) score by sound-based elastography at week 24, and (ii) the mean change from baseline in fasting serum triglyceride levels at week 24. To be eligible for this study, NAFLD patients must have a FibroScan® CAP score ≥ 248 dB/m within 8 weeks of randomization. Study completion is expected in December 2024.

**ZED1227** (Dr. Falk Pharma and Zedira) is a synthetic peptidomimetic compound designed by Zedira scientists to specifically inhibit the enzymatic activity of human tissue transglutaminase (TG2). Following the successful completion of a phase 2a study of ZED1227 in patients with celiac disease [Büchold et al., 2022], Dr. Falk Pharma and Zedira decided to expand its development to NAFLD. A phase 2a *proof of concept* study (NCT05305599, NormaliZED) commenced in 2022 to investigate the efficacy and safety of three doses of ZED1227 in NAFLD patients with significant liver fibrosis stages F2 or F3. Study completion was expected in June 2023, but recruiting status is assigned as ongoing in April 2024.

**Tirzepatide** (Eli Lilly) is a dual GLP1R/GIPR agonist and currently tested in a phase 2 trial for NASH (NCT04166773). Tirzepatide or placebo is administered subcutaneously once weekly over a period of 52 weeks. Participants must be willing to undergo baseline and endpoint liver biopsies. The primary endpoint is the percentage of participants with absence of NASH with no worsening of fibrosis on liver histology. Other outcome measures include the percentage of participants with ≥1 point decrease in fibrosis stage with no worsening of NASH on liver histology, mean absolute change from baseline in liver fat content by MRI-PDFF, and the mean change in body weight. Study completion was expected in February 2024. The study status is assigned as active, not recruiting in April 2024.

Another phase 2 trial (NCT05751720) commenced in October 2023, to evaluate the effects of Tirzepatide on NAFLD in approximately 30 patients with T2DM. The intervention group will receive GLP-1 analogues (subcutaneous Tirzepatide or oral Semaglutide). The dose of oral Semaglutide will be 3 mg daily for 30 days (initiation) and if dose then increased to 7 mg daily for 6 months while the dose of Tirzepatide is 0.25 mg once weekly for 4 weeks then 0.5 mg once weekly. The blood tests will be done at baseline, 3 months of treatment and at 6 months of treatment. Liver imaging (fibroscan and/or MRI fat measurement) will be done at baseline and at 6 months to see if there is any change. Study completion is expected in February 2025.

### *Phase 3*

**MSDC-0602K** (Cirius Therapeutics) is a novel insulin sensitizer designed to preferentially target the mitochondrial pyruvate carrier while minimizing direct binding to the transcriptional factor PPARγ (peroxisome proliferator-activated receptor gamma). First-generation insulin sensitizers are used to treat T2DM, but are associated with side effects including edema, bone fractures, and hypoglycemia. MSDC-0602K is a second-generation insulin sensitizer designed to reduce these side effects. As an improvement of glucose metabolism is supposed to reduce liver injury, MSDC-0602K is currently in development as treatment for individuals with NASH and T2DM.

The previous Phase 2b clinical trial (NCT02784444, EMMINENCE) investigated 402 NASH subjects with biopsy-confirmed fibrosis (F1-F3), approximately 50% of whom were also diagnosed with T2DM. MSDC-0602K did not demonstrate statistically significant effects on primary and secondary liver histology endpoints. However, effects on non-invasive measures of liver cell injury (Aspartate aminotransferase) and glucose metabolism (glycated hemoglobin) support further exploration of MSDC-0602K's safety and potential efficacy in patients with T2DM and liver injury [Harrison et al., 2020] (**Figure 15**). Cirius is now *preparing a Phase 3 trial* in T2DM patients with NAFLD/NASH (NCT03970031) that is assigned as not yet recruiting in April 2024.

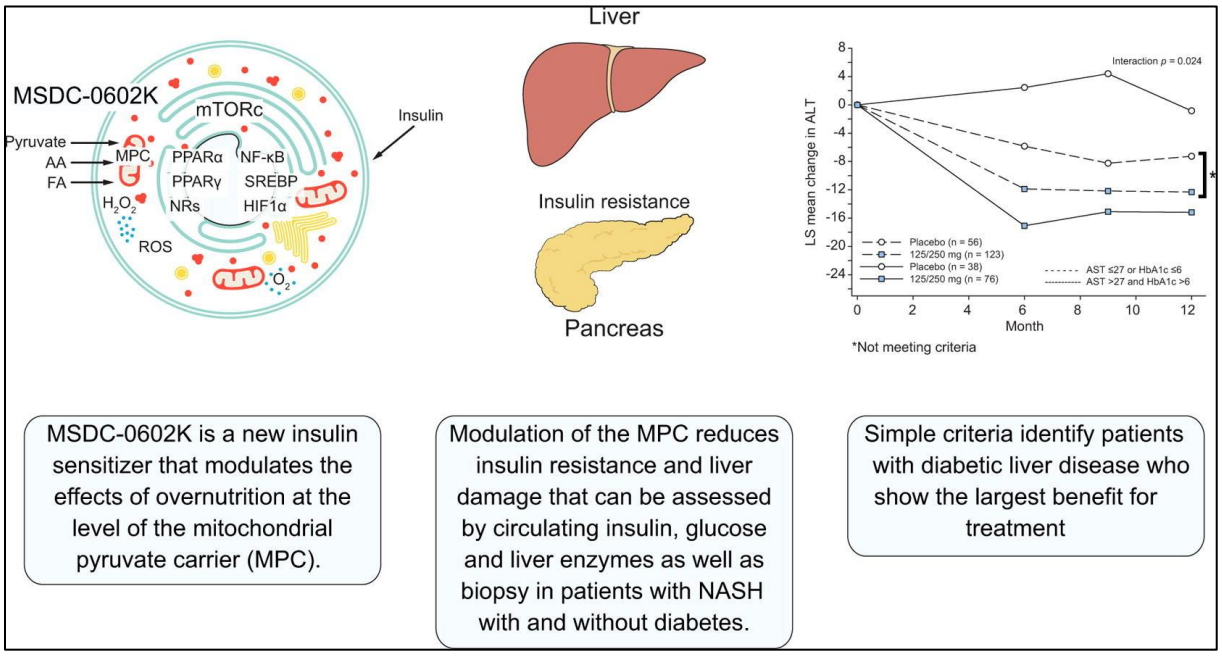



**Figure 15. Insulin sensitizer MSDC-0602K improved glucose metabolism and liver enzyme levels in patients with NASH, with or without T2DM**
*Source: Harrison et al., 2020*

**Lanifibranor** (Inventiva Pharma) is an orally available small molecule activating each of the three peroxisome proliferator-activated receptor (PPAR) isoforms PPAR α/δ/ɣ (pan-PPAR agonist). In the phase 2b NATIVE study Lanifibranor has demonstrated anti-fibrotic and anti-inflammatory action as well as beneficial effects on metabolism such as reduction of triglycerides, increase in HDL cholesterol and increased insulin sensitivity [Cooreman et al., 2022]. In a pre-clinical model of decompensated cirrhosis, it led to a marked improvement in fibrosis and portal hypertension.

Lanifibranor is currently tested in a phase 3 trial (NCT04849728, NATiV3) in adults with NASH and liver fibrosis histological stage F2 or F3. The main objective of the trial is to confirm the safety of

Lanifibranor in this patient population and to demonstrate its efficacy on NASH resolution and improvement of fibrosis assessed by liver histology. Study completion is expected in 2025/2026.

**Efruxifermin** (Akero Therapeutics) is an engineered Fc-FGF21 fusion protein that mimics the biological activity of fibroblast growth factor 21. Signaling through FGF21's receptors in liver and adipose tissue is known to reduce fat accumulation, but also core drivers of disease progression such as inflammation and fibrosis. This FGF21 agonist is engineered to provide an optimized pharmacokinetic and pharmacodynamic profile of the endogen hormone FGF21 (i.e., a longer half-life in humans of more than three days, and a higher binding affinity to the co-receptor, beta-klotho) [Kaufmann et al., 2020].

Positive phase 2 trial (NCT03976401) results were reported in patients with histologically confirmed NASH (fibrosis stages F1 to F4) after once weekly treatment with Efruxifermin at week 16: Efruxifermin significantly reduced liver fat and markers of liver injury, decreased fibrosis, improved glucose and lipid metabolism, and reduced hyperuricemia, with a trend to weight loss. Notable among these improvements was a 2-stage reversal of fibrosis in 11 of 22 (50%) patients with F2 or F3 NASH after only 16 weeks of treatment [Harrison et al., 2021; Harrison et al., 2023b]. In patients with compensated F4 fibrosis, improvements in markers of liver injury, fibrosis, and glucose and lipid metabolism were observed. At current, two phase 3 trials (NCT06161571 and NCT06215716) are ongoing to confirm these results. Study completion is expected in 2026 and 2027, respectively.

**Semaglutide** (Ozempic®, NovoNordisk) is a GLP1 receptor agonist, which has already been approved for therapy intensification in addition to metformin in T2DM patients with cardiovascular and/or renal diseases or risk factors thereof. Semaglutide has also received market approval by FDA (2021) and EMA (2022) as an efficacious therapy for weight reduction together with diet and exercise. GLP-1 is a physiological hormone that promotes glycemic control via several different mechanisms, including insulin secretion, slowing gastric emptying, and reducing postprandial glucagon secretion. In addition, Semaglutide is supposed to reduce liver fat and to have anti-fibrotic and anti-inflammatory actions via pleiotropic effects on glucagon receptor activation in NASH (for mechanism of action, please be referred to Figure 7)

In August 2020, the FDA granted breakthrough therapy designation for Semaglutide in NASH, and in April 2021 the phase 3 program Essence (NCT04822181) was initiated with the goal to include about 1200 NASH-patients with biopsy-confirmed fibrosis stages F2 or F3. In part 1 (double-blind), Semaglutide or placebo is administered subcutaneously once weekly over a period of 72 weeks, and in part 2 (sponsor unblinded) over a period of 240 weeks. Participants must be willing to undergo baseline and endpoint liver biopsies. The primary outcome measure in part 1 includes the number of participants with resolution of NASH and no worsening of liver fibrosis and the number of participants with improvement in liver fibrosis and no worsening of NASH. In part 2, the number of participants with cirrhosis-free survival is the primary endpoint. Study completion is expected in 2029.

### *Pipeline RNA-based drug developments for NAFLD/NASH*

There is emerging evidence that genetic susceptibility increases the risks of NAFLD, NASH and NASH-related cirrhosis. Certain single-nucleotide polymorphisms such as PNPLA3, TM6SF2, GCKR, MBOAT7 and HSD17B13 are clearly associated with NASH development or progression. These gene variants play different roles in lipid remodelling, in lipid droplets, hepatic very low-density lipoprotein (VLDL) secretion and de novo lipogenesis [Carlsson et al., 2020b]. Several single-nucleotide polymorphisms associated with NASH development or progression such as PNPLA3 and HSD17B13 are becoming a growing interest in drug development. RNA-based drugs like antisense oligonucleotides or double-stranded short interfering RNA can be used to modulate the expression of pathogenic gene variants.

The current RNA-based drug pipeline for NAFLD/NASH is summarized in **Table 1** at the end of this chapter. In the following, some of the novel RNA-based drug approaches will be described in more detail.

**ALN-PNP** (Regeneron) is a small interference RNA (siRNA) modulating the expression of the patatin-like phospholipase domain containing 3 protein (PNPLA3). Genome-wide association study (GWAS) revealed that a single nucleotide polymorphism in the human PNPLA3 gene—rs738409[G] (148M) is associated with hepatic fat accumulation and a broad spectrum of chronic liver diseases including NAFLD, NASH, fibrosis, cirrhosis, and hepatocellular carcinoma. Wildtype PNPLA3 turns over according to fasting/feeding cycles; however, the 148M mutant PNPLA3 is resistant to ubiquitin- or autophagy-mediated protein degradation. An abnormal accumulation of the PNPLA3(148M) variant on lipid droplets is linked to the impairment of lipid droplet metabolism: When PNPLA3(148M) variant proteins accumulate on lipid droplets, PNPLA3(148M) competes with adipose triglyceride lipase (ATGL, also called PNPLA2) for the interaction with abhydrolase domain containing 5 (ABHD5). As a result, the ATGL activity is reduced and lipid droplets are accumulated [Dong 2019]. (**Figure 16**).

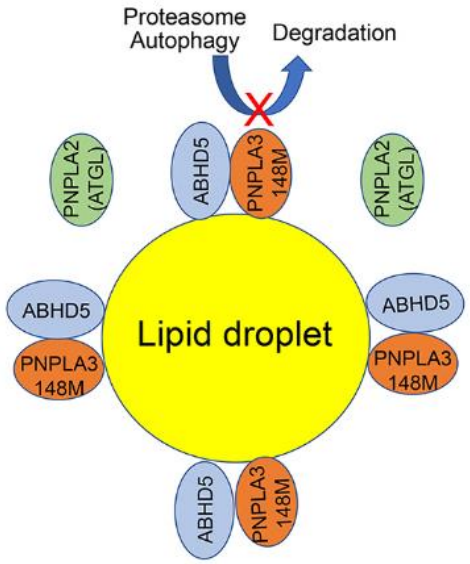



**Figure 16. A working model for the PNPLA3 function on lipid droplet.**
ATGL and ABHD5 normally interact to promote triglyceride breakdown from lipid droplets. The 148M mutation impairs the turnover of PNPLA3 protein by ubiquitin or autophagy mediated degradation. When PNPLA3(148M) variant proteins accumulate on lipid droplets, PNPLA3(148M) competes with ATGL for the interaction with ABHD5. As a result, the ATGL activity is reduced and lipid droplets are accumulated.
Abbreviations: ABHD5 = abhydrolase domain containing 5, ATGL = adipose triglyceride lipase, PNPLA3 = Patatin-like phospholipase domain-containing protein 3, PNPLA3 = Patatin-like phospholipase domain-containing protein 3
*Source: Dong 2019*

A phase 1 study (NCT06024408) will start in Q2/2024 to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics (reduction in liver fat content) of single and multiple doses of ALN-PNP siRNA in adult participants with NAFLD and a genetic risk factor, namely PNPLA3(148M).

**GSK4532990** (GSK), targets HSD17B13, a member of the hydroxysteroid dehydrogenase family involved in the metabolism of hormones, fatty acids, and bile acids. Published human genetic data indicate that a loss of function mutation in HSD17B13 provides strong protection against alcoholic hepatitis, cirrhosis, and NASH, with approximately 30-50% risk reduction compared to non-carriers.

A phase 2b Study (NCT05583344) is planned to evaluate the efficacy and safety of GSK4532990 in adults with pre-cirrhotic NASH with the goal of study completion in Q1/2025.

**ION224** (IONIS) is an investigational ligand-conjugated antisense (LICA) medicine designed to reduce the production of diacylglycerol acyltransferase 2 (DGAT2) to treat patients with NASH. DGAT2 is an enzyme that catalyzes the final step in triglyceride synthesis in the liver. In animal studies, antisense inhibition of DGAT2 significantly improved liver steatosis, lowered blood lipid levels and reversed diet-induced insulin resistance [Yu et al., 2005; Choi et al., 2007]. In clinical studies, the DGAT2 inhibitor PF-06865571 reduced liver fat (as measured by MRI–PDFF) after 2 weeks of treatment in adults with NAFLD. Clinical inhibition of DGAT2 alone [Saxena et al., 2019] or in combination with an ACC inhibitor [Calle et al., 2021] reduced steatosis in patients with NAFLD. In addition, in a previous study using DGAT2 antisense inhibition in T2DM patients, steatosis was reduced without causing hypertriglyceridemia [Loomba et al., 2019].

Based on these results, IONIS decided to investigate DGAT2 antisense inhibition by ION224 in patients with biopsy-proven NASH in a phase 2 study (NCT04932512). In March 2024, IONIS announced positive results from this phase 2 study, in which 160 patients with confirmed NASH received multiple doses of ION224 given for 49 weeks. The study met its primary endpoint at both doses (120 mg and 90 mg), achieving >2 point histologic improvement in NAS score without worsening fibrosis. The important secondary endpoint of NASH resolution without worsening of fibrosis, as measured by biopsy (p=0.039) was also met. ION224 was safe and well-tolerated in this study with once-monthly subcutaneous dosing.

*Table 1: NAFLD/NASH: Potential RNA-based Drugs*

| Drug candidate | Mechanism of Action/ Route of Administration | Design/Objectives | Registered Clinical Trials |
|---|---|---|---|
| **Phase 1** | | | |
| **ALN-PNP** (Regeneron) | RNAi targeting PNPLA3/ Subcutaneous (SC) injection | Safety, tolerability, PK and PD of single and multiple ascending doses of ALN-PNP compared to placebo in participants with NAFLD and who are carriers of the PNPLA3 148M risk allele | NCT06024408: Not yet recruiting |
| **AZD7503** (Astra Zeneca) | LICA inhibiting production of HSD17B13/ SC injection | Safety, tolerability, and PK of multiple ascending doses of AZD7503 compared to placebo in participants with suspected NASH | NCT05864391: Expected study completion in October 2024 |
| **LY3849891** (Eli Lilly) | RNAi targeting ANGPTL3/ SC injection | Safety, tolerability, PK and PD of single and multiple ascending doses of LY3849891 in participants with NAFLD who have the PNPLA3 I148M genotype | NCT05395481: Expected study completion in November 2024 |
| **Phase 2** | | | |
| **AZD2693** (Astra Zeneca) | PNPLA3 antisense oligonucleotide/ SC injection | Efficacy, safety and tolerability of repeat doses of AZD2693 in adult participants with non-cirrhotic NASH with fibrosis and who are carriers of the PNPLA3 148M risk allele | NCT05809934: Expected study completion in November 2025 |
| **ALN-HSD** (Regeneron Pharmaceuticals) | RNAi targeting HSD17B13/ SC injection | Efficacy and safety of ALN-HSD in adult participants with NASH with fibrosis F2 or F3 with genetic risk factors | NCT05519475: Expected study completion: September 2027 |
| **BMS-986263** (Bristol-Myers Squibb) | Lipid- nanoparticle siRNA targeting HSP47 mRNA/ Intravenous infusion | Safety and effectiveness of BMS-986263 in adults with compensated cirrhosis from NASH | NCT04267393: Terminated in February 2024 because of lack of efficacy in the short term acute phase |
| **GSK4532990** (GSK) | RNAi targeting HSD17B13/ SC injection | Efficacy and safety of GSK4532990 in adults with NASH and advanced fibrosis F3 or F4 on biopsy | NCT05583344: Expected study completion: December 2025 |
| **ION224** (Ionis) | LICA reducing the production of DGAT2/SC injection | Safety, Efficacy, and PK of Multiple Doses of ION224 administered once monthly in adult subjects with confirmed NASH | NCT04932512: Completed in February 2024 Positive top-line results announced in March 2024 |
| **Phase 3** | | | |
| None | | | |

ANGPTL3 = Angiopoietin-like protein 3, DGAT2 = Diacylglycerol acyltransferase 2, HSD17B13 = 17 β-Hydroxysteroid Dehydrogenase Type 13, LICA = ligand-conjugated antisense oligonucleotide, PNPLA3 = Patatin-like phospholipase domain-containing protein 3, RNAi: Ribonucleic acid interference

## Concluding remarks

**For** many years we tried to treat obesity and related diseases by correcting single findings or metabolic deficiencies. Obese people were stigmatized due to the common belief that obese people have insufficient self-discipline:

- If you have overweight – „Lose weight! Eat half!“
- If you have type-2 diabetes - “Eat less but regularly! Substitute insulin!”
- If you have heart failure - „Reduce salt intake! Take medication!“

**With** increasing knowledge, a modern concept of obesity and its adequate treatment has been elaborated that includes the following:

- Obesity is not an isolated symptom but part of a complex *metabolic syndrome* that puts people at higher risk for cardiovascular diseases (coronary heart disease and heart failure, or stroke), T2DM, and diseases related to fat accumulation in organs such as NAFLD/NASH.
- Obesity is now recognized as an autonomous chronic disease with a high tendency to re-occur. This change in status is inextricably linked to the right to treatment to achieve a clinical relevant body weight reduction.
- Obesity treatment consists of a comprehensive change in lifestyle risk factors including self-management education, nutritional counselling, increasing physical activity, and psychosocial counselling (if indicated). As lifestyle changes are often not sufficient to achieve a significant and long-lasting weight loss, adding pharmacological and/or surgical interventions is often indicated.
- Various innovative treatment strategies based on incretin mimetics are now in development as therapeutic options to treat obesity and its related diseases, such as glucagon like agonists on the peptide 1 receptor (GLP1R) or agonists on the glucose receptor (GCGR)
- Combinations of incretin analogues offer the potential for greater weight loss, as well as acting via synergistic mechanisms to enhance beneficial effects on liver injury caused by fatty liver disease such as NASH. The efficacy of combination therapy needs to be considered alongside the increased risk of side effects, drug-drug interactions and costs. Since those medications regulate essential metabolic pathways, the tolerability part in long-term treatment deserves great attention.
- The selective agonist of the THR-ß receptor Resmetirom showed improvement of structural changes in the liver and regulatory approval for NASH with liver scars due to fibrosis has just been granted.

The **Charité Research Organisation GmbH** is excited to watch, participate and contribute in a new era of medical science and clinical development. Stay tuned!

„Only those who regard healing as the ultimate purpose
of their endeavors can be called physicians.”
(Rudolph Virchow)

## References


**Adriaenssens** AE, Biggs EK, Darwish T, Tadross J, Sukthankar T, Girish M et al. (2019) Glucose-dependent insulinotropic polypeptide receptor-expressing cells in thehypothalamus regulate food intake. Cell Metab. 30, 987–996.

**Alkhouri** N, Lawitz E, Noureddin M, DeFronzo R, Shulman GI (2020) GS-0976 (Firsocostat): an investigational liver-directed acetylCoA carboxylase (ACC) inhibitor for the treatment of nonalcoholic steatohepatitis (NASH) Expert Opin Investig Drugs. 2020 February; 29(2): 135–141.

**Alkhouri** N, Herring R, Kabler H, Kayali Z, Hassanein T, Kohli A (2022) Safety and efficacy of combination therapy with semaglutide, cilofexor and firsocostat in patients with non-alcoholic steatohepatitis: A randomised, open-label phase II trial. J. Hepatol. 2022, 77, 607–618.

**Brand** MD (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Exp Gerontol. 35: 811-820.

**Büchold** C, Hils M, Gerlach U, Weber J, Pelzer C, Heil A, Aeschlimann D, Pasternack R (2022) Features of ZED1227: The First-In-Class Tissue Transglutaminase Inhibitor Undergoing Clinical Evaluation for the Treatment of Celiac Disease. Cells 11, 1667.

**Calle** RA, Amin NB, Carvajal-Gonzalez S, Ross TT, Bergman A et al. (2021) ACC inhibitor alone or co-administered with a DGAT2 inhibitor in patients with non-alcoholic fatty liver disease: two parallel, placebo-controlled, randomized phase 2a trials. Nature Medicine, VOL 27, 1836–1848.

**Carlsson** LMS et al. (2020a) Life expectancy after bariatric surgery in the Swedish Obese Subjects Study. N. Engl. J. Med. 383, 1535–1543.

**Carlsson** B. et al. (2020b) Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. Aliment. Pharmacol. Ther. 51, 1305–1320.

**Choi** CS, Savage DB, Kulkarni A, Yu XX, Liu Z-X, Morino K, Kim S, Distefano A, Samuel V T, Neschen S, et al. (2007) Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. J. Biol. Chem.282, 22678–22688.

**Cooreman** MP, Francque S, Dzen L, Huot-Marchand P, Junien JL, Broqua P, Abdelmalek MF (2022) The Pan-PPAR Agonist Lanifibranor Improves Nonalcoholic Steatohepatitis (NASH) and Glycemic Control. Diabetes, 71 (Supp 1): 830-P.

**Davison** BA, Harrison SA, Cotter G, Alkhouri N, Sanyal A. (2020) Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials. J. Hepatol. 73, 1322–1332.

**DELPHI Consensus 2023**: Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023 Jun 20:S0168-8278(23)00418-X.

**Devarbhavi** H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. Journal of Hepatology, August 2023. vol. 79, pp 516–537.

**Dong** XC (2019) PNPLA3—A Potential Therapeutic Target for Personalized Treatment of Chronic Liver Disease. Front. Med. 6:304.

**Elias** K, Plonowski A, Brown SD, McDonnell ND. 2022. Poster presentation at The Liver Meeting® of the American Association for the Study of Liver Diseases (AASLD).

**Eslam** M, Sanyal AJ, George J on behalf of the International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology 2020;158:1999–2014.

**German Interdisciplinary S3 Guideline (2014):** Obesity Society, German Diabetes Society, German Society for Nutrition e.V., German Society for Nutritional Medicine e.V. 2014. Interdisciplinary guideline of quality S3 on "Prevention and therapy of obesity" (currently being revised).

**Hanefeld** M, Leonhardt W (1981) Das metabolische Syndrom. Dt Gesundh Wesen 36: 545–551.

**Harrison** SA, Alkhouri N, Davison BA, Sanyal A, Edwards C et al. (2020) Insulin sensitizer MSDC-0602K in non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled phase IIb study. Journal of Hepatology vol. 72: 613–626.

**Harrison** SA, Abdelmalek MF, Neff G, Gunn N, Guy CD, Alkhouri N, et al. Aldafermin in patients with non-alcoholic steatohepatitis (ALPINE 2/3): A randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Gastroenterol Hepatol. 2022;7:603–16.

**Harrison** SA, Allen AM, Dubourg J, Noureddin M, Alkhouri N (**2023a**) Challenges and opportunities in NASH drug development. Nature Medicine, Volume 29, March 2023, 562–573.

**Harrison** SA, Ruane PJ, Freilich B, Neff G, Patil R, Behling C, Hu C et al. (**2023b**)A randomized, double-blind, placebo-controlled phase IIa trial of efruxifermin for patients with compensated NASH cirrhosis. JHEP Reports 2023 vol. 5 j 100563.

**Kaufman** A, Abuqayyas L, Denney WS, Tillman EJ, Rolph T (2020) AKR-001, an FcFGF21 analog, showed sustained pharmacodynamic effects on insulin sensitivity and lipid metabolism in type 2 diabetes patients. Cell Rep Med;1:100057

**Karim & Bansai** (2023) Resmetirom: An orally administered, small molecule, liver--directed, β-selective THR agonist for the treatment of Non-alcoholic Fatty Liver Disease and Non-alcoholic Steatohepatitis. touchReviews in Endocrinology. 2023;19(1):60-70.

**Lan** T, Morgan DA, Rahmouni K, et al. (2017) FGF19, FGF21, and an FGFR1/b-Klotho-activating antibody act on the nervous system to regulate body weight and glycemia. Cell Metab 2017;26:709–718.e3.

**Loomba** R et al. (2019) An international, randomized, placebo-controlled phase 2 trial demonstrates novel efects of DGAT2 antisense inhibition in reducing steatosis without causing hypertriglyceridemia in T2DM patients. J. Hepatol.70, e67–e68.

**Loomba** R (2021) MRI-proton density fat fraction treatment response criteria in nonalcoholic steatohepatitis. Hepatology 73, 881–883.

**Maddatu** J, Anderson-Baucum E, Evans-Molina C. Smoking and the risk of type 2 diabetes. Transl Res. 2017 Jun;184:101-107.

**Müller** TD, Blüher M, Tschöp MH, and DiMarchi RD (2022) Anti-obesity drug discovery: advances and challenges. Nature Reviews, DRUG DISCOVERY, Volume 21, March 2022.

**Noureddin** M, Khan S, Portell F, Jorkasky D, Dennis J et al. (2023) Lancet Gastroenterol Hepatol 8: 1094–105.

**National Diabetes Strategy 2020:** German Parliament - 19th electoral term; Printed matter 19/20619 - Motion: Launch of a National Diabetes Strategy - Targeted further development of health promotion and prevention in Germany and diabetes mellitus care - 01.07.2020

**Newsome PN and Ambery P**. Incretins (GLP-1 receptor agonists and dual/triple agonists) and the liverJournal of Hepatology, December 2023. vol. 79 j 1557–1565.

**Quek** J, Chan KE, Wong ZY, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. The Lancet Gastro And Hep. 2023;8(1):20-30.

**Rinella** ME, Lieu HD, Kowdley KV, Goodman ZD, Alkhouri N, Lawitz E et al. (2024) A randomized, double-blind, placebocontrolled trial of Aldafermin in patients with NASH and compensated cirrhosis. Hepatology. 2024;79:674–689.

**Rubino** D, Abrahamsson N, Davies M, Hesse D, Greenway FL, Jensen C, Lingvay I, Mosenzon O, Rosenstock J, et al. for the STEP 4 Investigators. JAMA. 2021;325(14):1414-1425.

**Sanyal** AJ, Ling L, Beuers U, DePaoli AM, Lieu HD, Harrison SA, et al. (2021) Potent suppression of hydrophobic bile acids by aldafermin, an FGF19 analogue, across metabolic and cholestatic liver diseases. JHEP Rep. 2021;3:100255.

**Samms** RJ, Coghlan MP and Sloop KW. Trends in Endocrinology & Metabolism, June 2020, Vol. 31, No. 6.

**Saxena** A, Chidsey K, Somayaji V, Ogden A & Duvvuri S (2019) Diacylglycerol acyltransferase 2 (DGAT2) inhibitor PF-06865571 reduces liver fat by MRI–PDFF afer 2 weeks in adults with NAFLD. Hepatology 70, 1260A.

**Tacke** F, Puengel T, Loomba R, Friedman SL. An integrated view of anti-inflammatory and antifibrotic targets for the treatment of NASH. Journal of Hepatology, August 2023. vol. 79 j 552–566.

**World Health Organization** (2024) Obesity and overweight: HTTPS://WWW.WHO.INT/NEWS-ROOM/FACT-SHEETS/DETAIL/OBESITY-AND-OVERWEIGHT (website accessed on 13February 2024).

**Yanai** H, Adachi H, Hakoshima M, Iida S and Katsuyama H. Metabolic-Dysfunction-Associated Steatotic Liver Disease-Its Pathophysiology, Association with Atherosclerosis and Cardiovascular Disease, and Treatments. Int J Mol Sci. 2023 Oct 23;24(20):15473.

**Younassi** ZM, Ratziu V, Loomba R, Rinella M, Anstee QM Goodman Z, Bedossa P et al. on behalf of the REGENERATE study investigators. (2019) Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019; 394: 2184–96.

**Yu** XX, Murray SF, Pandey SK, Booten SL, Bao D, Song XZ, Kelly S, Chen S, McKay R, Monia B P, Bhanot S (2005) Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. Hepatology 42, 362–371.