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Nonalcoholic fatty liver disease (NAFLD): emerging molecular targets for novel therapeutic strategies

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Abstract

Nonalcoholic fatty liver disease (NAFLD) — the most common chronic liver disease— encompasses a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NASH is projected to be the most common indication for liver transplantation in the next decade. The absence of an effective pharmacological therapy for NASH is boosting research into novel therapeutic approaches for this condition. These include modulation of nuclear transcription factors, agents that target oxidative stress, and modulation of cellular energy homeostasis, metabolism and the inflammatory response. Strategies to enhance resolution of inflammation and fibrosis could reverse the advanced stages of liver disease. Finally, we suggest areas where future research could lead to effective therapeutic agents for the treatment of NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world, affecting up to 30% of the adult population and 70-80% of obese and diabetic individuals[1]. NAFLD encompasses a histological spectrum, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), the latter with different degrees of fibrosis severity.

Although simple steatosis is considered to have a low potential for progression, NASH can progress to cirrhosis and end-stage liver disease. NASH the second leading etiology of liver disease among adults awaiting liver transplantation in the United States and is projected to become the most common indication for liver transplantation in the next decade[2]. Furthermore, NAFLD is an emerging risk factor for type 2 diabetes, cardiovascular disease and end-stage kidney disease[1, 3]. There are no approved pharmacological therapies for NASH[4], highlighting the urgent need to develop effective therapeutic strategies for this condition. Here we review recent advances in research into potential molecular targets for the treatment of NASH, focusing on their translational potential and on key challenges that must be overcome for the clinical development of investigational compounds.

A)Modulation of nuclear transcription factors

Nuclear transcription factors are molecules that, upon ligand binding, bind to response elements (REs) in the promoters of target genes to regulate their transcription. Several nuclear transcription factors are receiving considerable attention in light of their therapeutic potential for the treatment of NAFLD.

A1)Farnesoid X receptor (FXR)

Originally known for its function as a bile acid sensor in enterohepatic tissues, farnesoid X receptor (FXR) has recently emerged as a master regulator of lipid and glucose homeostasis and of

inflammatory and fibrogenic processes (**Table 1**). Several synthetic FXR agonists are being evaluated for the treatment of hepatic and metabolic disorders, including NAFLD[5, 6].

Two FXR-encoding genes have been identified, FXRα and FXRβ, although only FXRα senses bile acids in humans. FXRα is expressed mainly in the liver, intestine, kidney and adrenal glands, and at lower levels in adipose tissue.

FXR is constitutively bound to the 9-cis-retinoic acid receptor (RXR). This heterodimer binds FXR response elements (FXREs) and induces gene transcription. Upon ligand binding, FXR undergoes conformational changes to release co-repressors and recruit co-activators, including DRIP-205 (vitamin-D-receptor-interacting protein-205) and PGC-1α (peroxisome proliferator-activated receptor gamma coactivator-1α)[7].The mechanisms modulating recruitment of these co-activators by FXR ligands and the importance of these molecules to specific gene regulation by FXR ligands are being intensely investigated.

In patients with NAFLD, hepatic expression of FXR and of the bile acid biosynthetic enzymes CYP7A and CYP27A is down-regulated and inversely related to liver disease severity[8]. Consistent with this observation, FXR-deficient mice on a high fat diet exhibit massive hepatic steatosis, necrotic inflammation and fibrosis.[9] In rodent models of diet-induced NASH, FXR agonists prevent the development of NAFLD and can promote the resolution of steatohepatitis and fibrosis [10]. In the liver, FXR agonists enhance insulin sensitivity[11], increase triglyceride clearance and mitochondrial fatty acid β-oxidation, and suppress lipogenic gene transcription[12]. Furthermore, FXR receptor engagement decreases SREBP-1c expression[13] and upregulates apolipoprotein (apo) C-II and very-low-density lipoprotein receptor (VLDL-R) expression, which together enhance triglyceride-rich lipoprotein clearance and repress the expression of apolipoprotein AI[14].These effects on cholesterol metabolism could explain the 5% reduction in plasma HDL-C levels observed in patients treated with semi-synthetic bile acids[15]. FXR activation also directly inhibits hepatic stellate cell (HSC) activation and hepatic fibrogenesis[10], and has several beneficial extrahepatic effects, as it reverses adipose tissue dysfunction[16] and decreases gut microbiota-induced inflammation by attenuating

intestinal barrier dysfunction, endotoxin translocation and the hepatic nuclear factor (NF)-κB-mediated response to endotoxin[17,18], and by promoting intestinal fibroblast growth factor (FGF)-19 secretion[19].

Semi-synthetic bile acids also activate the G protein coupled receptor TGR5, which is ubiquitously expressed with the highest level of expression in the human placenta, spleen, liver, small intestine and adipose tissue: TGR5 activation may also potentially improve NASH by down-regulating NF-κB-mediated pathway activation in macrophages and Kupffer cells, enhancing mitochondrial biogenesis and function in muscle and adipose tissue, and increasing intestinal glucagon-like peptide(GLP)-1 secretion [20]. Mechanisms connecting FXR and TGR5 activation to improvement of liver disease and cardio-metabolic abnormalities in NASH are described in **Table 1.**

On this basis, potent semi-synthetic bile acid FXR agonists have been developed for the treatment of NASH (**Table 2**). Obeticholic acid (OCA, or 6-ethyl- chenodeoxycholic acid, or INT-747), a semi-synthetic derivative of chenodeoxycholic acid (CDCA) with a 80-fold higher potency at the human FXR (EC_{50}=0.033 μM) compared to endogenous CDCA[21], has been recently evaluated in the multicenter, double-blind, randomized "FXR Ligand NASH Treatment (FLINT)" trial[15]. Although OCA significantly improved the primary histological outcome (NAFLD activity score, NAS) and fibrosis score compared with placebo, NASH resolution occurred in only 22% of patients treated with OCA after 72 weeks (p=0.08 vs. placebo). Furthermore, the fraction of patients with resolution of advanced fibrosis did not significantly differ between arms (41% vs. 28%, p=0.30). Since the presence of NASH and of bridging fibrosis are strong predictors of liver disease progression and liver-related complications[1], the clinical relevance of the results of the FLINT trial requires further evaluation. Furthermore, OCA treatment did not significantly improve liver histology in non-diabetic patients (47% of study participants). The absence of effectiveness of OCA in non-diabetic individuals warrants confirmation, but may be related to different bile acid metabolism between diabetic and non-diabetic individuals[22]. Lastly, a 5% decrease in HDL-C levels

coupled with a 16% increase in LDL-C was observed with OCA as compared to placebo: the impact of these changes on long-term CVD risk in NAFLD is unknown.

In addition to OCA, other FXR agonists are currently being investigated, including the natural tea polyphenolic derivative epigallocatechin-3-gallate (EC_{50}=1 μM), that exhibited antioxidant, anti-inflammatory , anti-atherosclerotic and cholesterol-lowering properties preclinically [23], and non-steroidal synthetic derivatives of GW4064[21]. GW4064 is a trisubstituted isoxazole compound with large lipophilic groups at C3-position of the isoxazole and a C5-phenyl ring, additionally substituted in the ortho- position, both of which seem required for its pharmacological activity: GW4064 has a potency of 90 nM on FXR and has been patented in 1998, but never reached clinical use due to its poor bioavailability, photolability, and to the presence of the potentially toxic stilbene moiety[21]. For these reasons, many other non-steroidal isoxazole GW4064 derivatives have been synthesized Since early 2000s in an attempt to overcome the liabilities of the parent compound**:** to date only one of these molecules, the Px-104 (EC_{50}=122 nM)[21] has made it into the early stages of clinical development and is being evaluated in a phase IIa RCT in NAFLD (ClinicalTrials.gov Identifier: NCT0199910).

A2)Sterol regulatory binding protein-2(SREBP-2)

Growing evidence supports a role for the toxic accumulation of free cholesterol in the liver in the pathogenesis of NASH[24], and the therapeutic potential of unloading liver cells of their toxic cholesterol load is attracting considerable interest.

In mammals, the nuclear transcription factor sterol regulatory element-binding protein (SREBP)-2 is the master regulator of intracellular cholesterol homeostasis.[25]Low cellular cholesterol levels enhance trascription of SREBP-2, which regulates target genes involved in cholesterol synthesis, uptake, secretion and transport in order to increase intracellular cholesterol availability (**Table 1**)[26, 27]. In cholesterol-replete cells, SREBP-2 remains in the ER where it cannot induce transcription.

Alternative splicing of the SREBF-2 gene, which also encodes SREBP2, generates the microRNA miR-33a, which is processed from an intron within the SREBF2 primary transcript: miR-33a reduces cholesterol export, mitochondrial fatty acid β-oxidation and insulin signalling in hepatocytes[28, 29] and enhances TGFß-induced HSC activation[30], thereby promoting liver injury and fibrogenesis. Therefore, activation of SREBP-2 and transcription of miR-33a in low cholesterol conditions coordinately promote cholesterol synthesis and retention and the storage of neutral lipids, cholesteryl esters and triglycerides.

In the liver of patients with NASH, SREBP-2 and miR-33a are inappropriately up-regulated despite hepatic cholesterol overload and parallel the severity of liver histology[8]. Mechanisms for disruption of the physiological negative feedback by cholesterol stores and SREBP-2 upregulation in NASH may include: enhanced insulin-, cytokine- or mammalian target of rapamycin complex 1 (mTORC1)-mediated transcription of SREBF-2 gene[31],; downregulation of hepatic miR-122 (a suppressor of hepatic SREBP-2 expression); or genetic variation in SREBP-2 activity[32].

The pervasive effect of SREBP-2 and miR-33a upregulation on hepatic cholesterol metabolism and its inappropriate upregulation make modulation of its activity an attractive therapeutic target to tackle cholesterol-mediated liver injury in NASH. While selective SREBP-2 antagonists are under development, several natural antioxidants (curcumin[26], resveratrol[33] and proanthocyanidins[34]) repress hepatic SREBP-2 and miR-33a and their target genes and improved hepatic trigyceride infiltration and fibrogenesis activation in cellular and rodent models.

An alternative strategy could be the suppression of miR-33a expression with antisense oligonucleotides or their chemically modified versions — 2'-O-methyl-group (OMe)–modified oligonucleotides and locked nucleic acids (LNA) anti-miRs — which have yielded promising results in preclinical models[29]. Major issues with the therapeutic manipulation of these miRNAs, as with miRNAs in general, are to ensure their stability and organ-specific delivery and to test the long-term safety of this approach. miR-33a also regulates cell proliferation and cell cycle

progression in the liver and other organs, so safety is a particular concern with therapies targeting this miRNA[35, 36].

A3)pregnane X receptor (PXR)

Initially identified as a regulator of xenobiotic and drug metabolism and disposition, the pregnane X receptor (PXR) is also an important modulator of metabolic and inflammatory pathways at the hepatic and extrahepatic levels[37] and is therefore a potential therapeutic target for NASH(**Table 1**). Upon activation by a variety of ligands including drugs, insecticides, pesticides, and nutritional compounds, PXR heterodimerizes with RXR and induces transcription downstream of PXR response elements. PXR coordinates the expression of several genes that are critical to the metabolism and export of toxic xenobiotic compounds, including cytochrome P450 3A4 and 2B6 and multidrug resistance protein (MDR)1 and MRP2[38].

Recently, genetic screening and functional studies using PXR knockout and transgenic mice have shown that PXR modulates carbohydrate and lipid homeostasis, inflammation and fibrogenesis in NAFLD [37, 39] . PXR has been found to directly promote hepatic steatosis *in vitro* and *in vivo* [40,41] , through SREBP-1c activation and through SREBP-1c-independent pathways[40, 42, 43, 44 45].

PXR suppresses hepatic gluconeogenesis by competing with HNF-4 for the binding of PPARγ coactivator 1α (PGC-1α), thus attenuating hepatocyte nuclear factor-4 (HNF-4) signaling[46]. PXR also acts as a co-repressor of the transcription factor forkhead box–containing protein O subfamily-1 (FOXO1), another positive regulator of gluconeogenesis which was recently found to be overexpressed in NASH, decreasing the transcriptional activity of FOXO1 on the insulin response element (IRS)[47](**Table 1**).

In addition to its effects on metabolic regulation, PXR has potent anti-inflammatory and anti-fibrotic properties *in vitro* and *in vivo*: PXR activation suppressed hepatocyte apoptosis and NF-κB activation[48], enhanced hepatocyte autophagy[49] and abrogated proinflammatory and profibrogenic

responses to bacterial lipopolysaccharide (LPS)[50] in cultured hepatocytes and HSCs, ameliorating hepatic necrotic inflammation and fibrosis in rodent models of NASH[51 52](**Table 1**).

These data indicate that PXR may be a potential therapeutic target for NASH. However ,the role of PXR in xenobiotic metabolism suggests that targeting it could have unwanted drug-drug interactions, and PXR activation induced steatosis in preclinical models (**Table 1**). The translational and clinical relevance of this observation remain uncertain: for instance, there are no significant clinical or histological reports of hepatic steatosis, fibrosis, cirrhosis, or carcinoma induced by rifampicin, a potent PXR agonist widely used for the treatment of tuberculosis[53]. Strategies to overcome these unwanted steatogenic effects of PXR activation are being investigated: intriguingly, it has been shown that acetylation of PXR regulates its pro-lipogenic function independent of ligand activation[54], suggesting that PXR could be selectively regulated by manipulating its post-translational modifications.

A4) Peroxisome proliferators-activated receptor(PPAR)-α/δ agonists

Peroxisome proliferators-activated receptors (PPARs) belong to the nuclear receptor superfamily and they can be classified into 3 isotypes designated PPAR-α, PPAR-γ and PPAR-δ. PPARs form heterodimers with RXR[55]. The PPAR:RXR heterodimer regulates gene transcription by binding to PPAR response elements (PPRE).

Although the unwanted effects of PPAR-γ agonists — including weight gain, fluid retention, bone fractures, increased cardiovascular risk for rosiglitazone and increased risk of bladder cancer for pioglitazone — have limited their clinical use[56], several potent selective PPAR-α modulators (SPPARMs) and dual PPAR-α/δ modulators are currently under development for the treatment of NAFLD and cardio-metabolic disorders.

PPAR-α is expressed in the liver and other metabolically active tissues including striated muscle, kidney and pancreas where it upregulates numerous enzymes involved in mitochondrial and peroxisomal fatty acid β-oxidation and microsomal ω-oxidation, plasma fatty acid membrane

transporters, and ketogenesis[57, 58], thereby shifting hepatic metabolism toward lipid oxidation. PPAR-α activation also enhances plasma triglyceride clearance by up-regulating the expression of lipoprotein lipase (LPL) and down-regulating hepatic secretion of apo-CIII, a LPL inhibitor[59](**Table 1**). Another PPAR-α target, catalase, ameliorates hydrogen peroxide detoxification and protects hepatocytes from oxidative stress, which is believed to play a crucial role in liver injury in NASH(see below)[60].

PPAR-α enhances the transcription of FGF-21; FGF-21 seems to be crucial for the metabolic functions of PPAR-α, as FGF21 knockout mice fed a high fat-diet showed hepatic steatosis and impaired fatty acid oxidation and ketogenesis[57]. Therapeutic approaches to interfering with FGF-21 directly are discussed in more detail below.

PPAR-α also suppresses the acute phase inflammatory response via PPRE-binding-dependent[61, 62] and -independent mechanisms[63](**Table 1**): PPAR-α represses cytokine-induced and LPS-induced secretion of IL-1, IL-6 and TNF-α and the expression of adhesion molecules ICAM-1 and VCAM-1 *in vitro* and *in vivo*, independent of direct DNA binding[64,65]. Importantly, these PPRE-independent effects were sufficient to protect the liver from methionine-choline deficient diet(MCDD)-induced inflammation and fibrosis, without affecting fatty acid oxidation and lipid accumulation[60].

Fibrates, which are weak PPAR-α agonists (EC_{50} ranging 30,000 to 50,000nM for fenofibrate and bezafibrate, respectively), have hepatoprotective effects in rodent models of NASH.[66] However, the relatively weak potency of fibrates and other available PPAR-α agonists, the low expression level of PPAR-α in human liver relative to rodent liver[67] and the observation that PPAR-α expression decreases with progressive fibrosis may explain the contradictory results of PPAR-α agonists in randomized clinical trials (RCTs).[4] These results prompted the development of novel, more potent PPAR-α agonists, including the SPPARM-α K-877 (EC_{50} =1 nM) and the dual PPAR-α/δ agonist GFT505 (EC_{50} =6 nM), which activates both PPAR-α and PPAR-δ(**Table 2**).

PPAR-δ is ubiquitously expressed, with highest expression in liver and skeletal muscle, and has been implicated in lipid metabolism and energy homeostasis of various organs, including the liver[55].

In the liver, PPAR-δ is also expressed by hepatocutes and nonparenchimal cells where it exerts potent anti-inflammatory effects and polarizes macrophages from a pro-inflammatory M1 to an anti-inflammatory M2 phenotype[68]; furthermore, unlike PPAR-α, PPAR-δ is expressed also at extrahepatic sites, where it promotes fatty acid β-oxidation and adaptive thermogenesis[69](**Table 1**).

In preclinical models of NASH, PPAR-δ agonists enhanced hepatic lipid oxidation and insulin sensitivity and reduced steatosis, inflammation and fibrogenesis[70, 71]. MBX-8025, a potent SPPARM-δ (EC_{50} =2 nm) improved liver enzymes, inflammatory markers, insulin resistance and atherogenic dyslipidemia in overweight dyslipidemic patients[72].

Given the complementary effects and tissue distribution of PPAR-α and PPAR-δ, dual PPARα/δ agonists have been evaluated in NAFLD. GFT505 showed substantial hepatoprotective effects in rodent models of NASH[73], improved liver enzymes and hepatic and peripheral insulin sensitivity in abdominally obese subjects[74] and is currently being evaluated in a phase IIb RCT with histological endpoints in NASH (ClinicalTrials.gov ID: NCT01694849).

B)Targeting oxidative stress

Increased oxidative stress and impaired antioxidant defense have been extensively documented across progressive stages of human NAFLD and may contribute to liver injury[75]. Single antioxidant agent supplementation yielded often disappointing results, and the most extensively studied antioxidant — vitamin E — poses long-term safety issues[4]. For this reason, other approaches to enhance antioxidant defense are currently being investigated.

B1) Nuclear erythroid 2-related factor 2 (Nrf2) activation

Nrf2 is a member of the family of basic region leucine zipper (bZIP) transcription factors, and is expressed ubiquitously in human tissues, with highest expression in the key detoxification organs, particularly the liver[76]. Nrf2 regulates the expression of several antioxidant and detoxification enzymes by binding upstream antioxidant response elements (AREs)(**Table 1**). Under basal

conditions Nrf2 levels are low as Nrf2 is targeted for proteasomal degradation by Kelch-like ECH-associated protein 1 (KEAP1)[76]. The sulfhydryl groups in the cysteine residues of KEAP1 act as stress sensors: oxidation of these groups in response to stresses such as reactive oxygen species (ROS) and nitrogen species causes Nrf2 to dissociate from KEAP1 and induce target gene expression[77]. Mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC) and PKR-like endoplasmic reticulum kinase (PERK) also regulate Nrf2 signaling, although the exact mechanisms and relevance to NAFLD remain unclear[78].

In rodent models of diet-induced NAFLD, whole-body[79, 80,] or myeloid-derived cell[81] Nrf2 deletion promotes atherosclerosis and steatosis progression to NASH and fibrosis, whereas Nrf2 activation by oltipraz or NK-252 attenuated cultured human HSC activation[82] and protected against the development of NASH and fibrosis[83]. On this basis, numerous electrophilic small-molecule Nrf2 activators , including natural products (e.g., sulphoraphane, resveratrol, curcumin, epigallocatechin gallate and dimethyl fumarate) and synthetic compounds(e.g., oltipraz, anethole dithiolethione and bardoxolone methyl) are currently being evaluated: they are all electrophiles that covalently modify the cysteine sulfhydryl groups of KEAP1, thereby altering its conformation and preventing the KEAP1-Nrf2 interaction.

Preclinical data on these Nrf2 activators show promising results for the trratment of obesity-related disorders[84], and the dithiolethione oltipraz is being evaluated in a phase II RCT in NAFLD (clinicaltrials.gov ID: NCT01373554)(**Table 2**).

Since these electrophile thiol-containing Nrf2 activators bind nonselectively to cysteine-rich proteins, their low selectivity may elicit off-target effects on other thiol-rich molecules, which have been shown to be over 500 for some Nrf2 activators[85], with potentially unwanted effects. To address these concerns, newer, non-electrophilic Nrf2 activators with enhanced potency and selectivity for Nrf2 and potentially higher clinical effectiveness and safety. are being developed. Some of these compounds (such as NK-252 and tetrahydroisoquinoline THIQ) that have no thiol-reactive group directly interact with the Nrf2-binding site of KEAP1, the Kelch domain,

thereby preventing its interaction with Nrf2[83, 86]. Some (such as berberine) enhance transcription of Nrf2 by upregulating its related long noncoding RNA(lncRNA) MRAK052686[87]. Some (such as MG132) act at the level of proteasome and specifically and reversibly inhibit ubiquitination-proteosomal degradation of Nrf2, thus prolonging its half-life[78]. Others (such as tBHQ) also interact with critical cysteine thiol residues of Nrf2 causing its release from KEAP1[78]. Some of these compounds have been tested in cell cultures and diet-induced models of NASH and showed potent anti-inflammatory and anti-fibrotic effects[83]

B2)natural antioxidants: resveratrol, quercetin

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a polyphenolic compound found largely in the skin of red grapes, peanuts, and berries, that has been extensively studied due to its antioxidative, antiinflammatory, anticancer, antiobesity, antidiabetic, and antiaging properties[88,]. Resveratrol supplementation improved hepatic steatosis, insulin resistance and inflammation in rodent models of high fat-induced NAFLD[89, 90] by modulating several cellular metabolic pathways **(online supplementary Table 1 panel A).**

A core mechanism of action of resveratrol is the activation of sirtuin-1 (SIRT1), a nicotinamide adenine dinucleotide (NAD+)-dependent protein and histone deacetylase that modulates the activity of key enzymes and proteins involved in glucose and lipid metabolism and energy homeostasis. SIRT1 activation governs a complex array of signaling cascades in hepatocytes, myocytes and adipocytes, centering on AMPK activation and mimicking calorie restriction, which enhances insulin sensitivity, mitochondrial fatty acid oxidation and lipolysis and decreases de novo lipogenesis [91]. Resveratrol also upregulates autophagy[92] and the Nrf2-mediated antioxidant defense[93] and down-regulates the NF-κB-mediated inflammatory response in hepatocytes and adipocytes.

A major challenge to translate these promising preclinical findings into effective therapeutic agents is identifying the pharmacologically active and safe dose of resveratrol that should be used:

resveratrol is rapidly and extensively metabolized by intestinal and hepatic glucuronidases and sulfatases to conjugates with unclear biological activity, whose circulating levels are much higher than those of the parent compound[88]. Although it would be intuitive to administer large dosages to overcome this low bioavailability, a dose-response effect was not observed in preclinical studies, and the lower dose of resveratrol (0.005%) appeared to be more beneficial than the higher dose (0.02%)[94, 95]. Consistently, in the 4 small RCTs performed in NAFLD patients, the lower resveratrol dosages (150 and 500 mg/d) increased SIRT-1/AMPK activity and evoked a calorie-restriction-like response, improving metabolic, inflammatory and hepatic parameters[96,, 97], while the higher dosages (1500-3000 mg/d) adopted in the 2 negative RCTs failed to evoke these changes [98, 99] (**Table 2; online supplementary Table 1 panel B**). Importantly, the higher dosages achieved a ≈8-fold lower plasma resveratrol levels that the lower dosages, suggesting that repeated administration of high resveratrol doses may enhance the metabolism of parent compound to less active metabolites by highly inducible phase II enzymes glucuronidases and sulfatases.

Several strategies to enhance resveratrol bioavailability are in early stages of development and include resveratrol micronization or lipid-core nanocapsule formulations, combination with other polyphenols (piperine, quercetin) to inhibit drug-metabolizing enzymes, resveratrol prodrugs, alternative oral transmucosal or subcutaneous routes of delivery[100]. Clearly, a deeper knowledge of interspecies and inter-individual differences in resveratrol kinetics is needed to bring resveratrol into clinical use.

Quercetin is a natural flavonol typically present in broccoli, onions, and leafy green vegetables. In high fat diet-induced rodent models of NAFLD, quercetin supplementation improved insulin resistance and hepatic steatosis, and reduced inflammatory cell infiltration and portal fibrosis[101 ,102]. The molecular mechanisms of quercetin largely overlap with those of resveratrol, but quercetin also reduces cytochrome P450 2E1 (CYP2E1)-mediated ROS generation, which is believed to be a key factor in the pathogenesis of NASH[103], and enhances fatty acid ω-oxidation[104](**online supplementary Table 1 panel A**).

Similar to resveratrol, quercetin is extensively conjugated by intestinal Phase II systems and several strategies to improve its bioavailability are being investigated[105].

C)Targeting energy homeostasis and cellular metabolism

C1) Fibroblast Growth Faxtor(FGF)-21

FGF-21 is a 181 amino acid circulating protein that is expressed mainly in the liver but also in white adipose tissue (WAT), skeletal muscle, and the pancreas. FGF-21 transcription is up-regulated by ER stress[106], sirtuin-1[107] and by several transcription factors, including PPAR-α[57], PPAR-γ[108], retinoid acid receptor(RAR)-β[109], retinoic acid receptor-related orphan receptor(ROR)-α[110] and NuR77[111].

The activation of FGFR by FGF-21 requires the transmembrane protein cofactor β-Klotho, which is predominantly expressed in metabolic organs including liver, WAT, and pancreas and thus confers organ specificity to FGF-21[102, 112].

FGF-21 is a metabolic hormone as it is regulated by nutritional status and affects energy expenditure and glucose and lipid metabolism. FGF-21 increases adipose and hepatic insulin sensitivity by stimulating GLUT1 expression, enhancing insulin signaling in adipocytes[113] and suppressing hepatic gluconeogenesis and SREBP-1c mediated lipogenesis in the liver [114]. FGF-21 also increases energy expenditure, free fatty acid (FFA) oxidation and mitochondrial function by activating the AMPK-SIRT1-PGC-1α pathway and UCP1[115] and counteracts hepatocyte ER stress [103]. Furthermore, FGF-21 crosses the blood-brain barrier and its effects on the hypothalamus are believed to contribute substantially to its overall metabolic effects[116].

On this basis, activation of the FGF-21 axis has been explored as a method to treat obesity-associated disorders: pharmacological FGF-21 administration improved obesity and diabetes and reversed hepatic steatosis[111]. Most interestingly, FGF-21 administration limited

lipotoxicity and prevented liver disease progression in rodent models of diet-induced NASH[117].

Significant challenges exist for the therapeutic development of FGF-21. In obesity and NAFLD, circulating and tissue FGF-21 levels are increased rather than reduced, correlate with disease severity[118] and are normalized by therapeutic interventions[119], indicating the presence of FGF-21 resistance that is at least in part attributable to down-regulation of FGFR1 and β-Klotho expression in the liver and adipose tissue[119]. However, this resistance can be overcome by the administration of pharmacological doses of FGF-21. In light of the short half-life of endogenous FGF21 (0.5-5 hr), various strategies have been evaluated to maintain levels high enough to achieve therapeutic effects: conjugation with polyethylene glycol (PEG) reduces renal filtration and prolongs retention in the circulation[120]; recombinant mutant FGF-21 analogs conjugated to the Fc fragment of human IgG have 10-fold greater receptor binding and activation and less proteolytic degradation than native FGF-21[121]; adding disulfide bonds and replacing the FGF-21 C-terminal domain, which binds β-Klotho, with a more stable, higher affinity β-Klotho-binding domain increases FGF-21 stability and potency[122], and improved atherogenic dyslipidemia and insulin resistance together with increasing adiponectin levels in obese diabetic patients[123]; FGF-21-mimetic monoclonal antibodies activating the β-Klotho/FGFR1 complex with higher affinity and selectivity showed also promising results in preclinical models[124]. Whether one of these approaches confers higher therapeutic effectiveness and safety over the others has yet to be determined.

C2) 5-AMP activated protein kinase (AMPK) activators

Adenosine 5'-monophosphate(AMP)-activated protein kinase (AMPK) is a ubiquitous heterotrimeric serine/threonine kinase that functions as a fine cellular energy sensor and a key regulator of cellular metabolism. AMPK is activated during caloric restriction or high energy demands, which deplete cellular ATP stores and increase the AMP/ATP ratio. Conversely,

AMPK is inhibited under conditions of excess caloric intake, such as occurs in obesity[125]. Hence, agents mimicking calorie-restriction and/or physical exercise through AMPK activation are appealing treatment options for obesity-associated disorders.

In preclinical models of NAFLD, AMPK activators improved insulin resistance by enhancing oxidative glucose disposal and suppressing hepatic gluconeogenesis[125]. They also improve high-fat diet-induced NASH[126, 127], through the down-regulation of key factors in cholesterol and fatty acid synthesis, including SREBP-1c, 3-hydroxy-3-methylglutaryl-coenzyme A(HMG-CoA) reductase and acetyl-CoA carboxylase (ACC)[125]. Downregulation of ACC decreases malonyl-CoA levels, releasing the inhibition of mitochondrial fatty acid β-oxidation and enhancing oxidation of FFA. In hepatocytes, AMPK activation may also enhance mitochondrial biogenesis and activity and inhibit mTORC1, thus preventing excess-nutrient-induced hepatic lipid accumulation[128].

In addition to its metabolic effects, AMPK activation has also direct anti-inflammatory properties, as it induces the functional transition of macrophages from a pro-inflammatory M1 to an anti-inflammatory/restorative M2 phenotype[129], and antifibrotic effects by inhibiting HSC activation[130]((**Figure 1 panel A**).

Currently, several natural AMPK activators, including monascin and ankaflavin[127], quercetin[129] , berberin[130] , curcumin[131], are being tested preclinically in cell cultures and animal models of NASH. Of note, oltipraz, a Nrf2 activator discussed above that is being evaluated in non-cirrhotic NAFLD patients in a phase II RCT, is also a potent AMPK activator (clinicaltrials.gov ID: NCT01373554).

C3)Mammalian Target Of Rapamicin (mTOR)

mTOR is a large (~290 kDa) serine/threonine protein kinase that is a key regulator of cell metabolism and growth in response to nutritional and hormonal stimuli; mTOR deregulation has been implicated in many disease states, including diabetes, obesity and NAFLD[132].

mTOR associates with various companion proteins to form two distinct signaling complexes with distinct regulators, substrate preferences and signaling pathways: mTOR complex 1 (mTORC1) and mTORC2[132]. Among these companion proteins, regulatory-associated protein of mTOR (RAPTOR) and proline-rich AKT substrate of 40 kDa (PRAS40) are specific to mTORC1 and rapamycin-insensitive companion of mTOR (RICTOR), mSin1, and proline-rich protein 5 (PROTOR1/2) are specific to mTORC2[132].

mTORC1 promotes cellular anabolism by stimulating the synthesis of proteins, lipids, and nucleotides and blocking catabolic processes such as autophagy at the transcriptional and post-translational levels[132]. Growth factors such as insulin and IGF activate mTORC1 through the PI3K/Akt signaling pathway. Conversely, low cellular energy (signaled by a high AMP/ATP ratio) or hypoxia activates AMPK[133] and TSC2, both of which inhibit mTORC1. Other nutrients such as amino acids activate mTORC1.

The molecular mechanisms of mTORC2 regulation are less clear, with the only known upstream activator being the growth factor/PI3K signaling axis. Activated mTORC2 in turn phosphorylates Akt, thereby indirectly regulating mTORC1 activity [132].

Recent evidence indicates that mTOR is activated in NAFLD patients and may play a central role in lipid homeostasis and in NASH pathogenesis[134]. In animal models, mTORC1 inhibition improved experimental high fat diet-induced NASH[135, 136] through several potential mechanisms: in addition to regulating lipid metabolism, mTOR inhibition modulates macrophage polarization, the inflammatory response and autophagy. Mice with RAPTOR-deficient macrophages, which selectively disrupts mTORC1, had reduced ER stress, a shifted macrophage polarization phenotype (from a pro-inflammatory M1 to an M2 phenotype), improved NASH, hepatic and adipose tissue insulin resistance and atherosclerosis without changes in body fat storage[134, 135, 136]. These *in vivo* data are paralleled by data from cultured human monocytes, in which mTOR inhibition reduced the secretion of proinflammatory chemokines[137] (**Figure 1 panel B**).

mTOR inhibition may also improve NASH by restoring autophagy (**BOX 1**) [132, 138]. Autophagy is impaired in the liver of NASH patients[139] and defective autophagy promoted disease progression in diverse nutritional rodent models of NASH[140, 141] and enhanced adipose tissue macrophage recruitment and inflammation, leading to obesity and glucose intolerance[142, 143], alterations that were all reversed by autophagy activation.

mTORC1 activation downregulates PPAR-α-mediated fatty acid oxidation and ketogenesis[144] and upregulates lipogenesis both indirectly through Akt inhibition and directly through transcriptional[145] and posttranscriptional SREBP-1c upregulation (**Figure 1 panel B**). Accordingly, liver-specific RAPTOR deletion[146] or S6K1 inhibition[147] protected mice against high fat diet-induced NAFLD. In addition to fatty acid metabolism, mTORC1 activation also increases cholesterol synthesis and uptake by controlling SREBP-2 processing[31, 148], thereby disrupting negative feedback by cellular cholesterol stores and promoting toxic free cholesterol accumulation[135, 149](**Figure 1 panel B**).

mTORC2 also regulates lipid homeostasis, but the mechanisms are incompletely understood and the effects of mTORC2 activation on lipid metabolism appear to be tissue-dependent. Liver-specific deletion of RICTOR protects against high-fat-diet-induced NASH through reduced *de novo* lipogenesis and cholesterol overload but induces hepatic insulin resistance and increases gluconeogenesis as a result of impaired Akt-mediated insulin signaling[150] **(Figure 1 panel C).** Expression of constitutively active Akt during mTORC2 inhibition normalized insulin sensitivity and gluconeogenesis without affecting lipogenesis: mTORC1 activity toward Lipin-1 was decreased by mTORC2 inhibition and this was not rescued by constitutively active Akt[150]. Hepatic mTORC2 is therefore a critical Akt-dependent relay that separates the effects of insulin on glucose metabolism from those on lipid metabolism.

The effects of mTORC2 inhibition are tissue-dependent. Adipose-specific RICTOR knockout mice had fatty depositions in hepatic and muscle tissue and insulin resistance when fed a high-fat diet as

a result of unrestricted hormone-sensitive lipase activity and subsequent lipolysis in adipose tissue [151]((**Figure 1 panel C**).

Non-selective, dual mTORC1/2 inhibitors such as rapamycin are used clinically to prevent transplant rejection, but have side effects including hepatic insulin resistance and new-onset diabetes. Furthermore, experimental evidence suggests that persistent mTORC1/2 inhibition may enhance hepatic inflammation and tumorigenesis despite a transient reduction in steatosis[152]. These data suggest specific mTORC1 inhibitors[153, 154] may decrease these side effects. For the same reasons, direct inhibition of mTORC2 is undesirable, whereas targeting the mechanisms downstream of mTORC2 that regulate lipid metabolism without disturbing glucose homeostasis may be a prerequisite for safe clinical use of mTOR inhibitors.

D)targeting inflammation

D1) targeting inflammasome activation

Tissue injury and cell death induce an inflammatory response even in the absence of pathogens. This sterile inflammation plays an important role in a variety of pathologies, including NASH, where it can amplify liver damage after the initial insult. The development of sterile inflammation involves assembly and activation of a cytosolic multiprotein complex, termed the inflammasome, which converts two types of extracellular signals into an inflammatory response in immune cells, resulting in activation of caspase-1 and secretion of proinflammatory cytokines IL-1β and IL-18[155]. Signal 1 includes molecules such as TLR ligands [152, 156] . A diverse range of molecules can provide signal 2, including microparticles, uric acid, cholesterol crystals and other damage-associated and pathogen-associated molecular patterns (DAMPs, PAMPs); ATP and NAD via the purinergic 2X7 receptor (P2X7R); or ROS via thioredoxin-interacting protein (TXNIP). Both signaling pathways have to be activated to trigger inflammasone activation. (**Figure 2**).

With the exception of AIM2, which is a member of the HIN-200 family, inflammasomes are classified based on their NACHT domain into three subfamilies of proteins: NODs (NOD1–5), NLRPs or NALPs (NLRP/NALP 1–14), and IPAFs (IPAF, NAIP), with NLRP3 being the most extensively studied. The sensor that is activated by any one of these complexes, NLR, forms a complex with the effector molecule, pro-caspase-1, leads to auto-activation of pro-caspase-1 into caspase-1, which in turn cleaves pro-IL1ß and pro-IL18 to mature IL-1β and IL-18, allowing their secretion from the cells.

In the liver, inflammasome components are expressed prominently in Kupffer cells (the liver-resident macrophages) and sinusoidal endothelial cells, moderately in periportal myofibroblasts and HSCs, and at low levels in primary cultured hepatocytes[157]. Hepatic expression of inflammasome components is significantly increased in NAFLD patients and correlates with the severity of liver histology and the presence of NASH[158]. In diverse animal models of NASH, inflammasome activation promoted NASH and fibrosis development, which were reversed by genetic or pharmacological inhibition of inflammasome activation[159, 160] .

Two strategies to target the inflammasome in NAFLD have been developed: the first is to antagonize inflammasome activation by single DAMPs/PAMPs, including antagonizing cholesterol or uric acid crystals formation with cholesterol-lowering drugs or xanthine oxidase inhibitors [161, 162]; antagonizing saturated fatty acid (SFA)-induced TLR activation with ethyl pyruvate[163]; and antagonizing ATP-mediated P2X7R activation with the small molecule antagonist A438079[164].

The second, highly effective strategy targets the activation of the inflammasome constituents NLRP3 and capsase-1. Several potent NLRP3 inhibitors are currently being tested preclinically, with encouraging results, including isoliquiritigenin, a chalcone from Glycyrrhiza uralensis[165] , arglabin, a sesquiterpene lactone from Artemisia glabella[166], the thioredoxin reductase inhibitor auranofin[167], and N-methyl-d-aspartate(NMDA) receptor agonists[168]. The caspase 1, 8 and 9 inhibitor GS-9450 improved liver enzymes in a phase 2 RCT in NASH[169] (**Table 2).**

A critical point for clinical development of inflammasome-targeted therapies will be tissue selectivity: inhibition of inflammasome activation in the liver, and specifically in Kupffer cells, is central to NASH treatment, whereas intestinal inflammasome inhibition promotes gut dysbiosis, and enhances influx of TLR agonists into the portal circulation, resulting in steatosis progression to NASH[170]. A possible solution could be the conjugation of the drug with organic nanoparticles, including liposomes or polymers like hydroxypropyl methacrylamide (HPMA), which are cleared by macrophages and thus accumulate in the liver, where 80-90% of body macrophages can be found[171], thereby enhancing potency and selectivity of active compound.

D2) chemokine antagonists

Chemokines are small (8–13 kD) secreted proteins that regulate inflammation and leukocyte migration into tissues, tissue fibrosis, remodeling and angiogenesis[172]. The chemokine family includes nearly 45 chemokine ligands and 22 chemokine receptors that are differentially expressed by diverse cell types including leukocytes, hepatocytes, HSCs and adipocytes. The original concept of chemokine "redundancy" — due to the high chemokine-to-receptor ratio — has been discarded as different chemokines exert different and even opposite biological actions upon binding the same receptor[172].

Chemokines are categorized into 4 different families (CC, CXC, CX3C, C) based on the presence of N-terminal cysteine motifs. Upon binding their cognate receptors, G protein-coupled transmembrane proteins, chemokines cause Gα1 and Gβ-1 subunits to dissociate and activate phosphatidylinositol 3-kinase and Rho, which enhance cellular calcium influx and promote leukocyte adhesion and subsequent extravasation. Due to their high affinity for extracellular matrix (ECM) and endothelial surface glycosaminoglycans, secreted chemokines are locally immobilized and retained, creating a concentration gradient that directs leukocytes trafficking toward injured tissues[169].

Among the numerous chemokines involved in liver injury and wound healing processes, chemokine (C-C motif) ligand 2 (CCL2, also known as monocyte chemoattractant protein-1, MCP-1) and its

receptor CCR2, CCL5 (also known as regulated on activation, normal T cell expressed and secreted, RANTES) and its receptor CCR5, and the chemokine receptor CXCR3 with its ligands CXCL9 (MIG), CXCL10 (IP-10) and CXCL11(I-TAC) have been implicated in the pathogenesis of NASH[173].

Kupffer cells are central to the liver injury and hepatic metabolic changes that occur in NAFLD, as their depletion is sufficient to ameliorate diet-induced steatohepatitis[174] and hepatic insulin resistance[175]. Kupffer cells are activated by a variety of DAMPs and PAMPs and release proinflammatory cytokines, including IL-1 and TNF-α, which induce hepatocyte apoptosis and activates hepatic endothelial cells[176]; and chemokines including CCL2, which promotes hepatic accumulation of bone marrow-derived pro-inflammatory $Ly6C^+$ monocytes; CXCL1, CXCL2, CXCL8, which attract neutrophils via CXCR1/CXCR2; and CXCL16, which attracts NKT cells via CXCR6[177]. In addition to Kupffer cells, injured hepatocytes, activated HSCs and adipocytes in nearby adipose tissue also secrete CCL2, which further expands the local macrophage pool and promotes HSC activation, liver fibrosis[178] and adipose tissue inflammation and dysfunction[179]. The CCL2/CCR2 axis is upregulated in the liver and blood of patients with NASH[180] and genetic or pharmacologic inhibition of CCL2 or its receptor CCR2 reduced the macrophage pool by 80% in the liver and by 40% in adipose tissue[181], thereby ameliorating steatohepatitis, fibrosis, adipose tissue dysfunction and insulin resistance[182] in experimental models of NAFLD[174, 178] (**Figure 3**). Notably, CCR2 antagonism was more effective than CCL2 antagonism, possibly because CCR2 also binds other chemokines including CCL7, CCL8, CCL13[183]. CCL2/CCR2 antagonism also shifted the tissue macrophage equilibrium from a pro-inflammatory M1-polarized phenotype toward an anti-inflammatory, "restorative" M2-polarized phenotype; these cells express matrix metalloproteinases and elastase, which degrade the extracellular matrix and promote hepatic fibrosis regression in diet-induced NASH[184].

CCL5 and its receptors CCR1 and CCR5 have also been implicated in liver fibrosis and NASH. Both Kupffer cells and HSCs express CCR1 and CCR5; CCR1 predominantly promotes

fibrogenesis indirectly by activating macrophages whereas CCR5 does so directly by activating HSCs[185, 186]. In rodent models of diet-induced NASH, treatment with a modified version of CCL5 that acts as an antagonist (Met-CCL5) or the small-molecule CCR5 antagonist maraviroc (an FDA-approved inhibitor of CCR5-mediated entry of HIV into immune cells) ameliorated NASH and fibrosis[187, 188]. Cenicriviroc, a dual CCR2 and CCR5 antagonist with nanomolar potency, was safe and well-tolerated in the short-term in patients with mild-to-moderate hepatic impairment[189], had potent anti-inflammatory and anti-fibrotic activity in mouse models of NASH[190]. Cenicriviroc is currently being evaluated in the Phase IIb multicenter RCT "Cenicriviroc for the Treatment of NASH in Adult Subjects With Liver Fibrosis" (CENTAUR)(ClinicalTrials.gov ID**:** NCT02217475).

Recently, the CXCR3 chemokine receptor axis has also been implicated in the development of diet-induced NASH[173]. CXCR3 is expressed by Th1, Th17 and NK cells and its ligands CXCL9, CXCL10 and CXCL11 are secreted by hepatocytes, endothelial cells, HSCs and activated myofibroblasts upon IFN-γ induction[191] . CXCR3 activation contributes to NASH by mediating T-cell chemotaxis, and up-regulating *de novo* lipogenesis and impairing autophagy in hepatocytes [170]. Pharmacologic blockade of CXCR3 by the specific CXCR3 inhibitor NIBR2130 improved experimental NASH[170].

Despite promising preclinical results, several challenges have to be overcome to translate chemokine antagonists to clinical practice. Many chemokines bind multiple receptors and multiple receptors bind many chemokines; additionally, chemokines may have opposing biological actions by binding the same receptor on different cell lines. For example, CXCR6 deletion in NKT cells prevented their hepatic accumulation and improved liver inflammation and fibrosis[192], whereas CCR6 deletion in regulatory γδT cells aggravated hepatic fibrosis in experimental NASH models, as these cells promote HSC apoptosis and restrict hepatic fibrosis[193].

Similarly, activation of the CX3CL1-CX3CR1 axis in liver macrophages enhanced macrophage survival, promoted differentiation of an anti-inflammatory phenotype and improved hepatic inflammation and fibrosis[194]. Improved cell culture models will more accurately predict the *in vivo* effects of manipulating different chemokines and will facilitate translational efforts of this approach. To this aim, a deeper knowledge of the downstream intracellular pathways that are regulated by chemokines and control cell activation and migration, including Akt, focal adhesion kinase, and extracellular signal–regulated kinase (ERK)1-/2, may provide more predictable and effective strategies to modulate chemokine-induced signals[195].

E) Enhancing resolution of inflammation and fibrosis

Inflammation and fibrosis are key pathogenic features of NAFLD, and liver-related morbidity and mortality increase steeply in the presence of NASH and advanced fibrosis[1]. Accordingly, resolution of steatohepatitis and of advanced fibrosis are clinically relevant therapeutic targets (**Figure 4**). Although many therapeutic agents evaluated in RCTs showed substantial anti-fibrotic properties in preclinical models, none of them has yet reversed advanced fibrosis in NASH patients. This discrepancy may have several potential causes: in addition to biological and pharmacokinetic differences between animal models and man, the design of human trials also differs from that of most preclinical studies, where experimental drugs prevented NASH and fibrosis development in initially healthy livers challenged with genetically determined or environmental stressors. This design is most suitable for determining the preventive, not the therapeutic efficacy of experimental agents. It is now clear that despite the multiplicity and diversity of pathways that initiate liver disease, the liver responds to injuries with a stereotyped pattern of hepatocyte degeneration and cell death, which triggers inflammatory and regenerative programs to compensate for hepatocyte loss and to limit parenchymal damage[196]. Persistent injury leads to chronic activation of a wound-

healing process, which is morphologically characterized by the increased production of ECM components, formation of fibrous septae, regenerative nodules and consequently disruption of the liver architecture. Although correcting the initial, variegated stimuli that injure hepatocytes may prevent the development of NASH and fibrosis, targeting the pathways mediating inflammation and fibrogenesis may reverse more advanced stages of liver disease[197]. Recent experimental data in fact demonstrate that even cirrhosis is a dynamic process and may regress if the underlying fibrogenic stimuli are corrected[197].

E1) targeting inflammation resolution: annexin-A1 and resolvin D1

The mechanisms responsible for terminating the inflammatory response are being actively investigated as potential anti-inflammatory pharmacological targets. Resolution of acute inflammation is coordinated by numerous proteins and eicosanoids that downregulate leukocyte recruitment, promote clearance of tissue leukocytes and of DAMPs/PAMPs, and switch macrophages from a pro-inflammatory M1 to a pro-resolution M2 phenotype, thus favouring tissue healing. Among these pro-resolving factors, defective Annexin A1 (AnxA1) and resolving D1(RvD1) activity have been implicated in the pathogenesis of inflammation and fibrosis in NASH. AnxA1, previously known as lipocortin-1, is a calcium-phospholipid–binding protein which is expressed by immune cells (including neutrophils, monocytes/macrophages, and NKT cells), and by epithelial and endothelial cells[198, 199] and whose synthesis is stimulated by glucocorticoids. AnxA1 interacts with its receptor, formyl peptide receptor 2/lipoxin A4 receptor (FPR2/ALX) and inhibits the secretion of proinflammatory mediators including IL-6, nitric oxide and eicosanoids, reduces neutrophil migration to inflammatory sites, enhances DAMPs, PAMPs and apoptotic cells clearance (a process named efferocytosis) by macrophages[200], promotes epithelial repair[201] and counteracts tissue fibrosis[202].

Defective AnxA1 activity has been implicated in the pathogenesis of obesity and obesity-related NASH: hepatic and circulating AnxA1 levels are decreased in NASH and obese patients, and

inversely correlate with liver fibrosis, BMI and inflammatory markers[203, 204]. Furthermore, in models of diet-induced obesity, AnxA1-deficient mice have increased adiposity and adipose tissue inflammation, insulin resistance and enhanced hepatic inflammation and fibrosis, which is accompanied by increased hepatic pro-inflammatory M1 macrophage infiltration and increased macrophage expression of the pro-fibrogenic lectin galectin-3[200, 205]. This pro-inflammatory and pro-fibrogenic phenotype was reversed *in vitro* in isolated macrophages by the addition of AnxA1, but the effects of AnxA1 activation on NASH and fibrosis *in vivo* have not been evaluated yet. Innovative strategies to enhance AnxA1 biological activity, limit its proteolysis by neutrophil proteinase-3 and enhance its delivery to inflamed tissues include AnxA1-based cleavage-resistant peptides like CR-AnxA12–50[206], AnxA1-derived bioactive N-terminal peptide Ac2-26[206], AnxA1 conjugation to collagen IV-targeted nanoparticles[207], or ALX/FPR2 agonists[206]: these strategies induced resolution of inflammation and fibrosis in a range of inflammatory conditions, such as chronic pulmonary inflammation and fibrosis and myocardial ischemia-reperfusion injury [198, 199, 202].

Resolvin D1 (RvD1) is an eicosanoid which is physiologically synthesized from ω-3 docosahexaenoic acid (DHA) by numerous cell lines at inflammatory sites. RvD1exerts its pro-resolving actions through high affinity binding to phagocyte receptors ALX/FPR2 and the G-protein-coupled receptor GPR32 with high affinity (EC50=1.2 pM for ALX/FPR2; 8.8 pM for GPR32)[208]. RvD1 levels are reduced in adipose tissue and plasma of obese patients, likely as a result of upregulation of specific metabolizing enzymes (mainly eicosanoid oxidoreductase), and inversely correlate with the severity of tissue and systemic inflammation[209, 210]. The effectiveness of RvD1 administration has been evaluated in diverse animal disease models. RvD1 administration rescued adipose tissue inflammatory changes, normalized insulin sensitivity and glucose tolerance, restored adiponectin secretion, decreased the production of proinflammatory adipokines including leptin, TNF-α, IL-6, and IL-1β, and reduced adipose tissue MCP-1-induced macrophage accumulation[206].RvD1 administration enhanced inflammation resolution, limited fibrogenic

response and reduced infarct size, resulting in improved ventricular function, in rodent models of myocardial infarction[211].

In cultured hepatocytes, pretreatment with RvD1 attenuated ER stress-induced apoptosis, SREBP-1 expression and triglycerides accumulation[212]. In a murine model of high fat diet-induced NASH, the addition of RvD1to calorie restriction reversed established steatohepatitis[213], reduced liver macrophage infiltration and shifted macrophages from an M1 to an M2 phenotype, and normalized the pro-inflammatory adipokine pattern in adipose tissue. These effects were accompanied by specific changes in hepatic miRNA signatures, suggesting these small, noncoding RNAs may mediate the proresolution activity of RvD1 at the post-transcriptional level[213], and were absent in macrophage-depleted precision-cut liver slices, indicating a crucial role of these cells in mediating RvD1 actions[213]. Since RvD1 is rapidly inactivated by eicosanoid oxidoreductase (EOR), several strategies are being tested to prolong its biological activity, including the design of EOR-resistant synthetic RvD1 analogues, such as benzo-diacetylenic-17R-RvD1-methyl ester (BDA-RvD1)[214], and the incorporation of RvD1 into liposomes (Lipo-RvD1)[211], which are predominantly cleared by macrophages and may therefore accumulate in the liver, thereby enhancing potency and selectivity of RvD1[171]**(Table 2).**

E2) targeting fibrosis: Galectin-3 inhibitors

Galectin-3 is a member of the galectin family, which consists of 15 glycan-binding proteins (also known as lectins) defined by their specificity for binding β-galactoside carbohydrate units, such as N-acetyllactosamine, on cell surface glycoconjugates[215]

Galectin-3 is broadly expressed by immune and epithelial cells, where it is localized mainly in the cytoplasm, but it is also present in the nucleus, on the cell surface and in the extracellular space[215]. Galectin-3 exerts multiple and sometimes contrasting effects according to its cellular location, cell type and mechanism of injury. Cytoplasmic galectin-3 can inhibit T-cell apoptosis by binding to Bcl-2[216] and can interact with activated K-Ras (K-Ras-GTP) and affect Ras-mediated Akt

signaling[217, 218]. Nuclear galectin-3 is a pre-mRNA splicing factor and is involved in spliceosome assembly by forming protein complexes with Gemin4[219]. It also regulates gene transcription by enhancing the association of transcription factors with Spi1 and CRE elements in gene promoter sequences and by binding to β-catenin, a molecule involved in Wnt signaling pathway[220].

Extracellular galectin-3 interacts the β-galactoside units of ECM and cell surface glycoproteins : at the cellular surface, galectin-3 forms multimers driven by increasing concentrations of glycoprotein ligands, resulting in higher order lattices which trigger cell signaling and regulate cell adhesion and proliferation. These effects are mediated by cell surface adhesion molecules such as integrins and with the receptors of numerous growth factors, including epidermal growth factor(EGF), platelet-derived growth factor(PDGF), insulin-like growth factor(IGF), and FGFs[221]. By virtue of its interaction with β1-integrin, extracellular galectin-3 has been found to exert a pro-apoptotic action in activated T-cells[222], thereby opposing intracellular Galectin-3.

In the liver, *in vitro* and *in vivo* models suggest that extracellular and cell surface galectin-3 exert proinflammatory effects by promoting mononuclear, neutrophil and NKT cell adhesion and activation[223,224] and by mediating the uptake of advanced glycation end-products (AGEs) and advanced lipoxidation end-products(ALEs) by Kupffer and endothelial cells[225](**Figure 4**).

Hepatic galectin-3 is also upregulated in established human fibrosis and has pro-fibrogenic effects *in vivo* and *in vitro*[226]: galectin-3 stimulates myofibroblast and HSC proliferation and activation[226, 227] and promotes hepatic progenitor cell expansion and differentiation. These profibrogenic effects were reversed by genetic or pharmacologic galectin inhibition with thiodigalactoside (a potent inhibitor of b-galactoside binding) [228, 229]. In diet-induced NASH models, genetic deletion of galectin-3[225] or treatment with carbohydrate-based galectin inhibitors GR-MD-02 (galactoarabino-rhamnogalaturonan) or GM-CT-01 (galactomannan) prevented NASH and fibrosis development[230] and, most intriguingly, reversed established severe fibrosis and cirrhosis[231] .

The recently completed phase I RCT (ClinicalTrials.gov Identifier: NCT01899859) showed that administration of 2, 4 and 8 mg/kg lean body weight of GR-MD-02 intravenously for four doses

over 6 weeks was safe and well tolerated in patients with NASH with advanced fibrosis, and the highest dose improved a noninvasive marker of hepatic fibrosis[232].

Long-term extrahepatic safety of galectin-3 inhibition requires further evaluation: galectin-3 knockout mice fed a hyper-caloric diet developed increased adiposity, systemic and adipose tissue inflammation, glucose intolerance, atherosclerosis[233] and kidney damage[234], associated with upregulation of the receptor for advanced glycation end products(RAGE)[235, 236]. These findings suggest an important anti-inflammatory role of galectin-3 in extrahepatic tissues in response to overnutrition, through the mechanism remains unclear. It has been suggested that inhibition of AGE/ALE uptake by the liver, which clears >90% of these end-products from the circulation[237], promotes their systemic accumulation and uptake by RAGE at extrahepatic tissues, thereby enhancing their extrahepatic toxicity[225, 235, 236, 237,].

In addition to the tissue specificity of galectin-3 inhibition, it will also be important to assess if selective pharmacological inhibition of extracellular galectin-3 may reduce these unwanted pro-inflammatory effects, given the dual role of extracellular and intracellular role of galectin-3.

E3) targeting fibrosis: Lysyl oxidase-like 2 (LOXL2) inhibitors

The lysyl oxidase (LOX) family comprises five enzymes (LOX, lysyl oxidase-like 1 or LOXL1, LOXL2, LOXL3, and LOXL4), that catalyze the oxidative deamination of the ε-amino group of lysines and hydroxylysines in collagen and elastin to promote cross-linking of these molecules, which is essential for the tensile strength of ECM during fibrogenesis[238]. In addition to ECM remodeling, LOXL2 enhances fibrogenesis in NASH by inducing epithelial-to-mesenchymal transition (EMT)[239], a cellular process in which epithelial ductular-like cells disassemble cell-to-cell attachments that tether them to adjacent cells and acquire a mesenchymal phenotype that allows them to migrate into the stroma, proliferate and synthesize ECM in response to various growth factors and cytokines[240].

In the liver, HSCs, portal fibroblasts and hepatocytes are major sources for LOXL2[241] and hepatic overexpression has been observed in patients with various fibrotic conditions[242].

Treatment with a LOXL2-blocking antibody reduced TGF-ß signaling and fibroblast activation and reversed experimental liver fibrosis [242].

Simtuzumab (GS-6624, formerly AB0024), a humanized anti-LOXL2 monoclonal IgG4 antibody, reached safety and tolerability end-points in a phase I RCT enrolling patients with liver disease of diverse etiology[243] and its efficacy is currently being evaluated in 2 phase IIb, dose-ranging RCTs enrolling patients with NASH-related advanced non-cirrhotic liver fibrosis (ClinicalTrials.gov Identifier: NCT01672866) and cirrhosis (ClinicalTrials.gov Identifier: NCT01672879), respectively.

E4) targeting fibrosis: 5-lipoxygenase(5-LOX)/leukotriene pathway inhibitors

Leukotrienes(LT) are generated from arachidonic acid metabolism by the catalytic activity of the enzyme arachidonate 5-lipoxygenase (5-LOX)[244] and participate in inflammatory responses by promoting leukocyte recruitment and chemotaxis. In the liver, Kupffer cells constitutively express 5-LOX and synthesize LTB4 and cysteinyl-LT, the latter is also produced in hepatocytes by transcellular metabolism of LTA4 secreted by Kupffer cells[245]. 5-LOX-derived leukotrienes act in both paracrine and autocrine fashion to promote Kupffer cell viability and growth and HSC activation. A similar role for adipocyte 5-LOX in mediating adipose tissue inflammation and NAFLD has been found in experimental models of obesity[246]

Experimental data suggest a key role for 5-LOX in mediating liver inflammation and fibrosis: 5-LOX is heavily over-expressed in diverse experimental models of NASH, and genetic deletion or pharmacological inhibition of 5-LOX ameliorated the steatotic, inflammatory, and fibrotic responses[247, 248].

MN-001 (tipelukast) is a novel, orally bioavailable small molecule compound that exerts a potent anti-inflammatory and antifibrotic activity in preclinical models through several mechanisms, including 5-LOX inhibition, leukotriene (LT) receptor antagonism, and inhibition of phosphodiesterases (PDE) 3 and 4. Tipelukast reduced inflammation and fibrosis and down-regulated expression of proinflammatory and profibrogenic genes, including MCP-1, CCR2, tissue

inhibitor of metallopeptidase(TIMP)-1, collagen Type 1 and LOXL2 in an advanced NASH model[249] and was FDA-approved for a Phase IIa RCT in NASH patients with advanced fibrosis.[250]

E5) targeting fibrosis: Caspase inhibitors

Caspases are a family of cysteine proteases (cysteine aspartate-specific proteases) initiate and mediate apoptosis. Increased hepatocyte apoptosis has been consistently linked to the progression from simple steatosis to NASH and NASH-related cirrhosis in NAFLD patients and in cellular and animal models[251, 252]. A prevailing concept is that injured hepatocytes initiate the apoptotic process but fail to complete it, thereby providing a sustained source of apoptosis-associated molecular signals and cytokines that trigger liver inflammation, wound healing and fibrogenesis[251]. Inhibition of the initiator caspases (caspase-8, caspase-9 and caspase-2)[253, 254] or effector caspases (caspase-3 and caspase-7)[252] ameliorated necro-inflammation and fibrosis in experimental models of NASH[255, 256].

The irreversible, orally active oxamyl dipeptide pan-caspase inhibitor emricasan (IDN-6556) was safe and well-tolerated and reduced markers of apoptosis in a small, short-term phase I RCT enrolling patients with hepatic impairment[257] and in currently being evaluated in non-cirrhotic NAFLD patients (ClinicalTrials.gov ID: NCT02077374). Long-term safety of caspase inhibition needs to be assessed as many human cancers, including hepatocellular carcinoma, are characterized by uncontrolled cell survival and apoptosis suppression through endogenous caspase inhibitor production[258] .

E6) targeting fibrosis: Hedgehog signaling pathway inhibitors

Hedgehog (Hh) is a signaling pathway that regulates critical steps in cell fate, including differentiation, proliferation, migration and apoptosis, in tissue morphogenesis during fetal development[259]. In adult life the Hh pathway is inactive in healthy tissues, but is reactivated following injury to modulate wound healing in numerous tissues and organs, including the liver. In

the liver, Hh pathway activation induces expansion of hepatic progenitor cells, accumulation of inflammatory cells, and increased fibrogenesis and vascular remodeling, all of which are key events in the pathogenesis of cirrhosis. In addition, Hh signaling may play a role in primary liver cancers, including cholangiocarcinoma and hepatocellular carcinoma[259].

Hh pathway signaling is initiated by 3 families of palmitoyl- and cholesterol-modified ligand proteins named Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh), which are expressed by different types of cells and have functional specificity, partly regulated by their regulatory mechanisms and expression patterns[260]. In the canonical signaling pathway, these ligands interact with their cell surface receptor Patched (Ptch) that is expressed by Hh responsive target cells, resulting in disinhibition of another plasma membrane receptor, Smoothened(Smo), eventually culminating in changes in transcription [259, 260].Hh ligands are not expressed in healthy liver tissue, and Hh signaling is not activated in mature cholangiocytes or in hepatocytes. However, these cell types start to secrete Hh ligands when subjected to certain injury-associated cytokines, ER stress or oxidative stress; ballooned hepatocytes are a prominent source of Shh ligands in NASH patients; and NASH regression is associated with concomitant down-regulation of Hh pathway activity[261, 262].

Substantial evidence from animal models of NASH indicates that the Hh signaling pathway promotes fibrosis by enhancing activation and inhibiting apoptosis of HSCs[263], inducing EMT of immature ductular-type progenitor cells [240] and promoting the hepatic accumulation of pro-fibrogenic natural killer T(NKT) cells[264]. Hh antagonism by the small-molecule Smo inhibitors vismodegib (formerly GDC-0449) or cyclopamine reversed experimental NASH, advanced fibrosis and hepatocellular carcinoma[265, 266].

Given the robust experimental evidence supporting the importance of Hh pathway hyperactivation in NASH and the recent U.S. FDA approval of several Smo inhibitors for other indications(i.e., vismodegib for the treatment of basal cell carcinoma), hedgehog signaling pathway inhibitors could be evaluated for the treatment of NASH and fibrosis by future RCTs.

E7) targeting fibrosis: Induction of fibrogenic cell senescence

The reversibility of hepatic fibrosis, even at the cirrhotic stage, upon cessation of fibrogenic stimuli suggests the existence of endogenous mechanisms for the resolution of liver fibrosis. Liver fibrosis regression is associated with resorption of the fibrous scar and disappearance of HSC-derived collagen-producing myofibroblasts. These myofibroblasts can be inactivated by apoptosis[193], can revert to an quiescent-like, nonfibrogenic phenotype[267] or enter a state of senescence[268]. Although all these processes terminate ECM production, senescent myofibroblasts actively contribute to fibrosis regression by secreting molecules that decrease proliferation, downregulate ECM deposition and upregulate matrix-degrading enzymes (MMP2, MMP3 and MMP9) in neighbouring cells and promote the clearance of myofibroblasts by NK cells[269]. Therefore, the entry of myofibroblasts into senescence not only prevents further fibrosis deposition but also actively contributes to ECM degradation and clearance.

The matricellular protein Cysteine-rich protein 61 (CYR61), also known as CCN1 [CYR61, CTGF (connective tissue growth factor), and NOV (Nephroblastoma overexpressed gene)] is emerging as a key trigger of myofibroblast senescence and fibrosis resolution in the liver. CCN1 is not required for liver development or regeneration, and these processes are normal in mice with hepatocyte-specific *Ccn1* deletion. However, CCN1 expression is upregulated in human cirrhotic livers and in hepatocytes and HSCs during the early phase of liver injury, and its expression declines during prolonged phases of fibrogenesis[270, 271]. CCN1 limits liver fibrogenesis and promotes fibrosis regression by triggering senescence of activated HSCs and portal fibroblasts.

Mice with hepatocyte-specific *CCN1* deletion have exacerbated fibrosis with a concomitant deficit in myofibroblast senescence, whereas hepatic CCN1 over-expression reduces liver fibrosis and enhances cellular senescence. Furthermore, delivery of purified CCN1 protein or myofibroblast transfection with CCNI-overexpressing adenovirus accelerated fibrosis resolution in mice with

advanced fibrosis[270, 271]. Therefore, the CCN1 signaling pathway could be an attractive target for treating NASH-related advanced fibrosis.

Concluding remarks and perspectives

The prevalence of NAFLD in developed countries is constantly increasing, along with the obesity epidemic, and the health-related burden of NASH is concomitantly growing: NASH was the second leading aetiology of liver disease among adults awaiting liver transplantation in the United Network Organ Sharing(UNOS) registry during the years 2004-2013 and is projected to become the most common indication for liver transplantation in the next decade[2]. Therefore, effective treatments for this condition are eagerly awaited. Treatment of NAFLD is challenging, as progression from steatosis to NASH and fibrosis is likely a multi-factorial process, involving varied molecular pathways that may operate in different patient subsets, including insulin resistance, proinflammatory cytokine release from adipose tissues, altered redox balance, impaired lipid and cholesterol metabolism and gut microbial dysbiosis. Our knowledge of how to antagonize these pathways has substantially advanced and the development of a new pharmacological armamentarium is underway. A key challenge will be the selection of the optimal therapeutic strategy for each patient: bm this context it is likely that recent developments in metabolic phenotyping with metabolomics and systems biology technologies will substantially enable individualized treatment tailored to individual metabolic profile [272]

In parallel, the recognition that common effector mechanisms mediate inflammatory and fibrosis development has led to the development of antagonists of common effector mechanisms of inflammation. There is also growing interest in antifibrotic therapies in NASH, for several reasons: the increasing public health impact of NAFLD, which will soon replace other etiologies of liver disease as the leading cause of cirrhosis, our better understanding of the pathogenesis of hepatic fibrosis progression and regression, with preclinical data challenging the

longstanding conception of cirrhosis as an irreversible process, and the development of novel surrogates to assess fibrosis content and progression, which may hopefully enable short-term clinical studies in smaller, selected patient populations[273].

On the basis of data presented above, combination therapies targeting various cell types and pathways are also an attractive approach to be explored preclinically and in clinical trials.

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BOXES

BOX 1 Autophagy

Autophagy is the major cellular digestion process that removes damaged and dysfunctional macromolecules and organelles and recycles them to provide energy and molecular substrates in response to nutrient, oxidative or metabolic stress. Three types of autophagy exist in mammalian cells: macroautophagy, chaperone-mediated autophagy (CMA) and microautophagy. In macroautophagy, cytoplasmic material (*e.g.*, organelles or protein aggregates) is sequestrated in a double layer membrane structure, the autophagosome. This process initiates the formation of a phagophore (or isolation membrane), which subsequently lengthens to create an autophagosome. The autophagosome fuses with a lysosome to form an autolysosome where its content is degraded. When a small fraction of cytoplasm is engulfed directly by the lysosome, the term microautophagy is used. In CMA, proteins containing a special targeting motif, recognized by heat shock cognate protein 70 (HSC70) and co-chaperones, are selectively delivered to lysosomes where they are internalised *via* a lysosomal-associated membrane protein 2A (LAMP2A).

Among the three types of autophagy, macroautophagy plays the most important role in cell pathophysiology. Even though autophagy was initially believed to be a non-selective degradation pathway, selective forms such as "mitophagy" (selective autophagy of mitochondria), "peroxiphagy" (peroxisomes), "ribophagy" (ribosomes) or "xenophagy" (invading microbes) have been also recognized and defective mitophagy has been connected to the pathogenesis of NASH, since dysfunctional mitochondria produce reactive oxygen species and enhance oxidative stress[148].

The formation of autophagosomes is a dynamic process highly regulated at the molecular level by autophagy related (*Atg*) genes through different steps:

1)**Initiation and nucleation**: macroautophagy starts with the formation of a double-layered membrane, the phagophore (isolation membrane). Phagophore formation is regulated by the ULK1 complex (initiation), which is under control of mTOR complex, and by the beclin-1/VSP34(a class III PI3K)- interacting complex (nucleation).

mTORC1 phosphorylates the autophagy-related protein 13 (Atg 13), preventing it from entering the ULK1 kinase complex, which consists of Atg1, Atg17, and Atg101 This prevents the structure from being recruited to the preautophagosomal structure at the plasma membrane, inhibiting autophagy. Conversely, under conditions of low energy status, the AMP/ATP ratio increases, leading to adenosine 5'-monophosphateactivatedAMPK) activation and mTOR inhibition, thereby activating autophagy.

2)Elongation. Two ubiquitin-like conjugated complexes take care of elongation of the formed phagophore into an autophagosome: the ATG5-ATG12-ATG16L1 complex and light chain 3-Π(LC3-Π). An E1-like protein, ATG7, is necessary for formation of both elongation complexes. LC3 is the major mammalian orthologue of ATG8 and also one of the key regulators in autophagosome formation.

3)Fusion and degradation. The autophagosome fuses with a lysosome. The inner membrane of the autophagosome and the sequestered cytoplasm will be degraded and macromolecules can subsequently be (re-)used.

mTORC1 blocks autophagy by inhibiting the initiation of autophagosome formation through phosphorylation of UNC51-like kinase 1 (ULK1)(BOX 1), while its inhibition ameliorated autophagy and NAFLD development[138] .

FIGURE LEGENDS

Figure 1: effects of AMPK (panel A), mTORC1 (panel 1B) and mTORC2 (panel C) activation.

Abbreviations: ABCA1: **A**TP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase; AICAR: 5-Aminoimidazole-4-carboxamide-1-β-D- ribonucleoside; AMPK: adenosine-monophosphate kinase; CA: cholic acid; CDCA. chenodeoxycholic acid; Ccl3 : chemokine(C-C

motif ligand 3 ; CD36: cluster of differentiation-36; CHOP: C/EBP homologous protein; CPP: calciprotein particles; CPT-1: carnitine palmitoyltransferase-I**;** ; ER: endoplasmic reticulum; FAS: fatty acid synthase; FFA: free fatty acids; FXR: farnesoid X-receptor; GLUT: glucose transporter; HMG-CoAR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IL: interleukin; 11β-HSD1: 11β-hydroxysteroid dehydrogenase type 1; IRE1α : inositol requiring element 1α ; IRS-1: insulin receptor substrate-1; KLF: Kruppel-like factor; LDL: low-density lipoprotein; LDL-R: low-density lipoprotein receptor; MCP-1: monocyte chemotactic protein-1; NO: nitric oxide; NOX: NADPH oxidase; OCA: obeticholic acid; PCSK9 : proprotein convertasesubtilisin kexin 9 ; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1 α; ROS: reactive oxygen species; SCD-1: stearoyl-CoA desaturase-1; SOD2: superoxide dimutase-2; SR-A1: scavenger receptor-A1; SR-B1: scavenger receptor-B1; SREBP: sterol-responsive element binding protein; STAT3: signal transducer and activator of transcription; TGF-β: transforming growth factor- β; TLR: toll-like receptor; TNF: tumor necrosis factor; TZD: thiazolidinediones; VLDL: very low density lipoprotein; VSCMs: vascular smooth muscle cells;

Figure 2: the inflammasome and its involvement in initiation of inflammation.

The inflammasome is a cytosolic multiprotein complex that is essential for the initiation of many inflammatory responses in many cell types. The activation of 2 signaling pathways is required for full inflammasome activation and production of mature IL-1b and IL-18.

Signal-1: This results in the production of pro-IL-1b and pro-IL-18 through interaction of various DAMPs/PAMPs and cytokines like TNF-α and IL-1ß with TLRs, IL-1ß-R and TNF-R.

Signal-2: This leads to inflammasome activation through multiple signaling pathways. MSU and other crystals result in the formation of phagolysosomes. Other pathways for inflammasome activation include the P2X7 receptor and ROS-induced dissociation of thioredoxin-interacting protein (TXNIP) from thioredoxin: TXNIP can thereby interact with NLRP3and directly activate the inflammasome.

The activation of inflammasome results in the cleavage and activation of the proteases caspase-1 which subsequently cleaves pro-IL-1b and pro-IL-18 to mature IL-1b and IL-18, which are eventually secreted out of the cell.

Below is a classification of target molecules of signal 1 and signal 2, their activators and inhibitors

Signaling pathway	Target	Activators	Inhibitors
Signal 1	TLR-4	FFA, LPS	ethyl pyruvate, eritoran,
		HMGB1	anti-HMGB1 Abs
	P2X7 R	ATP, NAD	apyrase, A438079, etheno-NAD
	Phagosome	Uric acid crystals	allopurinol, febuxostat
Signal 2		Cholesterol crystals	statins, ezetimibe
	NLRP3	Phagosome, P2X7R activation	auranofin (TXNIP-mediated), NMDA agonists, isoliquiritigenin, arglabin
	Caspase-1	NLRP3 activation	GS-9450

Abbreviations: AIM2: absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a CARD; ATP, adenosinetriphosphate; DAMPs, damage associated molecular patterns; IL-1ß, interleukin-1beta; IL-18, interleukin-18; MSU: monosodium urate; NLRC4: NLR family CARD domain-containing protein 4; NLRP, Nod-like receptor proteins; PAMPs, pathogen associated molecular patterns; ROS, reactive oxygen species; TLRs, toll like receptors; TNF-a, tumor necrosis factor-alpha; TNFR, tumor necrosis factor receptor; TXN: thioredoxin; TXNIP: thioredoxin-interacting protein

Figure 3: chemokines in the pathogenesis of NASH

In NASH, monocytes are recruited from the bloodstream, predominantly via CCL2/CCR2. CXC chemokines such as CXCL2 contribute to neutrophil recruitment, whereas others, including CCL6/16, increase the inflow of T lymphocytes. These changes contribute to determine hepatic fatty degeneration, activation of Kupffer cells, which together with hepatocytes and stellate cells amplify inflammation via chemokines (CCL2 and CCL5), and recruitment of immune cells (eg,

monocytes) into the liver. Chemokines have also been directly implicated in the accumulation of lipids within hepatocytes.

Figure 4. Series of events occurring in a self-resolving inflammatory process in the liver.

Productive phase: during liver injury, molecular patterns (DAMPs and PAMPs) are recognized by resident cells (Kupffer cells, dendritic cells and sinusoidal cells) that produce pro-inflammatory mediators, including cytokines IL-1 and TNF-α, which induce hepatocyte apoptosis and hepatic endothelial cell activation, and chemokine production, including CCL2, which promotes hepatic accumulation of bone marrow-derived pro-inflammatory M1 monocytes, CXCL1, CXCL2, CXCL8, which attract neutrophils via CXCR1/CXCR2, and CXCL16, which attracts NKT cells via CXCR6. Sinusoidal endothelial cells express cell adhesion molecules (selectins and integrins) and present chemoattractant mediators which recruit leukocytes to the liver.

In this phase, Kupffer cells and macrophages secrete also galectin-3 which boosts bone marrow-derived cells accumulation in the liver, activates HSCs, myofibroblasts and hepatic progenitor cells(HPCs) to start extracellular matrix (ECM) and collagen deposition[215, 229].

Transition phase:

During the accumulation of leucocytes. the secretion of pro-resolving molecules (including AnxA1 and RvD1) starts triggering leukocyte apoptosis and phagocytosis of damaged cells by tissue macrophages (efferocytosis). During this phase, macrophage phenotype swithches from M1 to pro-resolving M2.

Resolving phase: efferocytosis by M2 macrophages is fully activated. Additionally, M2 macrophages produce anti-inflammatory (including IL-10) and pro-resolving mediators(including AnxA1 and RvD1), which attenuate leukocyte recruitment and promote monocyte migration and efferocytosis. M2 macrophages switch to Mresolution (Mres) phenotype, which exhibits reduced phagocytosis, but increased secretion of anti-fibrotic and anti-oxidant molecules, thereby limiting liver injury and fibrosis and restoring normal tissue homeostasis.

Table 1. Nuclear transcription factors FXR, SREBF-2/miRNA-33a, PXR, PPAR-α, -PPAR-δ in the pathogenesis and treatment of NAFLD.

Farnesoid X Receptor		
Cell type	**Molecular mechanism**	**Biological action**
hepatocyte	Activation of SREBP-1c-mediated lipogenesis and of PPAR-α-mediated FFA β-oxidation Downregulation of gluconeogenetic enzymes PEPCK G6-Pase, F-1,6-DP-ase Enhanced IRS-1 phosphorylation and coupling with the PI-3K Enhanced AdipoR2 expression [16] Enhanced CYP7A1 and ABCG5/G810 expression Reduced hepatic lipase activity and ApoC-III and apoA-1 synthesis Reduced VLDL secretion and HDL-C synthesis Increased ApoC-II synthesis and VLDLR-mediated uptake of VLDL Reduced NF-κB pzthway activation(IκB-α-mediated)	Reduced hepatic steatosis Enhanced insulin sensitivity Enhanced bile acid synthesis and cholesterol excretion into bile Reduced plasma HDL-C Reduced plasma Tg Reduced inflammation
Marophage Kupffer cell	Reduced IκB-α phosphorylation and NF-κB activation, resulting in reduced TGF-ß secretion	Reduced inflammation and fibrogenesis
HSC	Reduced MCP-1 secretion and TGF-ß-R expression	Reduced inflammation and fibrogenesis
Adipocyte	Increased PPAR-γ expression Increased adiponectin and AdipoR2 expression Reduced TNF-α secretion[16]	Improved adipose tissue dysfunction
Enterocyte	Enhanced gut barrier function and secretion of antibacterial factors angiogenin, iNOS, IL-18 Enhanced FGF-19 secretion[19]	Reduced bacterial endotoxemia Increased bile acid synthesis, EE and fat oxidation
G-protein-coupled receptor TGR5		
Cell type	**Molecular mechanism**	**Biological action**
Macrophage, Kupffer cell	Reduced NF-κB activation	Reduced inflammation

Intestinal L-cells	Increased GLP-1 secretion	Increased action of GLP-1
Skeletal miocyte, adipocyte	Increased PGC-1α expression Increased D2-mediated conversion of T4 to T3	Enhanced mitochondrial biogenesis and function Increased EE
Sterol regulatory binding protein-2 (SREBP-2)		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocytes adipocytes	Upregulaton of HMG-CoAR and squalene synthase[57,] Increased LDL-R expression	Increased cholesterol synthesis and uptake
	Upregulation of NPC1L1	Increased intestinal and biliary cholesterol reabsorption
	Reduced SR-BI expression[14,]	Reduced reverse cholesterol transport and biliary elimination
	Increased StARD4 expression[74]	Enhanced cholesterol accumulation into mitochondria
HSC	Enhanced LDL-R-mediated cholesterol uptake[23, 24]	HSC and fibrogenesis activation
Adipocytes	Increased secretion of proinflammatory adipokines (angiotensinogen, TNF-α, IL-6, chemerin[57, 62]	Adipose tissue dysfunction
miRNA33a		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocyte, adipocyte	Reduced ABCA1 expression	Reduced cholesterol excretion
	Reduced NPC-1 expression[21]	Enhanced cholesterol accumulation in LE/LY
	Reduced activation of AMPKα, CPT1A, CROT and mitochondrial trifunctional protein HADHB[25,26]	Reduced mitochondrial β-oxidation of fatty acids
	Reduced IRS-2 signalling [26]	Insulin resistance
HSC	Increased PI3K/Akt pathway activation Reduced PPAR-α expression [27]	Increased TGF-β-mediated HSC activation
Pregnane X receptor		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocyte	Increased *de novo* lipogenesis through: 1)SREBP-1c activation [38] 2)direct upregulation of lipogenic enzymes SCD-1,	Increased hepatic steatosis

	FAE, FAS and ATP citrate lyase Enhanced SLC13A5- and FAT/CD36-mediated uptake of citrate and FFA from plasma [37, 41] Reduced mitochondrial FA oxidation through 1)reduced PPAR-α expression 2) direct down-regulation of CPT1A[42]	
	↓ hepatic gluconeogenesis through reduced expression of PEPCK and G6-Pase Reduced hepatic FOXO1 transcription[44]	Improved hepatic insulin sensitivity
Hepacocyte, enterocyte	Reduced NF-κB-mediated secretion of IL-1, IL-6, COX-2, TNF-α[45] Increased Jak2-mediated pohosphorylation of STAT3, enhancing HO-1, Bcl-xL expression[46] Enhanced Beclin 1 and LC3B-I, -II expression [46]	Reduced inflammation Reduced apoptosis Enhanced autophagy
HSC	Reduced HSC transdifferentiation, proliferation and activation[48]	Reduced fibrosis
PPAR-α		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocyte, miocyte	Increased expression of : acyl-CoA synthetase, CPT1A, VLCAD/LCAD/MCAD, acyl-CoA dehydrogenase, trifunctional protein HADHB, ACOX1, L-bifunctional protein EHHADH Increased CYP4A and HMGCS[54]activity Increased FATP, CD36, L-FABP activity[55] Increased LPL activity and reduced apoC-III[56] Increased apo-AI/apo-AII synthesis	Increased mitochondrial and peroxisomal ß-oxidation Increased ω-oxidation Increased ketogenesis Enhanced FFA uptake Enhanced lipolysis of Tg Increased HDL-C levels
Hepatocyte	**PPRE-dependent regulation:** Enhanced p65 binding to NF-κB response element of C3 promote, rleading to reduced complement C3 secretion[57] Reduced NF-κB activation through IκBα upregulation[58] Enhanced CREBH-mediated FGF21 expression[54]	Reduced inflammatory response and endothelial dysfunction Enhanced metabolic effects of

	PPRE-independent regulation: Reduced expression of IL-6 **,** IL-1, TNF-α, ICAM-1, VCAM-1[60, 61, 70] Increased catalase activity[62]	PPAR-α Enhanced H_2O_2 detoxification
PPAR-δ		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocyte	Enhanced mitochondrial β-oxidation [67, 68]	Improved hepatic steatosis and insulin resistance
	Increased ABCA1 expression [68]	Increased HDL-C levels
Macrophage **Kupffer cell**	Increased M1/M2 phenotype ratio [65] Reduced NF-κB pathway activation and TGF-ß1 secretion[67]	Reduced inflammatory and /fibrogenesis
Adipocyte, miocyte	Enhanced PGC-1α-mediated mitochondrial biogenesis and ß-oxidation Increased mitochondrial UCP-1/3 expression Increased LPL expression [66]	Enhanced fat oxidation and EE Reduced plasma triglycerides
Enterocyte	Reduced NPC1L1 expression and cholesterol reabsorption from bile and intestine	Reduced cholesterol accumulation
Nuclear erythroid 2-related factor(Nrf2)		
Cell type	**Molecular mechanism**	**Biological action**
Hepatocyte, macrophage,	Increased expression of: 1) antioxidant proteins: Glt-R, Glt-Px, TXN-R, Cat 2) phase I oxidation, reduction and hydrolysis enzymes: ALDH3A1, EPHX1, NQO1 3)phase II detoxifying enzymes: GST, MGST: UGT, PSMB5 4) NADPH-generating Enzymes: G6PD 5))heme metabolizing enzymes: HO-1 6) protein degradative pathways: UbC, PSMB5	Reduced oxidative stress Xenobiotic detoxification
	Enhanced autophagy[76] Reduced NF-κB pathway activation(IκB-α-mediated) Reduced iNOS and COX-2 expression Increased FGF21 secretion [77]	Reduced inflammation and fat accumulation in liver and adipose tissue
HSC	Reduced Smad3-mediated TGF-β1 pathway activation Reduced PAI-1 expression [81]	Reduced fibrogenesis

Abbreviations: ABC. ATP-binding cassette; ACC: acetyl-CoA carboxylase , AKR1B10: aldo-keto reductase B10; ACOX1: straight-chain acyl-CoA oxidase; AdipoR2:adiponectin receptor 2; ALDH3A1 Aldehyde dehydrogenase 3A1; Apo: apolipoprotein; AMPKα: AMP kinase subunit-α; CREBH: cAMP-responsive element binding protein, hepatocyte specific; Cat: Catalase; CROT: carnitine *O*-octanoyltransferase; CYP7A1: Sterol 7α hydroxylase; CPT1A: carnitine palmitoyltransferase 1A; D2: Type II iodothyronine deionidase; EE: energy expenditure; EHHADH: enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase; *EPHX1* Microsomal epoxide hydrolase 1; F-1,6-DP-ase: fructose-1,6-biphosphatase ; FAE: fatty acid elongase; FAS: fatty acid synthase; FAT: fatty acid traslocase; FATP: fatty acid transport protein; FFA: free fatty acid; FGF: fibroblast growth factor; FOXO1: forkhead box–containing protein O subfamily-1; Glt-Px: Glutathione peroxidase; Glt-R: Glutathione reductase ; *G6PD:* Glucose-6-phosphate 1-dehydrogenase; G6-Pase: glucose-6-phosphatase; GLP-1: glucagon-like peptide-1; *GST:* Glutathione *S*-transferase; HADHB: hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase βsubunit; HMG-CoAR: 3-hydroxy-3-methyl-glutaryl-CoA reductase; HMGCS: 3-hydroxy-3-methyl-glutaryl-CoA synthase; HO-1: heme oxygenase-1; HSC: hepatic stellate cell; iNOS: inducible nitric oxide synthase; IRS: insulin-receptor substrate; Jak2: Janus kinase 2; LC3B: light chain 3B; LE: late endosome; L-FABP: liver fatty acid binding protein; LPL: lipoprotein lipase; LY: lysosome; LDL: low density lipoprotein; LDL-R: LDL receptor; MCP-1: monocyte chemotatic protein-1; *MGST:* Microsomal glutathione *S*-transferase*;* NF-κB: nuclear factor κB; NPC: Niemann-Pick C protein; NPC1L1: Niemann-Pick C1-like 1; *NQO1:* NAD(P)H:quinone oxidoreductase; PEPCK: phosphoenol-pyruvate carboxykinase; PGC-1α: PPARγ coactivator 1α; PI-3K: phosphoinositide 3-kinase; PPAR: peroxisome proliferator-activated receptor**;** *PSMB5:* Proteasome 26S PSMB5 subunit; SCD-1: stearoyl-CoA desaturase-1; SLC13A5: Solute carrier family 13 (sodium-dependent citrate transporter; SR-BI: scavenger receptor class B type I; SREBP: sterol regulatory element binding protein; StARD4: Steroidogenic acute regulatory protein D4; STAT3: Signal transducer and activator of transcription 3; T4: thyroxine; T3: triiodothyronine; TGF: transforming growth factor; *TXN-R:* Thioredoxin reductase; UbC: Ubiquitin C; UCP: uncoupling protein; *UGT:* UDP glucuronosyltransferase; VLCAD/LCAD(MCAD: very long-chain/long-chain/medium-chain acyl-CoA dehydrogenase; VLDL: very low density lipoprotein; VLDLR: VLDL receptor.

Table 2. Main molecular targets and developmental stages of drugs discussed in the article

Molecular mechanism of action	**Molecule**	**Developmental stage for NAFLD treatment**
FXR and TGR5 activators	Semisynthetic bile acid OCA (6-ethyl- CDCA, INT-747)	IIa [15]
	Synthetic non-steroidal isoxazole Px-104	IIa (ClinicalTrials.gov ID: NCT0199910)
	Natural polyphenol EGCG	Preclinical [23]
SREBP-2 and/or miR-33a inhibitors	Natural antioxidants (proanthocyanidins, resveratrol, curcumin) Synthetic anti-miR-33a oligonucleotides	Preclinical[26, 33, 34]
Pregnane X receptor activators	Natural compounds carapin, santonin and isokobusone	Preclinical[47]
PPAR-α/δ activators	Synthetic agonists: K-877, GFT505, GW501516, GFT505, L-165041	IIb (ClinicalTrials.gov ID: NCT01694849)
Nrf2 activators	Electrophilic compounds: -natural: sulphoraphane, resveratrol, curcumin, EGCG, dimethyl fumarate -synthetic: dithiolethiones(oltipraz, anethole dithiolethione), bardoxolone methyl	Preclinical [82, 83] IIa (clinicaltrials.gov ID: NCT01373554)
	Non-electrophilic compounds: -natural: berberine -synthetic: NK-252, THIQ, MG132, tBHQ	Preclinical [78, 83, 86, 87]
Natural antioxidants	Resveratrol	IIa [96-99]
	Quercetin	Preclinical [101-104]
FGF-21 analogues	PEGylated FGF-21, Fc-FGF21(RG), FGF21-mimetic monoclonal Ab mimAb1	Preclinical [120, 121, 124]
	Recombinant LY2405319	IIa[123]
AMPK activators	Natural: monascin, ankaflavin, quercetin, berberin, curcumin Synthetic: oltipraz	Preclinical[127, 129, 130, 131] IIa(clinicaltrials.gov ID: NCT01373554)
mTORC1/2 inhibitors	mTORC1/2 inhibitors: rapamycin, AZD3147	Preclinical [137, 138]
	mTORC1 inhibitors: Z1001, Rottlerin, XL388	Preclinical[134, 135, 136]

Inflammasome inhibitors	ethyl pyruvate, eritoran, apyrase, A438079, etheno-NAD, auranofin, NMDA agonists, isoliquiritigenin, arglabin,	Preclinical [161-168]
	GS-9450	IIa[169]
Chemokine antagonists	Dual CCR2/CCR5 antagonist cenicriviroc	IIb (ClinicalTrials.gov ID**:** NCT02217475)
	CCR5 antagonists: Met-CCL5, maraviroc	Preclinical [187, 188]
	CXCR3 antagonist: NIBR2130	Preclinical [170]
Annexin A1 analogues	CR-AnxA12–50, Ac2-26, Polymer-AnxA1, ALX/FPR2 agonists	Preclinical [206, 207]
Resolvin D1 analogues	BDA-RvD1, Lipo-RvD1	Preclinical [211, 214]
Galectin-3 inhibitors	GM-CT-01 (galactomannan)	Preclinical [227, 228]
	GR-MD-02 (galactoarabino-rhamnogalaturonan)	Phase I [229]
LOXL2 inhibitors	Simtuzumab	IIb (ClinicalTrials.gov ID: NCT01672866 and NCT01672879)
LT receptor antagonists	MN-001 (tipelukast)	FDA-approved for a IIa randomized trial[247]
Caspase inhibitors	emricasan (IDN-6556)	IIa (ClinicalTrials.gov ID: NCT02077374)
Hedgehog pathway inhibitors	Smo inhibitors: vismodegib (GDC-0449) and cyclopamine	Preclinical [262, 263]
CCN1/CYR61 analogues	purified CCN1 protein, CCNI-overexpressing adenovirus	Preclinical [266, 267]

Abbreviations: ALX/FPR2: lipoxin A4 receptor/formyl peptide receptor 2; AMPK: 5-AMP activated protein kinase; BDA-RvD1: benzo-diacetylenic-17R-RvD1-methyl ester; CCCN1: CYR61, CTGF (connective tissue growth factor), and NOV (Nephroblastoma overexpressed gene)]1; **CCR:** chemokine (C-C motif) ligand receptor; CYR61: Cysteine-rich protein 61; CDCA: chenodeoxycholic acid; EGCG: epigallocatechin-3-gallate; FGF: Fibroblast Growth Factor ; FXR: Farnesoid X receptor; Lipo-RvD1: Liposome-conjugated Resolvin D1; Nrf2: LOXL2: Lysyl oxidase-like 2; LT: leukotriene; mTORC1: mammalian target of rapamycin complex 1; NK-252: (1-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)-3-(pyridin-2-ylmethyl)urea).; NMDA: N-methyl-d-aspartate ; Nrf2: Nuclear erythroid 2-related factor; OCA: obeticholic acid; tBHQ: tert-

Butylhydroquinone THIQ: tetrahydroisoquinoline; ZJ001: 2-(3-benzoylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid

Supplementary material

Table 1: Mechanisms of action of natural antioxidants for the treatment of NAFLD (panel A) and main studies assessing the effect of resveratrol on markers of NAFLD (panel B).

A)Mechanisms of action of natural antioxidants		
Resveratrol		
Metabolism	**Cell type and molecular mechanisms of action**	**Biological effect**
Extensively metabolized by intestinal and hepatic sulfo- and glucuronosyl-transferases to Resv-3-O-glucuronide, Resv-4-O'-glucuronide, Resv-3-O-sulfate, whose biological activity is unclear	**Hepatocyte, miocyte. adipocyte** ↑ SIRT-1→↑AMPK phosphorylation by LKB1→↑AMPK activity [87] ↑ SIRT-1→↑Akt phosphorylation→Fox01 translocation to cytoplasm→↑ insulin sensitivity, ↓ gluconeogenesis [87] ↑ ACC and HMG-CoAR phosphorylation by AMPK→↓ de novo lipogenesis and cholesterol synthesis[87] ↓SREBP-1c→↓ACC, FAS→↓ de novo lipogenesis ↑PGC-1α→↑ mitochondrial biogenesis and function ↑PPAR-α→↑CPT-1A, ACO→↑fatty acid oxidation ↑ UCP-1→↑ thermogenesis ↑ Nrf2 activity→↑ antioxidant defense ↓ IκB-α phpsphorylation→ ↓ NF-κB activation→↓Il-1/TNF-α/IL-6 secretion [85, 86]	↑ insulin sensitivity ↓ hepatic steatosis ↓ oxidative stress ↓ inflammation
	Adipocyte ↓SREBP-1c, ACC, FAS→↓ de novo lipogenesis ↑ ATGL→↑lipolysis [87] ↑ autophagy ↑ UCP-1→↑ thermogenesis ↓PPAR-γ→↓ adipogenesis ↓Fox01 activity→↑ insulin sensitivity	↓ adpose tissue dysfunction

Quercetin		
Metabolism	**Cell type and molecular mechanisms of action**	**Biological effects**
Metabolized by intestinal and hepatic sulfo-, glucuronosyl- and methyl-transferases into phase II conjugates which are rapidly eliminated	**Hepatocyte,adipocyte** ↑ SIRT-1→↑AMPK activity [98] ↑ SIRT-1→↑Akt phosphorylation→↑ insulin sensitivity [98] ↓SREBP-1c→↓ACC, FAS→↓ de novo lipogenesis ↑ fatty acid ω-oxidation [100] ↓CYP2E1 activity→↓ ROS generation[99] ↑ Nrf2 activity→↑ antioxidant defense [97] ↓ IκB-α phpsphorylation→ ↓ NF-κB activation→↓Il-1/TNF-α/IL-6 secretion [97, 98]	↑ insulin sensitivity ↓ hepatic steatosis ↓ oxidative stress ↓ inflammation

B) Effects of resveratrol on NAFLD in placebo-controlled RCTs				
Author	**Participants,**	**Dose duration**	**Metabolic/inflammatory parameters**	**Liver disease**
Timmers 2011[92]	11 obese nondiabetic	Resv 150 mg 1 mo	↑ AMPK activation ↑ SIRT-1/PGC-1α ↓ HOMA-IR ↓ serum IL-1, TNF-α, leptin	↓ ALT ↓ steatosis (MRS)
Faghihzadeh 2014[93]	50 overweight nondiabetic NAFLD	Resv 500 mg 3 mo	↓ serum IL-6, CRP and TNF-α ↓ blood NF-κB activity	↓ liver enzymes ↓ steatosis (US) ↓ s-CK18
Poulsen 2013[94]	20 obese nondiabetic	Resv 1500 mg 1 mo	↔body/visceral fat ↔peripheral/hepatic/adipose IR (clamp) ↔REE/RQ ↔AMPK activation ↔SIRT-1/PGC-1α	↔liver enzymes ↔steatosis (MRS)
Chachay 2014[95]	20 obese NAFLD	Resv 3000 mg 2 mo	↔ serum IL-1, IL-6, IL-8, CRP and TNF-α ↔peripheral/hepatic/adipose IR (clamp) ↔body/visceral fat ↔oxidative stress markers ↔REE/RQ	↔liver enzymes ↔steatosis (MRS) ↔s-CK18

Abbreviations/symbols:
↓: significantly reduced as compared with placebo at the end of the trial;

↔: not significantly different from placebo at the end of trial;

AMPK: adenosine monophosphate-activated protein kinase; CRP: C-reactive protein;

IL-6: interleukin-6; IR: insulin resistance; MRS: magnetic resonance spectroscopy; NAS: NAFLD activity score; NF-κB: nuclear factor-κB; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1α; : RCT: randomized controlled trial; REE: resting energy expenditure; RQ: respiratory quotient; s-CK18: serum cytokeratin-18 fragments; TNF: tumor necrosis factor. US: ultrasonographic.

REFERENCES

[1]Chalasani N, et al.. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23

[2]Wong RJ, et al Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology. 2015;148: 547-55

[3] Musso G et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med. 2014;11: e1001680

[4] Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia. 2012;55: 885-904.

[5] Ding L, Pang S, Sun Y, Tian Y, Yu L, Dang N. Coordinated Actions of FXR and LXR in Metabolism: From Pathogenesis to Pharmacological Targets for Type 2 Diabetes. Int J Endocrinol. 2014; 2014:751859.

[6] Roda A, et al. Semisynthetic bile acid FXR and TGR5 agonists: physicochemical properties, pharmacokinetics, and metabolism in the rat. J Pharmacol Exp Ther. 2014;350: 56-68.

[7] Savkur RS, Thomas JS, Bramlett KS, Gao Y, Michael LF, Burris TP. Ligand-dependent coactivation of the human bile acid receptor FXR by the peroxisome proliferator-activated receptor gamma coactivator-1alpha. J Pharmacol Exp Ther. 2005; 312: 170–178

[8] Min HK, et al. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. Cell Metab. 2012;15:665-74.

[9]Kong B, , et al. Farnesoid X Receptor Deficiency Induces Nonalcoholic Steatohepatitis in Low-Density Lipoprotein Receptor-Knockout Mice Fed a High-Fat Diet. J Pharmacol Exp Ther. 2009; 328: 116–122,

[10]Fiorucci S, et al. A farnesoid x receptor-small heterodimer partner regulatory cascade modulates tissue metalloproteinase inhibitor-1 and matrix metalloprotease expression in hepatic stellate cells and promotes resolution of liver fibrosis. J. Pharmacol. Exp. Ther. 2005; 314: 584–595.

[11]Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J. Clin. Invest. 2006; 116: 1102–09

[12]Zhang Y, Castellani LW, Sinal CJ, Gonzalez FJ, Edwards PA. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) regulates triglyceride metabolism by activation of the nuclear receptor FXR. Genes Dev. 2004; 18: 157–169

[13]Watanabe M, et al.. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J. Clin. Invest. 2004; 113,:1408–1418

[14]Claudel T, et al. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. Gastroenterology. 2003; 125: 544–555

[15] Neuschwander-Tetri B, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial Lancet. 2015;385:956-65

[16] Xin XM, Zhong MX, Yang GL, Peng Y, Zhang YL, Zhu W. GW4064, a farnesoid X receptor agonist, upregulates adipokine expression in preadipocytes and HepG2 cells. World J Gastroenterol. 2014;20:15727-35.

[17]Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes NF-κB in hepatic inflammatory response. Hepatology 2008; 48:1632-1643

[18] Verbeke L, et al. The FXR Agonist Obeticholic Acid Prevents Gut Barrier Dysfunction and Bacterial Translocation in Cholestatic Rats. Am J Pathol. 2015 ;185:409-19

[19]Adams AC, et al. Fundamentals of FGF19 & FGF21 action in vitro and in vivo. PLoS One. 2012;7: e38438.

[20] Fiorucci S, Cipriani S, Baldelli F, Mencarelli A Bile acid-activated receptors in the treatment of dyslipidemia and related disorders Progress in Lipid Research 2010; 49: 171–185

[21] Carotti A, et al. Beyond bile acids: targeting Farnesoid X Receptor (FXR) with natural and synthetic ligands. Curr Top Med Chem. 2014;14: :2129-42

[22] Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol. 2014;28: 573-83

[23] Li G et al. A tea catechin, epigallocatechin-3-gallate, is a unique modulator of the farnesoid X receptor. Toxicol Appl Pharmacol. 2012;258:268-74

[24]Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. Prog Lipid Res. 2013;52: 175-91

[25] Bommer GT, MacDougald OA. Regulation of Lipid Homeostasis
by the Bifunctional SREBF2-miR33a Locus. Cell Metabolism 2011; 13: 241-47

[26] Kang Q, Chen A. Curcumin inhibits srebp-2 expression in activated hepatic stellate cells in vitro by reducing the activity of specificity protein-1. Endocrinology. 2009;150:5384-94

[27] Tomita K et al. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. Hepatology. 2014;59: 154-69.

[28]Gerin I, et al. Expression of miR-33 from an SREBP2 Intron Inhibits Cholesterol Export and Fatty Acid Oxidation. J Biol Chem. 2010;285:33652-61

[29] Dávalos A, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Proc Natl Acad Sci U S A. 2011;108:9232-7.

[30] Li ZJ, Ou-Yang PH, Han XP. Profibrotic effect of miR-33a with Akt activation in hepatic stellate cells. Cell Signal. 2014;26: 141-8.

[31] Liu J, et al. Activation of mTORC1 disrupted LDL receptor pathway: a potential new mechanism for the progression of non-alcoholic fatty liver disease. Int J Biochem Cell Biol. 2015 Jan 23. pii: S1357-2725(15)00021-7. doi: 10.1016/j.biocel.2015.01.011. [Epub ahead of print]

[32] Musso G, Cassader M, Bo S, De Michieli F, Gambino R.Sterol regulatory element-binding factor 2 (SREBF-2) predicts 7-year NAFLD incidence and severity of liver disease and lipoprotein and glucose dysmetabolism.Diabetes. 2013;62:1109-20.

33 Baselga-Escudero L,et al. Resveratrol and EGCG bind directly and distinctively to **miR-33a** and miR-122 and modulate divergently their levels in hepatic cells. Nucleic Acids Res. 2014;42: 882-92

34 Baselga-Escudero L, et al Chronic supplementation of proanthocyanidins reduces postprandial lipemia and liver miR-33a and miR-122 levels in a dose-dependent manner in healthy rats. J Nutr Biochem. 2014;25:151-6

[35] Cirera-Salinas D, et al Mir-33 regulates cell proliferation and cell cycle progression. Cell Cycle. 2012 ;11: 922-33.

[36] Zhu C, Zhao Y, Zhang Z, Ni Y, Li X, Yong H MicroRNA-33a inhibits lung cancer cell proliferation and invasion by regulating the expression of β-catenin. Mol Med Rep. 2014 Dec 24. doi: 10.3892/mmr.2014.3134. [Epub ahead of print]

[37]Banerjee M, Robbins D, Chen T Targeting xenobiotic receptors PXR and CAR in human diseases. Drug Discov Today. 2014 Nov 20. pii: S1359-6446(14)00456-5. doi: 10.1016/j.drudis.2014.11.011. [Epub ahead of print]

[38]Pascussi JM, Gerbal-Chaloin S, Duret C, Daujat-Chavanieu M, Vilarem MJ, Maurel P. The Tangle of Nuclear Receptors that Controls Xenobiotic Metabolism and Transport: Crosstalk and Consequences. Annu Rev Pharmacol Toxicol 2008 ;.48:1-32

[39] Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. Pharmacogenet Genomics. 2010;20: 1-8

[40]Zhou J, et al. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARγ in promoting steatosis. Gastroenterology 2008; .134: 556-567

[41] Bitter A, et al. Pregnane X receptor activation and silencing promote steatosis of human hepatic cells by distinct lipogenic mechanisms. Arch Toxicol. 2014 Sep 3. PMID: 25182422 [Epub ahead of print]

[42] Hariparsad N, et al Identification of pregnane-X receptor target genes and coactivator and corepressor binding to promoter elements in human hepatocytes. Nucleic Acids Res. 2009. 37:1160-73

[43] Moreau A, , et al. A novel pregnane X receptor and S14-mediated lipogenic pathway in human hepatocyte. Hepatology 2009.; 49: 2068-79

[44] Li L, et al SLC13A5 is A Novel Transcriptional Target of the Pregnane X Receptor and Sensitizes Drug-Induced Steatosis in Human Liver. Mol Pharmacol. 2015 Jan 27. pii: mol.114.097287. [Epub ahead of print]

[45] Hoekstra M, Lammers B, Out R, Li Z,Van Eck M, Van Berkel TJC. Activation of the Nuclear Receptor PXR Decreases Plasma LDL-Cholesterol Levels and Induces Hepatic Steatosis in LDL Receptor Knockout Mice. Mol Pharm 2009; 6: 182-189

[46]Miao J, Fang S, Bae Y, Kemper JK. Functional Inhibitory Cross-talk between Constitutive Androstane Receptor and Hepatic Nuclear Factor-4 in Hepatic Lipid/glucose Metabolism is Mediated by Competition for Binding to the DR1 Motif and to the Common Coactivators, GRIP-1 and PGC-1alpha. J. Biol. Chem. 2006; 281: 14537–46

[47]Valenti L, et al.. Increased Expression and Activity of the Transcription Factor FOXO1 in Nonalcoholic Steatohepatitis. Diabetes 2008; 57: 1355-1362

[48] Sun M, Cui W, Woody SK, Staudinger JL. Pregnane x receptor modulates the inflammatory response in primary cultures of hepatocytes. Drug Metab Dispos. 2015;43: 335-43

[49]Wang K, Damjanov I, Wan YJ. The protective role of pregnane X receptor in lipopolysaccharide/D-galactosamine-induced acute liver injury. Lab Invest. 2010;90:257-65.

[50] Kittayaruksakul S, et al Identification of three novel natural product compounds that activate PXR and CAR and inhibit inflammation. Pharm Res. 2013; 30:2199-208.

[51] Haughton EL, et al. Pregnane X receptor activators inhibit human hepatic stellate cell transdifferentiation in vitro. Gastroenterology. 2006;131:194-209.

[52] Marek CJ, , et al.. Pregnenolone-16alpha-carbonitrile inhibits rodent liver fibrogenesis via PXR (pregnane X receptor)-dependent and PXR-independent mechanisms. Biochem J 2005; 387 (Pt 3):601–8.

[53]Morere P, Nouvet G, Stain JP, Paillot B, Metayer J, Hemet J. Information obtained by liver biopsy in 100 tuberculous patients. Sem Hop. 1975; 51:2095–2102.

[54] Biswas A, Pasquel D, Tyagi RK, Mani S. Acetylation of pregnane X receptor protein determines selective function independent of ligand activation. Biochem Biophys Res Commun. 2011;406:371-6

[55] Sahebkar A, Chew GT, Watts GF. New peroxisome proliferator-activated receptor agonists: potential treatments for atherogenic dyslipidemia and non-alcoholic fatty liver disease. Expert Opin Pharmacother. 2014;15:493-503

[56] Hedrington MS, Davis SN. Discontinued in 2013: diabetic drugs. Expert Opin Investig Drugs. 2014;23:1703-11.

[57]Kim H, et al. Liver-enriched transcription factor CREBH interacts with peroxisome proliferator-activated receptor α to regulate metabolic hormone FGF21. Endocrinology. 2014;155:769-82. .

[58] Velkov T. Interactions between Human Liver Fatty Acid Binding Protein and Peroxisome Proliferator Activated Receptor Selective Drugs. PPAR Res 2013;2013:938401

[59] Qu S, et al. PPAR{alpha}mediates the hypolipidemic action of fibrates by antagonizing FoxO1. Am J Physiol Endocrinol Metab 2007;292:E421-434.

[60] Toyama T, et al. PPARalpha ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. Biochem Biophys Res Commun 2004;324:697-704.

[61] Mogilenko DA, et al. Peroxisome proliferator-activated receptor alpha positively regulates complement C3 expression but inhibits tumor necrosis factor alpha-mediated activation of C3 gene in mammalian hepatic-derived cells. J Biol Chem 2013;288:1726-1738.

[62] Kleemann R, Gervois PP, Verschuren L, Staels B, Princen HM, Kooistra T. Fibrates down-regulate IL-1-stimulated C-reactive protein gene expression inhepatocytes by reducing nuclear p50-NFkappa B-C/EBP-beta complex formation. Blood 2003;101:545-551.

[63] Pawlak M, et al. The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice. Hepatology. 2014;60:1593-606.

[64] Mansouri RM, Bauge E, Staels B, Gervois P. Systemic and distal repercussions of liver-specific peroxisome proliferator-activated receptor-alpha control of the acute-phase response. Endocrinology 2008;149:3215-3223.

[65] Delerive P, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J Biol Chem 1999;274:32048-32054.

[66] Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology 2004;39:1286-1296.

[67] Holden PR, Tugwood JD. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. J Mol Endocrinol 1999;22:1-8.

[68] Bojic LA, et al. PPARδ activation attenuates hepatic steatosis in Ldlr-/- mice by enhanced fat oxidation, reduced lipogenesis, and improved insulin sensitivity. J Lipid Res. 2014;55:1254-1266.

[69] Wang YX, et al. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 113: 159–170, 2003.

[70] Nagasawa T, et al. Effects of bezafibrate, PPAR pan-agonist, and **GW501516**, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. Eur J Pharmacol. 2006 Apr 24;536(1-2):182-91.

[71] Lim HJ, Park JH, Lee S, Choi HE, Lee KS, Park HY. PPARdelta ligand **L-165041** ameliorates Western diet-induced hepatic lipid accumulation and inflammation in LDLR-/- mice. Eur J Pharmacol. 2009 ;622:45-51

[72] Bays HE, et al. MBX-8025, a novel peroxisome proliferator receptor-delta agonist: lipid and other metabolic effects in dyslipidemic overweight patients treated with and without atorvastatin. J Clin Endocrinol Metab. 2011;96:2889-97

[73] Staels B, et al. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor **alpha**/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology. 2013 ;58:1941-52.

[74] Cariou B, et al. Dual peroxisome proliferator-activated receptor α/δ agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. Diabetes Care. 2013 ;36:2923-30

[75] Hardwick RN, Fisher CD, Canet MJ, Lake AD, Cherrington NJ. Diversity in antioxidant response enzymes in progressive stages of human nonalcoholic fatty liver disease. Drug Metab Dispos. 2010; 38:2293-301.

[76] Bae SH. et al. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. Cell Metab. 2013; 17: 73–84.

[77] Malloy MT, McIntosh DJ., Walters TS. Flores A, Goodwin JS. Arinze IJ. Trafficking of the transcription factor Nrf2 to promyelocytic leukemia-nuclear bodies: implications for degradation of NRF2 in the nucleus. J. Biol. Chem. 2013; 288: 14569-83

[78] Bhakkiyalakshmi E, Sireesh D, Rajaguru P, Paulmurugan R, Ramkumar KM.The emerging role of redox-sensitive Nrf2-Keap1 pathway in diabetes. Pharmacol Res. 2015;91C:104-114.

[79] Wang C, et al.Nrf2 deletion causes "benign" simple steatosis to develop into nonalcoholic steatohepatitis in mice fed a high-fat diet. Lipids Health Dis. 2013 Nov 4;12:165.

[80] Meakin PJ, et al. Susceptibility of Nrf2-null mice to steatohepatitis and cirrhosis upon consumption of a high-fat diet is associated with oxidative stress, perturbation of the unfolded protein response, and disturbance in the expression of metabolic enzymes but not with insulin resistance. Mol Cell Biol. 2014;34: 3305-20.

[81] Collins AR, et al. Myeloid deletion of nuclear factor erythroid 2-related factor 2 increases atherosclerosis and liver injury. Arterioscler Thromb Vasc Biol. 2012;32:2839-46

[82] Oh CJ, et al Sulforaphane attenuates hepatic fibrosis via NF-E2-related factor 2-mediated inhibition of transforming growth factor-β/Smad signaling. Free Radic Biol Med. 2012;52:671-82

[83] Shimozono R, et al. Nrf2 activators attenuate the progression of nonalcoholic steatohepatitis-related fibrosis in a dietary rat model. Mol Pharmacol. 2013;84:62-70

[84] Yu Z, et al. Oltipraz upregulates the nuclear factor (erythroid-derived 2)-like 2 [corrected](NRF2) antioxidant system and prevents insulin resistance and obesity induced by a high-fat diet in C57BL/6J mice. Diabetologia. 2011; 54:922-34.

[85]Cleasby A, et al. Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. PLoS One. 2014;9:e98896.

[86] Binding mode and structure-activity relationships around direct inhibitors of the Nrf2-Keap1 complex.

Jnoff E, Albrecht C, Barker JJ, Barker O, Beaumont E, Bromidge S, Brookfield F, Brooks M, Bubert C, Ceska T, Corden V, Dawson G, Duclos S, Fryatt T, Genicot C, Jigorel E, Kwong J, Maghames R, Mushi I, Pike R, Sands ZA, Smith MA, Stimson CC, Courade JP.

ChemMedChem. **2014** Apr;9(4):699-705. doi:

[87] Yuan X, Wang J, Tang X, Li Y, Xia P, Gao X Berberine ameliorates nonalcoholic fatty liver

disease by a global modulation of hepatic mRNA and lncRNA expression profiles J Transl Med. 2015;13: 24. [Epub ahead of print]

[88] Park EJ, Pezzuto JM.The pharmacology of resveratrol in animals and humans. Biochim Biophys Acta. 2015 Jan 31. pii: S0925-4439(15)00023-X. doi: 10.1016/j.bbadis.2015.01.014

[89] Andrade JM, et al. Resveratrol attenuates hepatic steatosis in high-fat fed mice by decreasing lipogenesis and inflammation. Nutrition. 2014;30:915-9

[90] Li L, et al. Resveratrol modulates autophagy and NF-jB activity in a murine model for treating non-alcoholic fatty liver disease. Food Chem Toxicol. 2014; 63:166-73

[91]Zhu W et al. Effects and mechanisms of resveratrol on the amelioration of oxidative stress and hepatic steatosis in KKAy mice. Nutr Metab (Lond). 2014;11:35.

[92] Konings E, et al. The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men. Int J Obes (Lond). 2014;38:470-3.

[93] Bagul PK., et al., Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. *Pharmacol. Res.* 2012, *66*, 260–268.

[94] Gomez-Zorita, S., et al., Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. *Br. J. Nutr.* 2012, *107*, 202–210.

[95] Cho, S. J., Jung, U. J., Choi, M. S., Differential effects of low-dose resveratrol on adiposity and hepatic steatosis in diet-induced obese mice. *Br. J. Nutr.* 2012, *108*, 2166–2175.

[96] Timmers S, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab. 2011; 14:612-22.

[97] Faghihzadeh F, Adibi P, Rafiei R, Hekmatdoost A. Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. Nutr Res. 2014;34:837-43

[98] Poulsen MM, et al. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes. 2013;62:1186-95

[99]Chachay VS, et al. Resveratrol does not benefit patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:2092–2103.

[100]Smoliga JM, Blanchard O. Enhancing the delivery of resveratrol in humans: if low bioavailability is the problem, what is the solution? Molecules. 2014 ;19:17154-72

[101] Panchal SK, Poudyal H, Brown L. Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. J Nutr. 2012 ;142:1026-32.

[102] Ying HZ, Liu YH, Yu B, Wang ZY, Zang JN, Yu CH. Dietary quercetin ameliorates nonalcoholic steatohepatitis induced by a high-fat diet in gerbils. Food Chem Toxicol. 2013 ;52:53-60.

[103] Surapaneni KM, Priya VV, Mallika J. Pioglitazone, quercetin and hydroxy citric acid effect on cytochrome P450 2E1 (CYP2E1) enzyme levels in experimentally induced non alcoholic steatohepatitis (NASH). Eur Rev Med Pharmacol Sci. 2014;18):2736-41.

[104] Hoek-van den Hil EF., et al., Quercetin induces hepatic lipid omega-oxidation and lowers serum lipid levels in mice. *PLoS One* 2013, *8*, e51588.

[105] Guo Y, Bruno RS. Endogenous and exogenous mediators of quercetin bioavailability. J Nutr Biochem. 2015;26:201-210..

[106] Jiang S, et al. Fibroblast growth factor 21 is regulated by the IRE1α-XBP1 branch of the unfolded protein response and counteracts endoplasmic reticulum stress-induced hepatic steatosis. J Biol Chem. 2014 ;289:29751-65.

[107] Li Y, et al Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. Gastroenterology. 2014 ;146:539-49.

[108] Wang H, Qiang L, Farmer SR. Identification of a domain within peroxisome proliferator-activated receptor gamma regulating expression of a group of genes containing fibroblast growth factor 21 that are selectively repressed by SIRT1 in adipocytes. Mol Cell Biol 2008;28:188-200

[109] Li Y, Wong K, Walsh K, Gao B, Zang M. Retinoic acid receptor beta stimulates hepatic induction of fibroblast growth factor 21 to promote fatty acid oxidation and control whole-body energy homeostasis in mice. J Biol Chem 2013;288:10490-504.
[110] Wang Y, Solt LA, Burris TP. Regulation of FGF21 expression and secretion by retinoic acid receptor-related orphan receptor alpha. J Biol Chem 2010;285:15668-73.
[111] Min AK, et al. Orphan nuclear receptor Nur77 mediates fasting-induced hepatic fibroblast growth factor 21 expression. Endocrinology. 2014;155:2924-31.

[112] Ogawa Y, et al. bKlotho is required for metabolic activity of fibroblast growth factor 21. PNAS 2007; 104 7432–37
[113] Lee DV, et al. Fibroblast growth factor 21 improves insulin sensitivity and synergizes with insulin in human adipose stem cell-derived (hASC) adipocytes. PLoS One. 2014;9: e111767.

[114] Xu J, et al Fibroblast growth facto21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 2009 58 250–259.
[115] Chau MD, et al. Fibroblast growth factor 21 regulates energy metabolism by activating the AMPK–SIRT1–PGC-1alpha pathway. Proc Natl Acad Sci U S A 2010; 28:12553–12558.
[116] Liang Q, et al. FGF21 maintains glucose homeostasis by mediating the cross talk between liver and brain during prolonged fasting. Diabetes. 2014; 63:4064-75.

[117] Fisher FM, et al. Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic fatty acid activation in mice on methionine and choline-deficient diets. Gastroenterology. 2014;147:1073-83

[118] Dushay J, et al. .Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology. 2010;139: 456-63.
[119] Fisher FM, , et al. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 2010; 11:2781–89.
[120]Ye X, et al. Long-lasting anti-diabetic efficacy of PEGylated FGF-21 and liraglutide in treatment of type 2 diabetic mice. Endocrine. 2015 Jan 4. [Epub ahead of print] PMID: 25557015

[121] Hecht R, et al. Rationale-Based Engineering of a Potent Long-Acting FGF21 Analog for the Treatment of Type 2 Diabetes. PLoS One. 2012;7:e49345

[122] Adams AC, et al. LY2405319, an Engineered **FGF21** Variant, Improves the Metabolic Status of Diabetic Monkeys. PLoS One. 2013;8: e65763.

[123] Gaich G, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab. 2013;18:333-40.

[124] Foltz IN, et al. Treating diabetes and obesity with an FGF21-mimetic antibody activating the βKlotho/FGFR1c receptor complex. Sci Transl Med. 2012;4:162ra153.

[125] Musso G, Gambino R, Cassader M. Emerging molecular targets for the treatment of nonalcoholic fatty liver disease. Annu Rev Med. 2010;61:375-92

[126] Castaño D, Larequi E, Belza I, Astudillo AM, Martínez-Ansó E, Balsinde J. Cardiotrophin-1 eliminates hepatic steatosis in obese mice by mechanisms involving AMPK activation J Hep 2014; ,60: 1017–25

[127] Hsu WH, Chen TH, Lee BH, Hsu YW, Pan I Monascin and ankaflavin act as natural AMPK activators with PPARα agonist activity to down-regulate nonalcoholic steatohepatitis in high-fat diet-fed C57BL/6 mice. Food Chem Toxicol. 2014; 64:94-103.

[128] Li H, et al. AMPK activation prevents excess nutrient-induced hepatic lipid accumulation by inhibiting mTORC1 signaling and endoplasmic reticulum stress response. Biochim Biophys Acta. 2014;1842:1844-54.

[129] Dong J, et al. Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKα1/SIRT1. J Lipid Res. 2014;55:363-74.

[130] Li J, et al. Hepatoprotective effects of berberine on liver fibrosis via activation of AMP-activated protein kinase. Life Sci. 2014;98:24-30

[131] Zhai X, et al. Curcumin regulates peroxisome proliferator-activated receptor-γ coactivator-1α expression by AMPK pathway in hepatic stellate cells in vitro. Eur J Pharmacol. 2015 ;746:56-62

[132] Kim YC, Guan KL. mTOR: a pharmacologic target for autophagy regulation. J Clin Invest. 2015;125: 25-32

[133] Gwinn DM, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008;30: 214–26.

[134] Sapp V, Gaffney L, EauClaire SF, Matthews RP. **Fructose** leads to hepatic steatosis in zebrafish that is reversed by mechanistic target of rapamycin (**mTOR**) inhibition. Hepatology. 2014;60:1581-92

[135] Jiang H, Westerterp M, Wang C, Zhu Y, Ai D. Macrophage mTORC1 disruption reduces inflammation and insulin resistance in obese mice. Diabetologia. 2014;57: :2393-404.

[136] Ai D, et al Disruption of mammalian target of rapamycin complex 1 in macrophages decreases chemokine gene expression and atherosclerosis. Circ Res. 2014 ;114: 1576-84

[137] Lin HY, et al. Effects of the mTOR inhibitor Rapamycin on Monocyte-Secreted Chemokines. BMC Immunol. 2014;15: 37

[138]Inokuchi-Shimizu S, et al TAK1-mediated autophagy and fatty acid oxidation prevent hepatosteatosis and tumorigenesis. J Clin Invest. 2014;124:3566-78.

[139]González-Rodríguez A et al. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis. 2014; 5:e1179.

[140] Wang L, et al. ALCAT1 controls mitochondrial etiology of fatty liver diseases, linking defective mitophagy to steatosis. Hepatology. 2015;61:486-96.

[141] Liu K, et al.Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. Autophagy. 2015 Feb 4:0. [Epub ahead of print] PMID: 25650776

[142] Yin J, Gu L, Wang Y, Fan N, Ma Y, Peng Y. Rapamycin Improves Palmitate-Induced ER Stress/NF κ B Pathways Associated with Stimulating Autophagy in Adipocytes. Mediators Inflamm. 2015;2015:272313

[143]Müller TD et al. p62 links β-adrenergic input to mitochondrial function and thermogenesis. J Clin Invest. 2013;123:469-78.

[144] Kim, K., et al. S6 kinase 2 deficiency enhances ketone body production and increases peroxisome proliferator-activated receptor alpha activity in the liver. Hepatology 2012; 55, 1727–1737.

[145] Peterson T.R., et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 2011; 146, 408–420.

[146]Sengupta S., Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature 2010; 468, 1100–1104.

[147] Li S, et al. Role of S6K1 in regulation of SREBP1c expression in the liver. Biochem Biophys Res Commun 2011; 412:197–202.

[148] Wang C, et al. Inflammatory stress increases hepatic CD36 translational efficiency via activation of the mTOR signalling pathway. PLoS One. 2014 Jul 21;9(7):e103071

[149] Ma KL, et al. Activation of mTOR modulates SREBP-2 to induce foam cell formation through increased retinoblastoma protein phosphorylation. Cardiovasc Res. 2013 1;100:450-60

[150] Yuan, M., et al. Identification of Akt-independent regulation of hepatic lipogenesis by mammalian target of rapamycin (mTOR) complex 2. J. Biol. Chem. 2012; 287, 29579–29588.

[151] Kumar A, et al. Fat cell-specific ablation of rictor in mice impairs insulin-regulated fat cell and whole-body glucose and lipid metabolism. Diabetes 2010; 59: 1397–1406.

152 Umemura A, et al. Liver damage, inflammation, and enhanced tumorigenesis after persistent mTORC1 inhibition. Cell Metab. 2014; 20:133-44.

[153]Torricelli C, et al. Phosphorylation-independent mTORC1 inhibition by the autophagy inducer Rottlerin. Cancer Lett. 2015 Feb 4. pii: S0304-3835(15)00074-9. doi: 10.1016/j.canlet.2015.01.040. [Epub ahead of print]

[154] Zhang J, et al. 2-(3-Benzoylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid ameliorates metabolic disorders in high-fat diet-fed mice. Acta Pharmacol Sin. 2015;36:483-96.

[155] Ozaki E, Campbell M, Doyle SL. Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives. J Inflamm Res. 2015 ; 8:15-27

[156] Csak T, et al. Both bone marrow-derived and non-bone marrow-derived cells contribute to **AIM2** and NLRP3 inflammasome activation in a MyD88-dependent manner in dietary steatohepatitis. Liver Int. 2014;34:1402-13

[157] Boaru SG, Borkham-Kamphorst E, Tihaa L, Haas U, Weiskirchen R. Expression analysis of inflammasomes in experimental models of inflammatory and fibrotic liver disease. J Inflamm (Lond) 2012;9:49.

[158]Wree A et al NLRP3 inflammasome activation is required for fibrosis development in NAFLD. J Mol Med (Berl). 2014; 92:1069-82.

[159] Wree A, et al. NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. Hepatology. 2014;59:898-910

[160] Dixon LJ, Flask CA, Papouchado BG, Feldstein AE, Nagy LE. Caspase-1 as a central regulator of high fat diet-induced non-alcoholic steatohepatitis. PLoS One. 2013;8:e56100

[161]Ioannou GN et al. Cholesterol-lowering drugs cause dissolution of cholesterol crystals and disperse Kupffer cell structures during. crown-like resolution of NASH. J Lipid Res 2015;56:277-85.

[162] Xu C, et al. Xanthine oxidase in nonalcoholic fatty liver disease and hyperuricemia: One stone hits two birds. J Hepatol. 2015 Jan 23. doi: 10.1016/j.jhep.2015.01.019

[163]Miura K, et al. Toll-like receptor 2 and palmitic acid cooperatively contribute to the development of nonalcoholic steatohepatitis through inflammasome activation in miceHepatology. 2013;57:577-89..

[164] Toki Y, et al. Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1β, HMGB1, and PGE2, decreased MHC class I expression and necrotic

cell death. Biochem Biophys Res Commun. 2015 Feb 11. pii: S0006-291X(15)00225-9. doi: 10.1016/j.bbrc.2015.02.011.

[165]Honda H et al. Isoliquiritigenin is a potent inhibitor of NLRP3 inflammasome activation and diet-induced adipose tissue inflammation. Leukoc Biol. 2014;96:1087-100.

[166] Abderrazak A et al Anti-Inflammatory and Anti-Atherogenic Effects of the Inflammasome NLRP3 Inhibitor, Arglabin, in ApoE2.Ki Mice Fed a High Fat Diet. Circulation. 2015 Jan 22. pii: CIRCULATIONAHA.114.013730. [Epub ahead of print]

[167] Isakov E, Weisman-Shomer P, Benhar M. Suppression of the pro-inflammatory NLRP3/interleukin-1β pathway in macrophages by the thioredoxin reductase inhibitor auranofin. Biochim Biophys Acta. 2014;1840:3153-61

[168] Farooq A, et al..Activation of N-methyl-d-aspartate receptor downregulates inflammasome activity and liver inflammation via a β-arrestin-2 pathway.Am J Physiol Gastrointest Liver Physiol. 2014;307:G732-40

[169]Ratziu V, et al.A phase 2, randomized, double-blind, placebo-controlled study of GS-9450 in subjects with nonalcoholic steatohepatitis. Hepatology. 2012 ;55:419-28.

[170] Henao-Mejia J et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity Nature. 2012;482:179-85.

[171] Bartneck M, Warzecha KT, Tacke F.Therapeutic targeting of liver inflammation and fibrosis by nanomedicine. Hepatobiliary Surg Nutr. 2014;3:364-76

[172] Kufareva I, Salanga CL, Handel TM. Chemokine and chemokine receptor structure and interactions: implications for therapeutic strategies. Immunol Cell Biol. 2015 Feb 24. doi: 10.1038/icb.2015.15.

[173]Zhang X et al. Hepatic CXCR3 Promotes Non-Alcoholic Steatohepatitis Through Inflammation, Lipid Accumulation and Autophagy Deficiency Gastroenterology 2014; 146(S1): 922

[174] Miura K, Yang L, van Rooijen N, Ohnishi H, Seki E. Hepatic recruitment of macrophages promotes nonalcoholic steatohepatitis through CCR2. Am J Physiol Gastrointest Liver Physiol. 2012 ;302:G1310-21

[175] Huang W, et al. Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. Diabetes 2010;59:347–57.

[176]Tosello-Trampont AC, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS. Kuppfer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-α production. J Biol Chem. 2012;287:40161-72

[177] Wehr A, et al. Pharmacological inhibition of the chemokine CXCL16 diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. PLoS One. 2014;9:e112327.

[178] Karlmark KR, et al. Hepatic recruitment of the inflammatory Gr1þ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology 2009;50:261–274.

[179] Rouault C, et al. Roles of chemokine ligand-2 (CXCL2) and neutrophils in influencing endothelial cell function and inflammation of human adipose tissue. Endocrinology 2013;154:1069–79

[180] Haukeland JW, et al. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. J Hepatol 2006; 44:1167–74.

[181] Oh DY, et al. Increased macrophage migration into adipose tissue in obese mice. Diabetes 2012;61:346–354.

[182] Obstfeld AE , et al. C-C chemokine receptor 2 (CCR2) regulates the hepatic recruitment of myeloid cells that promote obesity-induced hepatic steatosis. Diabetes. 2010;59:916-25..

[183] Kirk EA, Sagawa ZK, McDonald TO, O'Brien KD, Heinecke JW. Monocyte chemoattractant protein deficiency fails to restrain macrophage infiltration into adipose tissue [corrected]. Diabetes. 2008;57:1254-61.

[184] Baeck C, et al. Pharmacological inhibition of the chemokine C-C motif chemokine ligand 2 (monocyte chemoattractant protein 1) accelerates liver fibrosis regression by suppressing Ly-6C(+) macrophage infiltration in mice. Hepatology 2014; 59: 1060-72

[185] Seki E, et al. CCR1 and CCR5 promote hepatic fibrosis in mice. J Clin Invest 2009;119:1858-70.

[186] Chu PS et al. C-C motif chemokine receptor 9 positive macrophages activate hepatic stellate cells and promote liver fibrosis in mice. Hepatology 2013;58:337-350.

[187] Pérez-Martínez L, et al. Maraviroc, a CCR5 antagonist, ameliorates the development of hepatic steatosis in a mouse model of non-alcoholic fatty liver disease (NAFLD). Antimicrob Chemother. 2014;69:1903-10.

[188] Berres ML, et al. Antagonism of the chemokine Ccl5 ameliorates experimental liver fibrosis in mice. J Clin Invest 2010;120:4129-40.

[189]Lefebvre E et al. Pharmacokinetics and safety of multiple-dose cenicriviroc, a novel, oral, once-daily CCR2 and CCR5 antagonist, in adults with mild or moderate hepatic impairment. Hepatology 2014; 60(S1): 1275A

[190] Lefebvre E et al. Anti-fibrotic and anti-inflammatory activity oft h edual CCR2 and CCR5 antagonist cenicriviroc in a mouse model of NASH. Hepatology 2013; 58(S1): 221A-222A

[191] Van Raemdonck K, Van den Steen PE, Liekens S, Van Damme J, Struyf S. CXCR3 ligands in disease and therapy. Cytokine Growth Factor Rev. 2014 Nov 22. pii: S1359-6101(14)00163-4. doi: 10.1016/j.cytogfr.2014.11.009.

[192]Wehr A, et al. Chemokine receptor CXCR6-dependent hepatic NK T Cell accumulation promotes inflammation and liver fibrosis. J Immunol. 2013 ;190: 5226-36

[193] Hammerich L, et al Chemokine receptor CCR6-dependent accumulation of γδ T cells in injured liver restricts hepatic inflammation and fibrosis. Hepatology. 2014;59:630-42

[194] Karlmark KR, et al. The fractalkine receptor CX(3)CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. Hepatology 2010;52:1769-1782.

[195] Patel J, Channon KM, McNeill E. The downstream regulation of chemokine receptor signalling: implications for atherosclerosis. Mediators Inflamm 2013; 2013:459520.

[196]Czaja AJ. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. World J Gastroenterol. 2014;20: 2515-32.

[197] Rius B , et al. Resolution of inflammation in obesity-induced liver disease. Front Immunol. 2012;3:257.

[198] Bozinovski S, Anthony D, Vlahos R.Targeting pro-resolution pathways to combat chronic inflammation in COPD. J Thorac Dis. 2014;6: 1548-56.

[199] Qin C, et al Cardioprotective potential of annexin-A1 mimetics in myocardial infarctionPharmacol Ther. 2015;148C:47-65

[200] Vago JP,et al: Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. J Leukoc Biol 2012, 92:249-258.

[201] Leoni G, et al. Annexin A1, formyl peptide receptor, and NOX1 orchestrate epithelial repair. J Clin Invest 2013;123:443-454

[202] Trentin PG , et al. Annexin A1-mimetic peptide controls the inflammatory and fibrotic effects of silica particles in mice. Br J Pharmacol. 2015 Feb 9. doi: 10.1111/bph.13109. [Epub ahead of print]

[203] Locatelli I , et al. Endogenous annexin A1 is a novel protective determinant in nonalcoholic steatohepatitis in mice. Hepatology. 2014;60:531-44.

[204] Kosicka A, et al.. Attenuation of plasma annexin A1 in human obesity. FASEB J 2013;27:368-378.

[205] Akasheh RT, Pini M, Pang J, Fantuzzi G. Increased adiposity in annexin A1-deficient mice. PLoS One. 2013;8:e82608

[206] Dalli J, et al. Proresolving and tissue-protective actions of annexin A1-based cleavage-resistant peptides are mediated by formyl peptide receptor 2/lipoxin A4 receptor. J Immunol 2013; 190: 6478–87

[207] Kamaly N, et al. Development and in vivo efficacy of targeted polymeric inflammation-resolving nanoparticles. Proc Natl Acad Sci U S A 2013; 110, 6506–11.

[208]Krishnamoorthy S, et al. Resolvin D1 binds humanphagocytes with evidence for proresolving receptors. Proc Natl Acad Sci USA2010;107: 1660–5.

[209] Claria J, Dalli J, Yacoubian S, Gao F, Serhan CN: Resolvin D1 and resolvin D2 govern local inflammatory tone in obese fat. J Immunol 2012, 189:2597-2605

[210] Psychogios N, et al.: The human serum metabolome. PLoS ONE 2011, 6:e16957.

[211] Kain V, et al. Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol. 2015; 84:24-35.

[212] Jung TW, et al. Resolvin D1 reduces ER stress-induced apoptosis and triglyceride accumulation through JNK pathway in HepG2 cells. Mol Cell Endocrinol. 2014;391: 30-40.

[213] Rius B et al. Resolvin D1 primes the resolution process initiated by calorie restriction in obesity-induced steatohepatitis. *FASEB J. 2014;* 28: 836–48

[214]Orr SK, Colas RA, Dalli J, Chiang N, Serhan CN. Proresolving actions of a new **resolvin D1** analog mimetic qualifies as an immunoresolvent. Am J Physiol Lung Cell Mol Physiol. 2015;308:L904-11

[215] Pugliese G, Iacobini C, Pesce CM, Menini CM. Galecin-3; an emerging all-out player in metabolic disorders and their complications. Glycobiology 2015; 25: 136-50

[216] Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc Natl Acad Sci USA* 1996; **93**: 6737-6742

[217] Elad-Sfadia **G**, Haklai R, Balan E, Kloog Y. Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates
ERK but not phosphoinositide 3-kinase activity. *J Biol Chem* 2004; **279**: 34922-34930

[218] Oka **N**, et al.. Galectin-3 inhibits tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by activating Akt in human bladder carcinoma cells. *Cancer Res* 2005; **65**: 7546-7553

[219] Park JW, Voss PG, Grabski S, Wang JL, Patterson RJ. Association of galectin-1 and galectin-3 with Gemin4 in complexes containing the SMN protein. Nucleic Acids Res 2001; 29: 3595-3602

[220] Shimura T, et al. Implication of galectin-3 in Wnt signaling. Cancer Res 2005; 65: 3535-37

[221] Lau KS, et al. Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. Cell. 2007; 129:123–134.

[222] Fukumori T, et al. CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. Cancer Res 2003; 63:8302–8311.

[223] Gao X, Balan V, Tai G, and Raz A. Galectin-3 induces cell migration via a calcium-sensitive MAPK/ERK1/2 pathway. Oncotarget 2014; 5:2077–84.

[224] Volarevic V, et al.Gal-3 regulates the capacity of dendritic cells to promote NKT-cell-induced liver injury. Eur J Immunol. 2015;45:531-43

[225] Iacobini C et al. Galectin-3 ablation protects mice from diet-induced NASH: A major scavenging role for galectin-3 in liver. J Hep 2011; 54: 975–983

[226] Henderson NC et al. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. Proc Natl Acad Sci U S A. 2006;103: 5060-5.

[227] DePeralta DK, et al Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology 2014; 59:1577–90.

[228]Maeda N, et al. Stimulation of proliferation of rat hepatic stellate cells by galectin-1 and galectin-3 through different intracellular signaling pathways. J Biol Chem 2003; 278:189-44

[229] Hsieh WC et al. Galectin-3 regulates hepatic progenitor cell expansion during liver injury. Gut 2015;64: 312–321.

[230] Traber PG and Eliezer Zomer E. Therapy of Experimental NASH and Fibrosis with Galectin Inhibitors. PLoS ONE 2013; 8: e83481

[231] Traber PG et al. Regression of Fibrosis and Reversal of Cirrhosis in Rats by Galectin Inhibitors in Thioacetamide-Induced Liver Disease. PLoS ONE 2013; 8: e75361

[232] Harrison SA, et al. Early phase 1 clinical trial results of GR-MD-02, a galectin-3 inhibitor, in patients having non-alcoholic steatohepatitis (NASH) with advanced fibrosis. Hepatology 2014; November 7-11, 2014; Boston, Massachusetts. Abstract 57 Hepatology 2014; 60(S1): 225A

[233] Iacobini C, et al. Accelerated lipid-induced atherogenesis in galectin-3-deficient mice: role of lipoxidation via receptor-mediated mechanisms. Arterioscler Thromb Vasc Biol. 2009; 29:831–836.

[234] Iacobini C, et al. Advanced lipoxidation end-products mediate lipid-induced glomerular injury: role of receptor-mediated mechanisms. J Pathol. 2009; 218:360–69.

[235] Pejnovic N, et al.. Galectin-3 Deficiency Accelerates High-Fat Diet Induced Obesity and Amplifies Inflammation in Adipose Tissue and Pancreatic Islets. Diabetes. 2013;62:1932-44.

[236] Pang J, et al. Increased Adiposity, Dysregulated Glucose Metabolism and Systemic Inflammation in Galectin-3 KO Mice. PLoS One. 2013; 8:e57915.

[237] Smedsrød B, Melkko J, Araki N, Sano H, Horiuchi S. Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. Biochem J. 1997; 322:567–73.

[238] Moon HJ, Finney J, Ronnebaum T, Mure M. Human lysyl oxidase-like 2. Bioorg Chem. 2014;57: 231-41

[239] Moon HJ, Finney J, Xu L, Moore D, Welch DR, Mure M. MCF-7 cells expressing nuclear associated lysyl oxidase-like 2 (LOXL2) exhibit an epithelial-to-mesenchymal transition (EMT) phenotype and are highly invasive in vitro. J Biol Chem. 2013;288:30000-8.

[240] Syn WK,et al. Hedgehog-mediated epithelial-to-mesenchymal transition and fibrogenic repair in nonalcoholic fatty liver disease. Gastroenterology. 2009;137:1478-88.

[241] Perepelyuk M, et al. Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. Am J Physiol Gastrointest Liver Physiol. 2013;304:G605-14

[242] Barry-Hamilton V, et al. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. Nat Med. 2010;16:1009-17.

[243] Talal AH, et al. Simtuzumab, an antifibrotic monoclonal antibody against lylyl oxidase-like 2(LOXL2) enzyme, appears safe and well-tolerated in patients with liver disease of diverse etiology. J Hep 2013; 58: S532

[244]Rådmark O, Werz O, Steinhilber D, Samuelsson B. 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. Biochim Biophys Acta. 2015;1851:331-339

[245] Titos E, et al. Inhibition of 5-lipoxygenase induces cell growth arrest and apoptosis in rat Kupffer cells: implications for liver fibrosis. FASEB J. 2003 ;17:1745-7.

[246] Horrillo R, et al. 5-lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity.J Immunol. 2010;184:3978-87

[247] Martinez-Clemente M, et al. 5-lipoxygenase deficiency reduces hepatic inflammation and tumor necrosis factor alpha-induced hepatocyte damage in hyperlipidemia- prone ApoE-null mice. Hepatology 2010;51:817–27.

[248] Titos E, et al. Protection from hepatic lipid accumulation and inflammation by genetic ablation of 5-lipoxygenase. Prostaglandins Other Lipid Mediat 2010;92:54–61.

[249]Matsuda K, Iwak Y; MN-001 (tipelukast), a novel, orally bioavailable drug, reduces fibrosis and inflammation and down-regulates TIMP-1, collagen Type 1 and LOXL2 mRNA overexpression in an advanced NASH (nonalcoholic steatohepatitis) model Hepatology 2014; 60(S1): 1283A

[250]American Association for the Study of Liver Diseases (AASLD) and Industry Colloquium - March 20, 2015, Durham, California, US. http://globenewswire.com/news-release/2015/02/25/709915/10122037/en/MediciNova-s-MN-001-tipelukast-NASH-with-Advanced-Fibrosis

[251]Hirsova P, Gores GJ. Death Receptor-Mediated Cell Death and Proinflammatory Signaling in Nonalcoholic Steatohepatitis. Cell Mol Gastroenterol Hepatol. 2015;1:17-27

[252] Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology. 2006;44: 27-33.

[253] Hatting M et al. Hepatocyte caspase-8 is an essential modulator of steatohepatitis in rodents. Hepatology 2013; 57: 2189-2201

[254] Machado MV et al. Reduced lipoapoptosis, hedgehog pathway activation and fibrosis in caspase-2 deficient mice with non-alcoholic steatohepatitis. Gut 2014 Jul 22. pii: gutjnl-2014-307362. doi: 10.1136/gutjnl-2014-307362. [Epub ahead of print]

[255] Thapaliya S, et al. Caspase 3 inactivation protects against hepatic cell death and ameliorates fibrogenesis in a diet-induced NASH model. Dig Dis Sci. 2014;59: 1197-206

[256] Barreyro FJ, et al. The pan-caspase inhibitor Emricasan (IDN-6556) decreases liver injury and fibrosis in a murine model of non-alcoholic steatohepatitis. Liver Int. 2015;35:953-66.

[257] Spada Ap et al. Rapid and statistically significant reduction of markers of apoptosis and cell death in subjects with mild, moderate and severe hepatic impairment treated with a single dose of the pan caspase inhibitor, emricasan. Hepatology 2014; 60(S1): 1277A

[258]Xiao Z, Ko HL, Goh EH, Wang B, Ren EC. hnRNP K suppresses apoptosis independent of p53 status by maintaining high levels of endogenous caspase inhibitors. Carcinogenesis. 2013;34:1458-67.

[259] Hu L, Lin X, Lu H, Chen B, Bai Y.An overview of hedgehog signaling in fibrosis. Mol Pharmacol. 2015;87:174-82

[260] Pathi S, et al. Comparative biological responses to human Sonic, Indian, and Desert hedgehog. Mech Dev 2001; 106:107–17.

[261] Choi SS, et al**.** Leptin promotes the myofibroblastic phenotype in hepatic stellate cells by activating the hedgehog pathway. J Biol Chem. 2010;285:36551-60

[262] Guy CD, Suzuki A, Abdelmalek MF, Burchette JL, Diehl AM; NASH CRN. Treatment response in the PIVENS trial is associated with decreased Hedgehog pathway activity. Hepatology. 2015;61: 98-107

[263] Choi SS, et al. Activation of Rac1 promotes hedgehog-mediated acquisition of the myofibroblastic phenotype in rat and human hepatic stellate cells. Hepatology. 2010;52:278-90

[264] Syn WK, et al.Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. Hepatology. 2010;51:1998-2007

[265]Philips GM , et al. Hedgehog signaling antagonist promotes regression of both liver fibrosis and hepatocellular carcinoma in a murine model of primary liver cancer. PLoS One. 2011;6:e23943.

[266] Hirsova P, Ibrahim SH, Bronk SF, Yagita H, Gores GJ. Vismodegib suppresses TRAIL-mediated liver injury in a mouse model of nonalcoholic steatohepatitis. PLoS One 2013;8:e70599.

[267] Kisseleva T, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc. Natl. Acad. Sci. U. S. A. 2012; 109:9448 –9453.

[268]Kim KH, Chen CC, Monzon RI, Lau LF. Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. Mol Cell Biol. 2013;33: 2078-90.

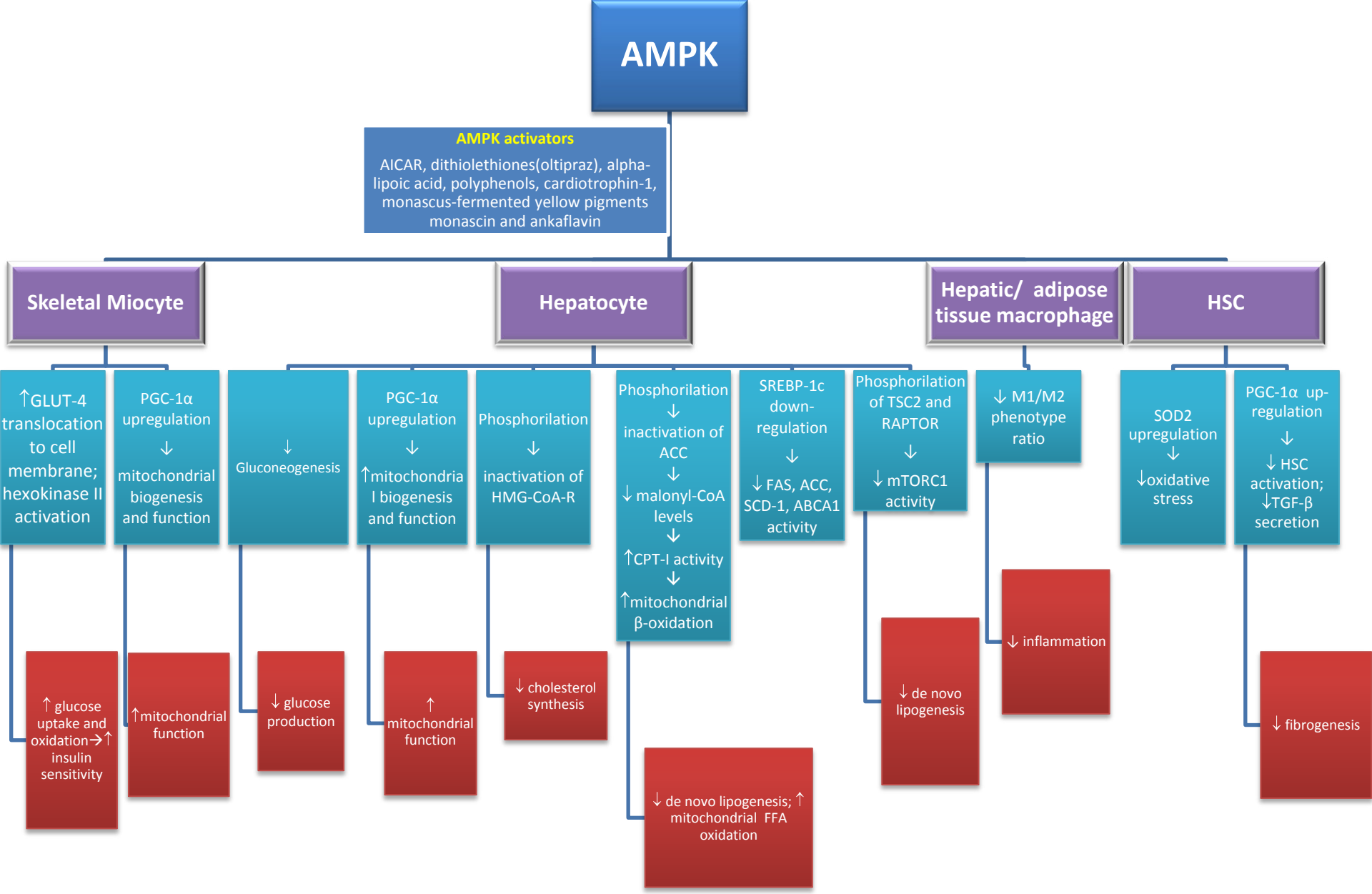
[269] Jun JiL. Lau F, Cellular senescence controls fibrosis in wound healing, Aging 2010; 2: 627–631.

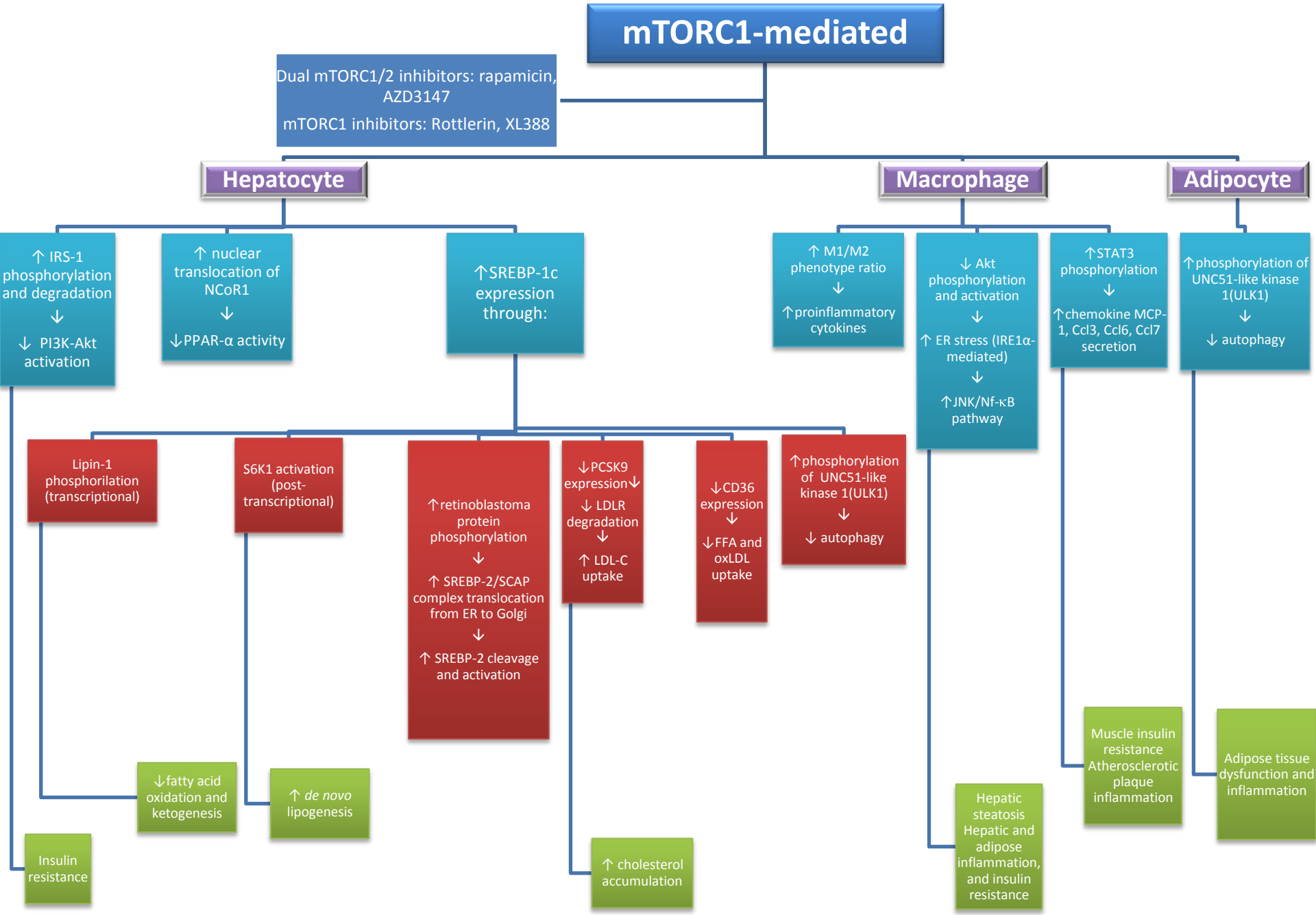
[270] Kim KH, Chen CC, Monzon RI, Lau LF. Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. Mol Cell Biol. 2013;33:2078-90.

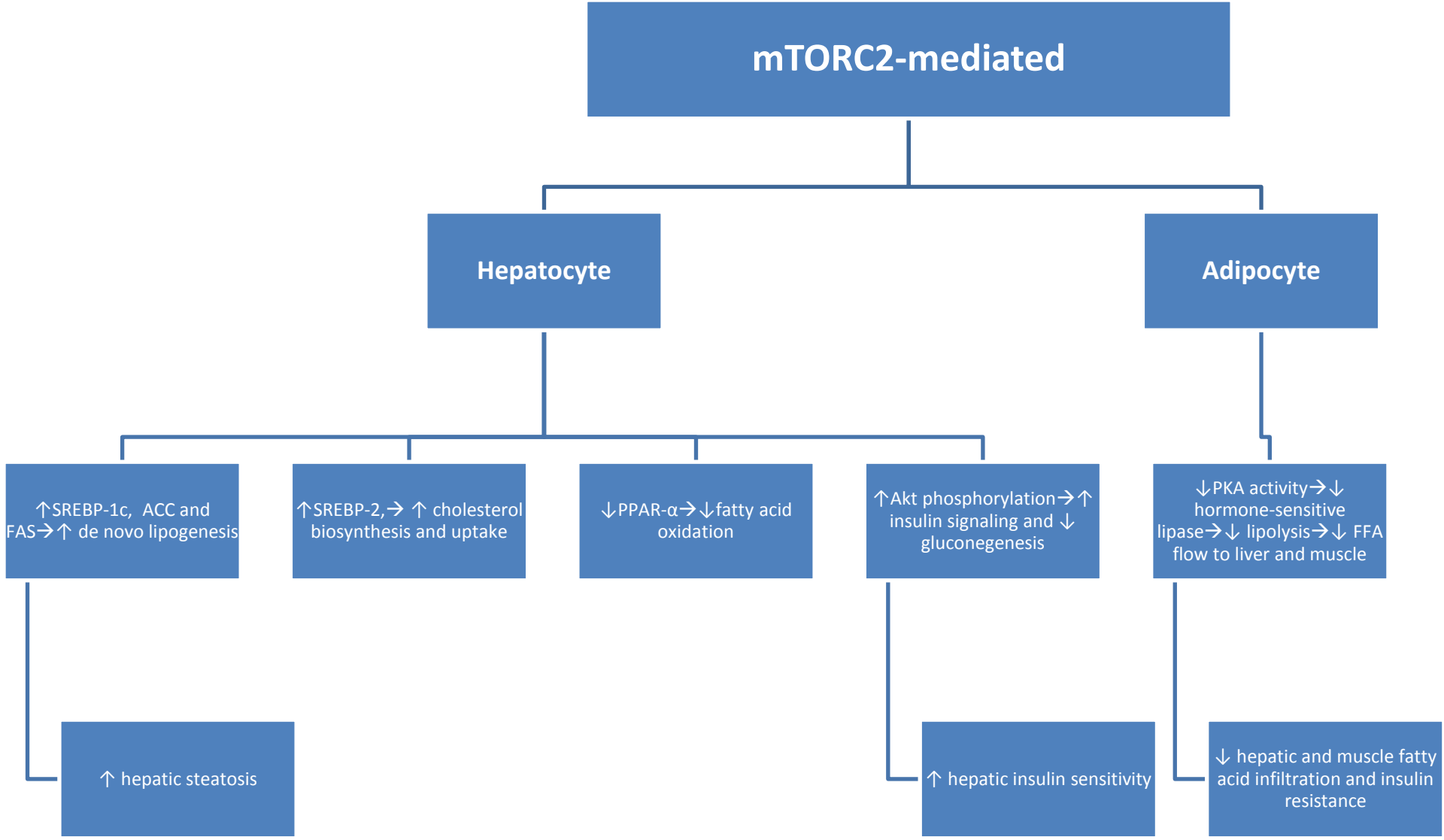
[271]Borkham-Kamphorst E et al. The anti-fibrotic effects of CCN1/CYR61 in primary portal myofibroblasts are mediated through induction of reactive oxygen species resulting in cellular senescence, apoptosis and attenuated TGF-β signaling. Biochim Biophys Acta. 2014;1843:902-14.

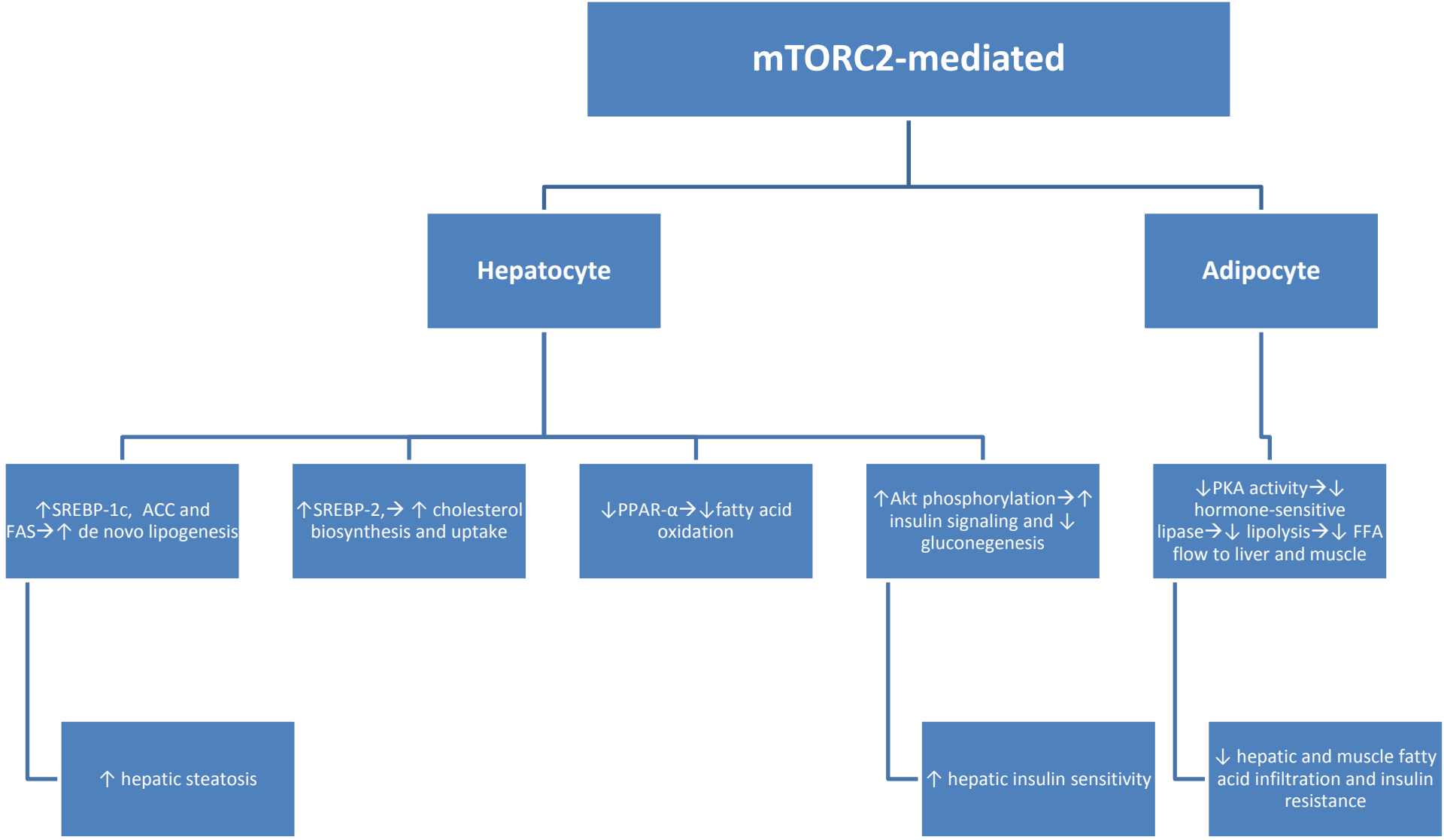
[272] Stiuso P, et al.. Serum oxidative stress markers and lipidomic profile to detect NASH patients responsive to an antioxidant treatment: a pilot study. Oxid Med Cell Longev. 2014;2014:169216.

[273]Torok NJ, Dranoff JA, Schuppan D, Friedman SL Strategies and endpoints of antifibrotic drug trials: Summary and recommendations from the AASLD Emerging Trends Conference, Chicago, June 2014 Hepatology 2015; published online : 10 Mar 2015, DOI: 10.1002/hep.27720


AMPK
AMPK activators
AICAR, dithiolethiones(oltipraz), alpha-lipoic acid, polyphenols, cardiotrophin-1, monascus-fermented yellow pigments monascin and ankaflavin
Skeletal Miocyte
Hepatocyte
Hepatic/ adipose tissue macrophage
HSC
↑GLUT-4 translocation to cell membrane; hexokinase II activation
PGC-1α upregulation ↓ mitochondrial biogenesis and function
↓ Gluconeogenesis
PGC-1α upregulation ↓ ↑mitochondrial biogenesis and function
Phosphorylation ↓ inactivation of HMG-CoA-R
Phosphorylation ↓ inactivation of ACC ↓ ↓ malonyl-CoA levels ↓ ↑CPT-I activity ↓ ↑mitochondrial β-oxidation
SREBP-1c down-regulation ↓ ↓ FAS, ACC, SCD-1, ABCA1 activity
Phosphorylation of TSC2 and RAPTOR ↓ ↓ mTORC1 activity
↓ M1/M2 phenotype ratio
SOD2 upregulation ↓ ↓oxidative stress
PGC-1α up-regulation ↓ ↓ HSC activation; ↓TGF-β secretion
↑ glucose uptake and oxidation→↑ insulin sensitivity
↑mitochondrial function
↓ glucose production
↑ mitochondrial function
↓ cholesterol synthesis
↓ de novo lipogenesis; ↑ mitochondrial FFA oxidation
↓ de novo lipogenesis
↓ inflammation
↓ fibrogenesis


mTORC1-mediated
Dual mTORC1/2 inhibitors: rapamicin, AZD3147
mTORC1 inhibitors: Rottlerin, XL388
Hepatocyte
Macrophage
Adipocyte
↑ IRS-1 phosphorylation and degradation
↓
↓ PI3K-Akt activation
↑ nuclear translocation of NCoR1
↓
↓PPAR-α activity
↑SREBP-1c expression through:
↑ M1/M2 phenotype ratio
↓
↑proinflammatory cytokines
↓ Akt phosphorylation and activation
↓
↑ ER stress (IRE1α-mediated)
↓
↑JNK/Nf-κB pathway
↑STAT3 phosphorylation
↓
↑chemokine MCP-1, Ccl3, Ccl6, Ccl7 secretion
↑phosphorylation of UNC51-like kinase 1(ULK1)
↓
↓ autophagy
Lipin-1 phosphorilation (transcriptional)
S6K1 activation (post-transcriptional)
↑retinoblastoma protein phosphorylation
↓
↑ SREBP-2/SCAP complex translocation from ER to Golgi
↓
↑ SREBP-2 cleavage and activation
↓PCSK9 expression↓
↓ LDLR degradation
↓
↑ LDL-C uptake
↓CD36 expression
↓
↓FFA and oxLDL uptake
↑phosphorylation of UNC51-like kinase 1(ULK1)
↓
↓ autophagy
↓fatty acid oxidation and ketogenesis
↑ de novo lipogenesis
Insulin resistance
↑ cholesterol accumulation
Hepatic steatosis Hepatic and adipose inflammation, and insulin resistance
Muscle insulin resistance Atherosclerotic plaque inflammation
Adipose tissue dysfunction and inflammation


mTORC2-mediated
Hepatocyte
Adipocyte
↑SREBP-1c, ACC and FAS→↑ de novo lipogenesis
↑SREBP-2,→ ↑ cholesterol biosynthesis and uptake
↓PPAR-α→↓fatty acid oxidation
↑Akt phosphorylation→↑ insulin signaling and ↓ gluconegenesis
↓PKA activity→↓ hormone-sensitive lipase→↓ lipolysis→↓ FFA flow to liver and muscle
↑ hepatic steatosis
↑ hepatic insulin sensitivity
↓ hepatic and muscle fatty acid infiltration and insulin resistance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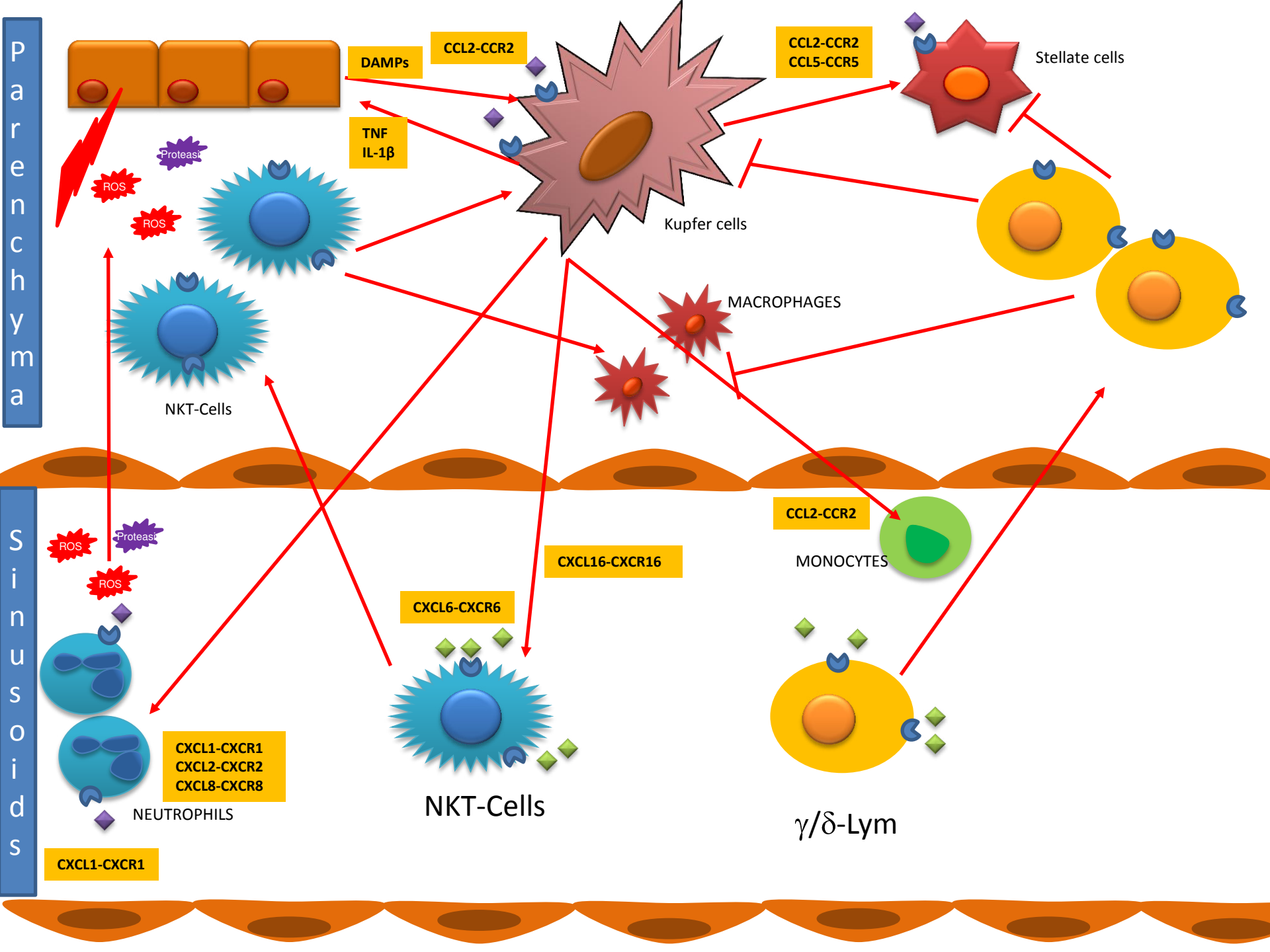
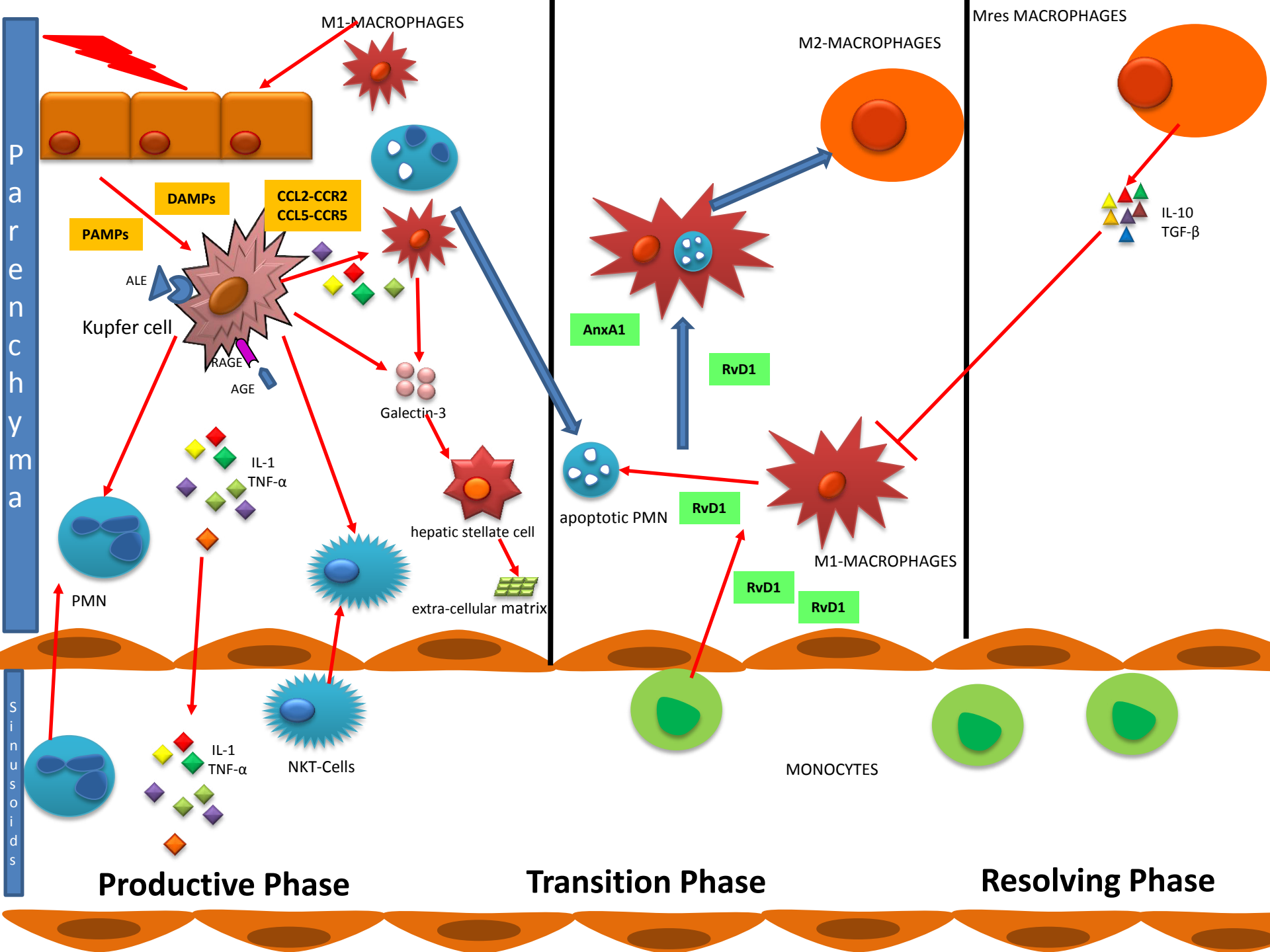

Parenchyma
Sinusoids
DAMPs
CCL2-CCR2
TNF
IL-1β
CCL2-CCR2
CCL5-CCR5
Stellate cells
Kupfer cells
Proteasi
ROS
ROS
NKT-Cells
MACROPHAGES
CCL2-CCR2
MONOCYTES
ROS
Proteasi
ROS
CXCL16-CXCR16
CXCL6-CXCR6
CXCL1-CXCR1
CXCL2-CXCR2
CXCL8-CXCR8
NEUTROPHILS
NKT-Cells
γ/δ-Lym
CXCL1-CXCR1


Parenchyma
Sinusoids
M1-MACROPHAGES
DAMPs
PAMPs
CCL2-CCR2
CCL5-CCR5
ALE
Kupfer cell
RAGE
AGE
Galectin-3
IL-1
TNF-α
hepatic stellate cell
extra-cellular matrix
PMN
IL-1
TNF-α
NKT-Cells
Productive Phase
M2-MACROPHAGES
AnxA1
RvD1
apoptotic PMN
RvD1
M1-MACROPHAGES
RvD1
RvD1
MONOCYTES
Transition Phase
Mres MACROPHAGES
IL-10
TGF-β
Resolving Phase