New Pharmacologic Agents That Target Inflammation and Fibrosis in Nonalcoholic Steatohepatitis-Related Kidney Disease

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Running title: NASH and CKD

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Abstract

Epidemiological data set an association between the prevalence and severity of NAFLD and the incidence and stage of chronic kidney disease(CKD); furthermore, NASH-related cirrhosis has a higher risk of renal failure, a greater necessity for simultaneous liver-kidney transplantation(SLKT) and a poorer renal outcome than cirrhosis of other etiologies even after SLKT. These data suggest NASH and CKD share common proinflammatory and profibrotic mechanisms of progression, which are incompletely targeted by current treatments.

We reviewed therapeutic approaches at late preclinical/early clinical stage of development in NASH and/or CKD, focusing on anti-inflammatory and anti-fibrotic treatments, which could retard progression of both disease conditions.

Renin inhibitors and ACE2 activators are new renin-angiotensin axis modulators that showed incremental advantages over ACEIs/ARBs in preclinical models. Novel, potent and selective agonists of peroxisome proliferator-activated receptors and of farnesoid X receptor, designed to overcome limitations of older compounds, gave promising results in clinical trials.

Epigenetics, heat stress response and common effectors of redox regulation are also subjected to intensive research, while the gut is targeted by several approaches, including synbyotics, anti-LPS antibodies, Toll-Like Receptor-4 antagonists, incretin mimetics and Fibroblast Growth Factor(FGF)19 analogs.

Promising anti-inflammatory therapies include inhibitors of NLRP3 inflammasome, of NF-κB, and of Vascular Adhesion Protein(VAP)-1, chemokine antagonists, and solithrmycin, while approaches targeting common pro-fibrogenic pathways operating in the liver and the kidney include galectin-3 antagonists, and inhibitors of Rho-associated Protein Kinase and of Epidermal Growth Factor activation.

The evidence, merits and limitations of each approach fot the treatment of NASH and CKD are discussed

Key words: CKD, renal function, eGFR, NASH, fibrosis

Abbreviations

ABCA1**: A**TP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase; ADMA asymmetric dimethylarginine;: **CAT:** cationic amino acid transporter**;** AP-1: activation protein-1**; CA:** cholic acid; **CDCA.** chenodeoxycholic acid; CD36: cluster of differentiation-36; CHOP: C/EBP homologous protein; DDAH: dimethylarginine dimethylaminohydrolases ; EMT: epithelial-to-mesenchimal transition; HSC: hepatic stellate cell; IRS: insulin receptor substrate; JNK**:** c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; PDCD4: programmed cell death protein 4; RECK. Reversion-inducing cysteine-rich protein with Kazal Motifs; Smad**:** Mothers against decapentaplegic homolog ;Trx: thioredoxin; VLDLR: VLDL receptor.

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; 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; NLRP3: NOD-like receptor family, pyrin domain containing 3; NO: nitric oxide; NOX: NADPH oxidase; OCA: obeticholic acid; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1 α; RAGE: receptor for advanced glycation end-products; ROCK. Rho-associated Protein KinaseM; ROS: reactive oxygen species; SCD-1: stearoyl-CoA desaturase-1; SR-A1: scavenger receptor-A1; SR-B1: scavenger receptor-B1; SREBP: sterol-responsive element binding protein; TGF-β: transforming growth factor- β; TLR: toll-like receptor; TNF: tumor necrosis factor; TZD: thiazolidinediones; VAP-1: Vascular Adhesion Protein-1; VLDL: very low density lipoprotein; VSCMs: vascular smooth muscle cells;

Introduction: the need for more effective therapeutic approaches in NASH-related chronic kidney disease (CKD)

Growing epidemiological and experimental evidence links non-alcoholic fatty liver disease(NAFLD) to CKD and suggest NAFLD accelerates the development and progression of CKD, independently of traditional risk factors[1,2]. More importantly, longitudinal data set an association between the severity of liver histology and the incidence and stage of CKD, suggesting overlapping mechanisms contribute to the onset and progression of liver and kidney injury in NAFLD2. Conversely, CKD adversely affects health-related outcomes in NAFLD: in the National Health and Nutrition Survey (NHANES)-III, the presence of CKD increased by 4.8-fold overall mortality in female patients with NAFLD[3].

Further evidence for an aetiological association between NAFLD and CKD comes from transplant medicine, where NASH-related cirrhosis has a higher risk of renal failure than other aetiologies and is the most rapidly growing indication for simultaneous liver-kidney transplantation (SLKT)[4]. Remarkably, even after SLKT, SLK recipients for NASH-related cirrhosis have a higher risk of kidney graft loss compared to cirrhosis of other etiologies4.

These findings suggest NAFLD and CKD may be part of the same multisystem condition and prompt exploring potential therapeutic targets to improve outcomes of both diseases.

Renin inhibitors and angiotensin converting enzyme(ACE)-2 activators: new Renin-Angiotensin Axis (RAS) modulators for the treamtent of NASH and CKD

Although the RAS contributes to the pathogenesis of obesity-related disorders, including NAFLD and CKD, a considerable proportion of CKD patients on ACE-inhibitors(ACEIs) and Angiotensin Receptor Blockers(ARBs) develop ESRD or CVD, and available RAS inhibitors do nor improve all-cause mortality in CKD, while randomized trials with ACEIs/ARBs in NASH are scarce and controversial[5,6]. Therefore, current research focuses on different steps in RAS axis to retard NAFLD and CKD progression, including renin activity and ACE2-Angiotensin(1-7)-Mas receptor axis.

Aliskiren, the first direct renin inhibitor to be approved for clinical use, binds to the renin active site

that is responsible for the hydrolysis of angiotensinogen to angiotensin I, thereby blocking the renin enzymatic activity and decreasing angiotensin I and II generation.

In diet-induced NASH, aliskiren improved hepatic steatosis, inflammation and fibrosis and systemic insulin resistance by down-regulating lipogenic pathways and pro-inflammatory nuclear factor (NF)-κB activation, while enhancing lipid and glucose oxidation[7,8]. While there are no human data on the effects of aliskiren on NAFLD, this drug attenuated oxidative stress and improved tubular status in non-diabetic patients with CKD[9].

The ACE2-Angiotensin(1-7)-Mas receptor axis is a key endogenous counter-regulatory mechanism of the ACE-AngII-AT_1 receptor pathway that is attracting considerable interest[10]. ACE2 degrades AngII to generate Ang(1-7), a peptide with opposing biological activity to AngII: accordingly, the net RAS activity depends on the balance between the ACE-AngII-AT_1 receptor and the ACE2-Ang(1-7)-Mas receptor axis(**supplementary Table 1**). Therefore, approaches enhancing ACE2 activation may offer incremental advantages over ACE/ARBs by enabling both AngII catabolism and Ang(1-7) peptide production.

Consistent with these premises, NASH and CKD are associated with reduced ACE2 expression, which has been implicated in hepatic, renal and cardiovascular disease[11,12]; while Ang(1-7) analogues or ACE2 activators xanthenone and diminazene ameliorated systemic inflammation and liver and kidney injury in animal models of NASH and CKD[13, 14,15].

"Old" drugs with new potential: pentoxyfilline and cysteamine bitartrate

Pentoxifylline is a methylxanthine-derived non-specific phosphodiesterase inhibitor that also antagonizes the adenosine receptor and inhibits TNF-α expression, therefore having the potential to improve NASH and CKD through its antiinflammatory and haemodinamic actions[16]. Emerging human data support the effectiveness of pentoxifylline in NAFLD and CKD. A meta-analysis of small RCTs showed that pentoxifylline significantly reduced serum aminotransferase, BMI, fasting glucose, steatosis, lobular inflammation, and fibrosis in NAFLD[17], while in the large Pentoxifylline for Renoprotection in Diabetic Nephropathy (PREDIAN) trial pentoxifylline added to to maximized RAS

blockade slowed eGFR decline and reduced albuminuria and urine TNF-α excretion in stage 3–4 diabetic nephropathy[18]. The renal benefits of pentoxifylline were confrmed in a recent meta-analysis of 26 studies (1516 participants)[19]. Further longer and larger RCTs with clinical outcomes are needed to confirm these data in diabetic and nondiabetic patients with NAFLD-associated CKD.

Cysteamine bitartrate is a small aminothiol molecule approved for the treatment of nephropatic cystinosis, a lysosomal storage disorder characterized by cystine accumulation and early ESRD: cysteamine effectively traverses the cellular and organelle membranes and removes accumulated cystine from lysosomes. Additional mechanisms of action of cysteamine could benefit non-cystinotic CKD and NASH: increase of the cellular thiol and free thiol tri-peptide glutathione (GSH) pool, reactive oxygen species (ROS) scavenging, and decreased lipoperoxidation and glutathione peroxidase activity. Furthermore, cysteamine may also directly increase adiponectin multimerization and showed TGFß–independent renal antifibrotic properties in experimental CKD via blockade of myofibroblast activity[20,21] .

While enteric-coated cysteamine bitartrate improved noninvasive markers of liver disease in paediatric NAFLD[22], its impact on liver histology and renal function decline in non-cystinotic CKD remain unassessed.

Nuclear transcription factors in the pathogenesis of NAFLD and CKD

Ectopic lipid deposition is a hallmark of NASH and CKD and contributes to disease progression through cellular energy depletion and accumulation of toxic lipid intermediates, leading to lipoperoxidative stress, cell apoptosis, inflammation and fibrosis[23,24]. In NASH and CKD, ectopic lipid accumulation is subtended by an extensive derangement in multiple steps in triglyceride and cholesterol metabolism, including defective catabolism and increased synthesis and uptake from circulating lipoproteins[25]. On this basis, a strategy targeting nuclear transcription factors, which coordinately regulate multiple steps in lipid metabolism, inflammation and fibrosis, including peroxisome proliferator-activated receptors(PPARs) and Farnesoid X-receptor(FXR), might be more effective than targeting single enzymatic steps[26].

Fibrates and thiazolidinediones (TZDs), which are PPAR-α and PPAR-γ agonists, resèpectively, were first evaluated in NASH and CKD5[,26]. More recently, activation of PPAR-δ has been found to complement the beneficial metabolic and anti-inflammatory actions of PPAR-α activation [27]**(supplementary Table 2).**

Fibrates improved liver disease in rodent models of NASH, but their weak potency, their low expression in human liver relative to rodents, which further decreases across progressive NAFLD stages, explain the disappointing results in randomized clinical trials (RCTs) in NASH5. In diabetic nephropathy, fibrates decreased albuminuria and slowed eGFR decline independently of their lipid-lowering properties, but the overall effect was small and their interference with creatinine excretion can raise serum creatinine[28]. The novel, more potent and selective PPAR-α, PPAR-δ and dual PPAR-α/δ agonists, including K-877, GW501516, MBX-8025 and elafibranor, reversed lipotoxicity, and improved metabolic profile, inflammation, fibrosis and renal function decline in diet-induced models of NASH and CKD[29,30,31,32]. Some of these compounds advanced into early phase of clinical trials: the PPAR-δ agonist MBX-8025 improved liver enzymes, insulin resistance, inflammatory markers and atherogenic dyslipidemia in overweight subjects[33], and the dual PPARα/δ agonist elafibranor induced significant steatohepatitis resolution(19% vs 12% with placebo) and fibrosis improvement in NASH patients[34].

TZDs improved steatohepatitis and slowed liver fibrosis progression in NASH patients5; furthermore, TZDs improve proteinuria in diabetic nephropathy[35]. Unfortunately, the clinical use of TZDs is limited by their unwanted effects5. For these reasons, compounds are being developed to overcome the side effects of TZDs, while keeping their effectiveness, including selective non-TZD PPARγ modulators (SPPARMs), mitochondrial target of thiazolidinediones (mTOT) modulators, stabilized R-enantiomer of pioglitazone DRX-065, and dual PPAR-α/γ agonists.

Upon PPAR*γ* binding, PPAR*γ* undergoes conformational changes that favor its association with the retinoid X receptor (RXR), as well as with a range of cofactors (coactivators and corepressors) in different cell types, which determine the overall spectrum of therapeutic and unwanted effects[36]. In

the case of PPAR*γ* activation by the full agonists TZDs, the dose-response curve for the therapeutic effects overlaps with that of side effects, such that increasing doses produce both greater therapeutic benefits and more pronounced side effects.

SPPARM-γ are PPAR*γ* ligands that induce alternative conformational changes of the bound receptor heterodimer complex, which recruits different patterns of cofactors, gene transcription, and cellular responses than a full agonist. SPPARM-γ, like INT131 besylate, induce a right shifted dose–response curve for unwanted effects as compared with the curve of therapeutic effects, such that at any given concentration the ligand induces therapeutic effects without side effects.

Another approach stems from the discovery that the insulin-sensitizing properties of TZDs are largely mediated by the PPARγ-independent inhibition of mitochondrial pyruvate carrier (MPC) proteins MPC1 and MPC2, which perform facilitated pyruvate transport across the mitochondrial inner membrane, a critical step in carbohydrate, amino acid, and lipid metabolism. Mitochondrial target of thiazolidinediones (mTOT)-modulating insulin sensitizers are novel TZD analogs that bind and inhibit MPC protein but have very low affinity for PPARγ[37].

Some of the above-mentioned compounds advanced to clinical/late preclinical stage: INT131 and MSDC-0160 (formerly mitoglitazone), a prototype mTOT modulator, induced similar glycemic and metabolic improvement than pioglitazone, but with less weight gain or fluid retention in diabetic patients[36,37] , while the mTOT modulator MSDC-0602 improved steatohepatitis and fibrosis in diet-induced rodent NASH[38].

DRX-065 is a stabilized deuterated R-enantiomer of pioglitazone. Pioglitazone is a mixture of two mirror-image compounds (the R- and S-enantiomers) that rapidly interconvert *in vivo*. While d-S-pioglitazone (d-S-pio) accounts for all of the PPAR*γ* agonist activity observed with racemic pioglitazone, d-R-pioglitazone (DRX-065) is not a PPAR*γ* agonist and has pharmacological properties desirable for the treatment of NASH and CKD, including improvement of mitochondrial dysfunction and respiratory chain deficiency, non-steroidal anti-inflammatory effects, and insulin sensitizing effects, without the PPARγ-related weight gain side effects[39].

Dual PPAR-α/γ agonsists combine the lipid- oxidizing and lipid-lowering properties of PPAR-α agonists with the insulin sensitizing effects of PPAR-γ agonists. Saroglitazar improved diet-induced NASH[40] and markers of NAFLD in diabetic patients[41] without inducing weight gain or fluid retention and is being evaluated in biopsy-proven NASH patients (CTRI registration no.: CTRI/2010/091/000108), while aleglitazar slowed eGFR decline in stage 3 diabetic CKD[42].

Farnesoid X-receptor(FXR) is abundantly expressed in hepatic and renal cells as well as in adipocytes and macrophages, where its activation restores physiological lipid and glucose homeostasis and endothelial function and shows anti-inflammatory and anti-fibrotic properties[43,44,45](**supplementary Table 2**). After the observation that FXR expression is down-regulated and inversely related to disease severity in NAFLD and CKD, potent semi-synthetic bile acid FXR agonists have been developed[26]. Obeticholic acid (OCA, INT-747), a potent semi-synthetic derivative of chenodeoxycholic acid, reversed renal lipid accumulation, proteinuria, and tubulo-interstitial inflammation and fibrosis in diet-induced experimental CKD[43,44] and improved NAFLD histological activity and fibrosis score in the multicenter, randomized "FXR Ligand NASH Treatment (FLINT)" trial[46].The impact of OCA on stage 2-3 fibrosis in NASH is currently being evaluated in the 6-year, multicenter REGENERATE (Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment) trial **(**ClinicalTrials.gov.ID: NCT02548351), which is expected to enroll 2000 NASH patients at up to 300 centers. and will allow evaluaton of long-term clinical safety and effectiveness of OCA[47].

MicroRNA modulation for the treatment of liver and kidney injury

MicroRNAs (miRNAs) are small noncoding RNAs transcribed as ~70 nucleotide precursors in the nucleus and subsequently processed into mature ~22 nucleotide miRNAs[48] . miRNAs have regulatory roles through complementarity to messenger RNA (mRNA): they can silence their cognate target genes by inhibiting mRNA translation or degrading the mRNA molecules by binding to their 3′-untranslated region (UTR) or, less commonly, can upregulate gene translation and expression[48].

A single miRNA can regulate an array of genes and over 800 miRNAs have been identified in the human genome, so that over two-thirds of all human genes are regulated by microRNAs.

New data point to a critical role of miRNAs in the regulation of diverse cellular processes, including cell metabolism, differentiation and proliferation, and implicate miRNA dysregulation in the pathogenesis of various diseases, including NASH and CKD[48]. Consequently, normalization of dysregulated miRNAs by antisense oligonucleotides (ASOs), short locked nucleic-acid-modified oligonucleotide ('LNA-anti-mir') or synthetic miRNA mimics represents an attractive therapeutic tool. Among dysregulated miRNAs involved in he pathogenesis of liver and kidney disease, the modulation of miRNA-103/107 and miRNA-21 is at more advanced stage of development.

miRNA-103/107 are potent regulators of insulin signaling and glucose homeostasis, and their upregulation in the liver and adipose tissue can promote insulin resistance, glucose intolerance and NASH though modulation of several biological pathways[49,50](**supplementary Table 3**). Following the demonstration that ASO-mediated inhibition of miRNA-103/107 improved insulin resistance, glucose homeostasis and steatosis, RG-125 (AZD4076), a GalNAc-conjugated anti-miRNA-103/107 oligonucleotide, has entered phase I clinical development for the treatment of diabetic/prebiabetic NASH patients(ClinicalTrials.gov ID: NCT02612662).

miRNA21 is another key miRNA whose overexpression is believed to contribute to the development and fibrotic progression of NASH and CKD[51, 52, 53](**supplementary Table 3**). In experimental models, miRNA21 overexpression promoted TGF-ß1-induced adipogenesis and fibrogenesis through modulation of diverse molecular pathways involved in lipid metabolism and redox balance (**supplementary Table 3**)[51-53], which were all normalized by anti-microRNA-21 ASOs[51-54,55].

PPAR-α activation seems central for the effects of antagomir-21, which were abrogated by PPAR-α deletion and mimicked by PPAR-α overexpression[55].

Challenges remain to bring miRNA-based therapeutics into the clinic: long-term safety profile requires to be more firmly established, because serious side effects, including unwanted (on-target effects) or unintended (off-target effects) gene regulation, potential toxicity of chemical modifications of the oligonucleotides, may limit miRNA-based therapeutic applications. As an example, miRNA-107

functions as a tumor suppressor gene in renal carcinoma and potential pro-oncogenic effects of miRNA-107 downregulation in the long-term are unknown[56].

Sensing cellular stress: role of Heat Shock Proteins(HSPs) in liver and kidney injury

HSPs are highly conserved proteins whose cellular expression, physiologically low, is upregulated in response to various stresses, including heat, surgical stress, hypoxia, infections, nutritional deficiencies (e.g. glucose deprivation), conditions where HSPs account for up to 4-6% of cell proteins, thereby constituting the heat shock response(HSR)[57]. The HSR helps maintaining cellular homeostasis under stress and allowing the cell to survive to lethal conditions, through several mechanisms: as molecular chaperones, HSPs catalyze proper protein folding, transport, translocation and assembly, especially helping in the refolding of misfolded proteins, or assist proteasomal degradation of irreversibly damaged proteins, thus allowing autophagy and cell survival. Furthermore, HSPs are potent anti-apoptotic proteins, associating with key effectors of the apoptotic machinery, and thereby interfering with this cell death process.

Mammalian HSPs have been classified into six families according to their molecular size: HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs(15-30 kDa).

High molecular weight HSPs are ATP-dependent chaperones, whereas smaller HSPs act in an ATP-independent fashion. This has therapeutic implications, as most inhibitors of high molecular weight HSPs block the ATPase activity and prevent the conformational changes necessary for the loading of co-chaperones and client proteins, while inhibitors of smaller molecular weight HSPs (like HSP47) act in alternative ways.

Inappropriate upregulation of HSPs have been recently connected to NASH and CKD progression and pharmacological inhibition of two of these HSPs(HSP90, HSP47) has potential therapeutic implications[58, 59].

HSP90 is one of the most abundant HSPs and acts as a molecular chaperone critical to the folding, stability and activity of over 200 client proteins, including key intracellular regulators of

inflammation and fibrogenesis like NF-κB, TGF-β and Signal Transducer of Activation and Transcription (STAT), that are upregulated in NASH and CKD[58,59,60]. Furthermore, HSP90 exerts antiapoptotic effects towards HSC and renal tubulointerstitial cells, key players in fibrogenesis[59]. (**supplementary Table 3**). HSP90 inhibitors, which are at a clinical developmental stage for cancer therapy, ameliorated fibrosis and renal function in rodent models of NASH and CKD[59,60,61] .

HSP47 is an ATP-independent ER-glycoprotein which plays a critical role in collagen maturation, biosynthesis and secretion by preventing newly formed procollagen chains from aggregation and ER-mediated degradation, promoting the stability of the triple-helical region of procollagen and aiding collagen secretion. Consistently, HSP47 overexpression correlates with the severity of fibrosis in different hepatic and renal diseases[62] and HSP47 inhibition by specific siRNAs conjugated with cationized gelatin microspheres, which prolong siRNA inhibitory effects from 7 days to 14 days, suppressed renal fibrosis in the Unilateral Ureteral Obstruction model[62].

ND-L02-s0201, a vitamin A-coupled lipid nanoparticle containing siRNA targeted against HSP47, proved safe and well tolerated in a phase 1b/2 RCT enrolling patients with NASH/HCV-related moderate-to-advanced liver fibrosis (www.clinicaltrials.gov ID: NCT02227459) and was granted FDA fast-track designation for the treatment of NASH patients with fibrosis.

Effectors of redox regulation in the pathogenesis of NASH and CKD: role of NADPH Oxidase(NOX)1/4, Apoptosis Signal-Regulating Kinase 1(ASK1) and Nuclear Erythroid 2-related Factor 2 (Nrf2).

Increased oxidative stress is believed to play a major role in liver and renal disease progression in NASH and CKD[26,29], but strategies using single antioxidant agent supplementation proved elusive5. Therefore approaches targeting common effectors of redox regulation, including Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), apoptosis signal-regulating kinase 1 (ASK1) and nuclear erythroid 2-related factor 2 (Nrf2), are being investigated.

NOX is a multimeric transmembrane enzyme complex that forms superoxide and hydrogen peroxide from molecular oxygen using NADPH as an electron donor[63]. The mammalian NOX family comprises seven isoforms: NOX1-5, and the dual oxidases (Duox) Duox1 and -2[64]. Beside being expressed by neutrophils and phagocytes, where NOX2 isoform participates in microbial killing, NOX isoforms (predominantly NOX4, 1, and 2) are consitutively expressed in liver and renal cells **(supplementary Table 4)**, where they produce ROS in response to a wide range of stimuli, including hyperglycemia, cytokines, and AGEs, which operate in NASH and CKD and expalin hepatic and renal NOX1/4 is upregulation in these two conditions[64,65]. The increased ROS production leads to necro-inflammation and fibrosis in the liver and to podocyte loss, glomerlosclerosis and tubulointerstitial fibrosis in the kidney **(supplementary Table 4)**. Genetic or pharmacological NOX1/4 inhibition protected from liver and kidney disease progression[65,66] and on this basis small-molecule inhibitors have been developed: GKT137831, a potent dual pyrazolopyridine dione NOX1/4 inhibitor, ameliorated hepatic inflammation and fibrosis, albuminuria and renal function in preclinical models[67,67], has been evaluated in diabetic albuminuric patients (clinicaltrials.gov ID: NCT02010242; results not available yet) and is scheduled for a phase 2a RCT in NASH patients with fibrosis.

Although the results are encouraging to date, potential safety issues deriving from inhibition of normal physiological function of NOX remain: NOX4 protects the vasculatore from ischemic or inflammatory damage by enhancing eNOS and heme oxygenase-1 expression and NO production[68]. A better understanding of the physiological functions of NOX isoforms in different tissues and strategies to enhance selective delivery of NOX inhibitors to target organs may enhance potency and safety of NOX inhibitors.

ASK1is a serine/threonine kinase belonging to the mitogen-activated protein kinase kinase kinase .(MAP3Ks) family, which, upon activation by pro-inflammatory stimuli[69], activates downstream terminal MAPK kinases p38 and c-Jun N-terminal kinase (JNK), resulting in production of inflammatory cytokines/chemokines, matrix remodeling and fibrosis, apoptotic and necrotic cell death, aberrant cell proliferation, and metabolic perturbations[70] (**supplementary Table 4**).

Preclinical data suggest ASK1-MAPK activation is a key mediator of the pathogenic consequences of oxidative stress in NASH and CKD and that genetic or pharmacological ASK1deletion may prevent hepatic steatosis and fibrosis development[70] and CKD progression[71, 72]. On this basis, GS-4997, a potent, selective oral ASK1 inhibitor is being evaluated in 2 phase IIa RCTs, enrolling NASH patients with moderate-advanced (stage F2-F3) fibrosis and patients with stage 3/4 diabetic nephropathy(ClinicalTrials.gov ID: NCT02466516, NCT02177786). The impact of ASK1 inhibitors in nondiabetic CKD is currently unknown.

Nrf2 belongs to the family of basic region leucine zipper (bZIP) transcription factors, expressed ubiquitously in human tissues and most abundantly in the liver, where it regulates the expression of several antioxidant and detoxification enzymes and has anti-inflammatory and pro-autophagic actions involving NF-κB, p53, mammalian target of rapamycin (mTOR), HSPs, and Fibroblast growth factor(FGF)-21[26](**supplementary Table 4**).

Under basal conditions Nrf2 is kept transcriptionally inactive through binding to its inhibitor, Kelch-like ECH-associated protein 1 (KEAP1), which targets Nrf2 for proteasomal degradation[26].

Oxidative or electrophilic cellular stresses, including ROS and reactive nitrogen species (RNS), interact with cysteine residues of KEAP1, which changes its conformation and dissociates from Nrf-2, which translocates to the nucleus and binds its antioxidant response element (ARE) in the promoter region of its target genes.

Substantial data support a key protective role for Nrf2 against NASH and CKD development: in diet-induced NASH models, Nrf2 deletion enhanced fibrosis progression, which was prevented by Nrf2 activators[73,74]. Similarly, Nrf2 activation ameliorated oxidative stress, inflammation, and kidney function in experimental CKD, while Nrf2 deletion enhanced CKD progression[75].

Several natural and synthetic small-molecule Nrf2 activators are currently evaluated preclinically**:** older compounds are thiol-containing electrophiles that relatively non-selectively bind cysteine-rich proteins, including KEAP1, thereby altering its conformation and preventing KEAP1-Nrf2 protein–protein interaction. Newer Nrf2 activators act by different mechanisms and have enhanced selectivity and potency for Nrf2 activation, which may translate in different clinical effectiveness and safety.

A phase 2 RCT is currently evaluating the synthetic triterpenoid Nrf2 activator bardoxolone methyl in diabetic nephropathy (clinicaltrials.gov ID: NCT02316821), while another RCT is evaluating oltipraz in NAFLD(clinicaltrials.gov ID: NCT01373554).

Targeting effectors of inflammation in NASH and CKD

Together with fibrosis, inflammation is regarded as a key contributor to the progression of both NAFLD and CKD toward organ failure[26] and therapeutic approaches targeting different steps of the inflammatory cascade are being investigated.

Targeting the NLRP3 inflammasome

The NOD-like receptor NLRP3 (NACHT, LRR and PYD domains-containing protein 3) inflammasome is a cytosolic multiprotein complex that converts extracellular signals into an inflammatory response, resulting in activation of caspase-1 and secretion of proinflammatory cytokines IL-1β and IL-18[26,76] .

Hepatic expression of inflammasome components is upregulated in NASH and correlates with liver disease severity, while pharmacological blockade of NLRP3 inflammasome with the potent, selective, small-molecule MCC950 prevented or reversed diet-induced NASH and fibrosis[77].

In CKD, the NLRP3 inflammasome is activated in both immune and intrinsic kidney cells, including renal tubular epithelium and podocytes, and NLRP3 inflammasome inhibition ameliorated diverse models of CKD[77]. Beside NLRP3 inflammasome inhibition, another therapeutic approach antagonizes downstream effectors of inflammasome activation, including IL-1β, with the anthraquinone diacerein and the anti-IL-1β recombinant mAb gevokizumab, currently under phase II clinical development in diabetic NAFLD(ClinicalTrials.gov ID**:** NCT02242149) and with diabetic nephropathy**(**EudraCT Number**:** 2013-003610-41).

NF-κB inhibitors

The NF-kB family comprises five different isoforms (p65/Rel-A, c-Rel, Rel-B, p50 and p52) of transcription factors that play a central role in regulating inflammatory and immune

responses, cell survival, proliferation and differentiation[78]. NF-κB is a "rapid-acting", inducible transcription factor: it is present in cells in an inactive state and does not require new protein synthesis in order to become activated, as NF-κB dimers are sequestered in the cytoplasm by protein inhibitors called Inhibitor of κB (IκBs). NF-κB activation is regulated by two main pathways: in the canonical pathway, which has been involved in NASH and CKD, NF-κB activation is initiated by the signal-induced degradation of IκB proteins via activation of the IκB kinase(IKK). Upon activation by a variety of signals, the IκB kinase phosphorylates IκB, which is degraded by ubiquitination, leaving the NF-κB free to enter the nucleus and upregulate gene transcription of proinfammatory chemokines and cytokines, including the chemokine (C-C motif) ligand 2 (CCL2, or monocyte chemoattractant protein-1, MCP-1) and IL-12ß[79]. A broad range of stimuli in metabolically-induced inflammation can activate NF-κB through this pathway, including TNFα, IL-1β, LPS, ROS, NEFAs and oxidized lipoproteins: consistently, inappropriate NF-κB signaling activation in monocytes and in the liver, adipose tissue and kidney has been implicated in the pathogenesis of NASH and of diabetic and obesity-associated CKD[79, 80].

NF-κB inhibitors, including the synthetic chalcone L2H17 and the small-molecule indazolic derivative bindarit. prevent phosphorylation and ubiquitination of IκB, reduce proinflammatory cytokine and chemokine expression and retarded experimental CKD progression[79,81]. While these agents have yet to advance to clinical stage in NASH, bindarit reduced albuminuria in a small phase 2a RCT in diabetic nephropathy(clinicaltrials.gov ID: NCT 01109212).

Anti-inflammatory macrolides: solithromycin

Solithromycin is the first fluoroketolide antibiotic belonging to the macrolide family, currently approved for bacterial community-acquired pneumonia and sexually transmitted infections. Solithromycin has higher antibacterial activity and prominent antiinflammatory properties than older macrolides. It achieves high hepatic concentrations, with 78% of an oral dose being metabolized in the liver and its metabolites maintain antiinflammatory properties and undergo renal excretion[81]. These antiinflammatory and kinetic properties prompted evaluation of solithromycin in NASH: in a high fat

diet-induced rodent model of NASH and hepatocellular carcinoma, 20 weeks of solythromycin improved glycemia, plasma triglycerides, histological NAFLD activity score and fibrosis and decreased tumor nodules[82]. Several potential mechanisms may underlie these benefits: solithromycin treatment did not alter gut microbiota composition, but potently inhibits NF-κB activity and hepatic MCP-1 expression and upregulates histone deacetylase 2, thereby suppressing pro-inflammatory cytokine production from hepatocytes and mononuclear cells[83]. Furthermore, solithromycin treatment suppressed expression of hepatic gluconeogeentic enzymes, thereby explaining glycemic improvement[83].

Following these encouraging preclinical results a phase IIa clinical trial has been started, assessing the effects of solithromycin for 13 weeks on liver histology in noncirrhotic NASH patients(clinicaltrials.gov ID: NCT02510599)

Despite the interest of this first, proof-of-concept, human study, concerns on long-term safety of antibiotic therapy remain, as the longer duration of treatment required for chronic conditions such as NAFLD and CKD would increase the risk of selecting resistant bacterial strains. For this reason, the company has developed a chemical synthesis program to modify solithromycin to find a new compound with antiinflammatory properties but devoid of antibacterial activity.

Targeting chemokines, extracellular mediators of inflammation

Chemokines are small molecules (MW ranging 8–13 kd) regulating leukocyte migration, inflammation, and fibrosis[26]. Chemokine (C-C motif) ligand 2 (CCL2, or MCP-1) and its receptor CCR2 and CCL5/CCR5 axis have been implicated in NASH and CKD development and fibrotic progression by attracting pro-inflammatory cells, activating HSC and inducing myofibroblast-like activation of tubule cells[26,84,85]. Consistently, cenicriviroc, a dual chemokine receptor CCR2/CCR5 antagonist, improved inflammatory and fibrotic changes, albuminuria and kidney function in diet-induced models of NASH and CKD[86], and has been recently evaluated in the Phase IIb multicenter RCT "Cenicriviroc for the Treatment of NASH in Adult Subjects With Liver Fibrosis" (CENTAUR)(ClinicalTrials.gov ID**:** NCT02217475) with encouraging results. CCX140-B, an oral

small molecule CCR2 antagonist, added on top ofstandard of care reduced albuminuria and slowed eGFR decline in diabetic nephropathy[87].

Other dual CCR2/CCR5 chemokine antagonists, BMS-813160 and PF-04634817, are being evaluated in phase IIa RCTs enrolling diabetic patients with different CKD stages (ClinicalTrials.gov ID**:** NCT01752985**,** NCT01712061).

Several issues, however, remain: due to chemokine functional redundancy (i.e., many chemokines bind multiple receptors and multiple receptors bind many chemokines), single chemokine antagonism may not be sufficient to produce clinically relevant benefit; additionally, chemokines may have even opposite biological actions by binding the same receptor on different cell lines[88]. These limitations mat be overcome by a deeper knowledge of the downstream intracellular signaling pathways regulated by chemokines, relevant for cell activation and migration, including Akt and ERK1-/2,

Targeting gut-derived mediators for the treatment of NAFLD and CKD

The ability of the gut to modulate host metabolism and inflammatory response and its contribution to obesity-related complications, including NAFLD and CKD, has been increasingly recognized[27] and various gut-oriented approaches to treat NASH and CKD are undev evaluation, including modulation of gut microbiota and of gut-derived peptide incretins and fibroblast growth factor(FGF)19.

Two main strategies are being evaluated to counteract host adverse effects of dysregulated gut microflora: the first involves modulation of gut microbiota composition, the second is direct antagonization of microbial pro-infoammatory mediators.

Gut microbiota manipulation with probiotics, prebiotics or synbiotics improved surrogate markers of NAFLD in small RCTs of short duration[89] and its impact on renal function in CKD is being investigated in the SYNbiotics Easing Renal failure by improving Gut microbiologY (SYNERGY) trial[90].

Pharmacological antagonization of microbial proinflammatory mediators currently focuses on the LPS-Toll-like Receptor 4(TLR4) axis activation and includes LPS-neutralizing antibodies and TLR-4 antagonists: IMM-124E is composed of anti-LPS bovine hyperimmune colostrum, harvested from

Australian dairy cows, which are immunised with LPS vaccine. A phase I/IIa RCT conducted in type 2 diabetic patients with NASH demonstrated IMM-124E is safe, exerts an antiinflammatory effect, mediated by increased Tregs and an increased serum GLP-1 and adiponectin levels, and improves insulin resistance and markers of liver injury[91]. A multicenter RCT is currently investigating the effect of IMM-124E on liver disease in patients with NASH (clinicaltrials.gov ID: NCT02316717).

TLR4 is a membrane receptor for LPS and for other molecules, including FFAs: upon ligand binding, TLR4 activation initiates an intracellular signaling cascade eventually leading to the degradation of the IKK complex, which frees the transcription factor NF-κB. NF-κB then moves into the nucleus and enhances transcription of pro-inflammatory and pro-fibrotic cytokines[92] . A critical role for TLR4 axis activation in triggering hepatic inflammation and fibrosis in NASH and renal tubulointerstitial inflammation and fibrosis in diabetic nephropathy has been recently recognized[98].

On this basis, the long-acting small molecule TLR-4 receptor antagonist JKB-121 has been developed. JKB-121 is a non-selective opioid antagonist which neutralized LPS-induced release of inflammatory cytokines, inhibited Kupffer cell and HSC proliferation and activation and prevented LPS-induced inflammatory liver injury in a dietary model of NASH[93]. A phase IIa RCT is currently investigating the safety and efficacy of JKB-121 in biopsy-proven NASH patients (ClinicalTrials.gov ID: NCT02442687).

Incretin mimetics include glucagon-like peptide-1 receptor(GLP-1R) analogs and dipeptidyl peptidase-4 (DPP-4) inhibitors. Incretin mimetics have the potential to improve NASH and CKD through multiple mechanisms (reviewed in [27]). On this basis, their impact on liver disease in NAFLD has been recently evaluated in two small phase IIa RCTs, with contrasting results: while liraglutide induced significant weight loss and NASH resolution and improved markers of lipotoxicity, inflammation and metabolic dysfunction[94], sitagliptin did not induce weight loss or improved radiological and biochemical markers of steatosis, fibrosis and insulin resistance in diabetic/prediabetic NAFLD patients[95].

A potential explanation for these discrepancies is that in addition to inactivating GLP-1, DPP-4 cleaves multiple other peptides, including glucose-dependent insulinotropic polypeptide(GIP), which

has opposite effects than GLP-1 on energy expenditure, adiposity and inflammation and may promote NAFLD[96,97]. Regarding the renal impact of incretin mimetics, both GLP-1R agonists and DPP-4 demonstrated potential for nephroprotection in diabetic nephropathy[98,99] .

FGF19 belongs to the endocrine FGF subfamily, which includes FGF19/21/23: FGFs bind to four cell surface tyrosine kinase FGF receptors (FGFRs 1–4) and use single-pass transmembrane proteins of the Klotho family as co-receptors to activate FGFR signaling[100]. FGF19 is secreted by enterocytes and regulates the bile acid enterohepatic circulation, glucose and lipid metabolism, and phosphate/vitamin D homeostasis.

FGF19 inhibits bile acid synthesis and transportation in a negative feed-back: bile acids bind to FXR in enterocytes, and induce intestinal secretion of FGF19, which in turn inhibits hepatic expression of *Cyp7a1*, the rate-limiting enzyme in bile acid synthesis in hepatocytes[106]. Beside bile acid homeostasis, FGF19 is a key metabolic regulator in the liver, adipose tissue and brain: FGF19 is an insulin-sensitizing hormone through enhanced hepatic glycogen synthesis and gluconeogenesis suppression and has also central nervous system-mediated, insulin-independent glucose- and weight-lowering proterties[106].

FGF19 increases also FFA oxidation and metabolic rate, leading to a reduction in fat mass[106]. The therapeutic potential of FGF19 in the treatment of metabolic disorders has been confirmed in obese and diabetic animal models, where genetic of pharmacological FGF19 activation ameliorated adiposity, glucose intolerance and insulin resistance[101,102]. In patients with NAFLD or CKD, circulating FGF19 levels are reduced and correlate inversely with metabolic parameters and disease severity[103, 104]. On this basis , recombinant FGF19 analog NGM282 is currently being evaluated in a phase IIa RCT in patients with NASH(clinicaltrials-gov ID: NCT02443116).

One major concern relating to the potential use of FGF19 is its mitogenic function, implicated in HCC development and progression, which was atrributed to STAT3 pathway activation by native FGF19 and has been eliminated in NGM282[105].

Targeting Vascular Adhesion Protein-1(VAP-1): a liver-derived amine oxidase with dual pro-inflammatory and pro-oxidative function

VAP-1 is a copper-dependent membrane-bound semicarbazide-sensitive amine oxidase(SSAO) expressed by hepatic endothelium and adipocytes. The soluble form of VAP-1 (sVAP-1) is largely derived from cleavage of membrane-bound VAP-1 from the hepatic vessels and adipocytes under pro-inflammatory stimuli[106]. Soluble VAP-1 levels are increased in chronic inflammatory conditions, including NAFLD and CKD and correlate with disease severity[107]; most importantly, increased VAP-1/ SSAO activity may contribute to NASH and CKD pathogenesis due to its dual pro-inflammatory and prooxidative functions(**supplementary Table 5**). Following pro-inflammatory stimuli, VAP-1 is rapidly translocated from the intracellular storage vesicles to the hepatic endothelial cell surface, where it functions as an adhesion molecule and contributes to extravasation and migration of immune cells to the site of inflammation[106,107]. Beside mediating inflammatory cell adhesion , VAP-1 catalyzes oxidative deamination of primary amines with the generation of potent pro-adhesive, pro-inflammatory and pro-fibrogenic endproducts, boosting local inflammation and fibrogenesis (**supplementary Table 5).** The comparison of models lacking both adhesion and enzymatic activity of VAP-1 with models lacking either catalytic or pro-adhesive activity of this protein demonstrated that both the proadhesive and enzymatic activity of VAP-1 promote tissue injury, but blockade of the enzymatic activity is sufficient to ameliorate NASH and fibrosis[107]. This may partially explain the proinflammatory and profibrotic effects of s-VAP-1 at distant extrahepatic sites like the kidney[108]. On this basis, two different strategies targeting the dual, enzymatic and proadhesive functionality of VAP-1 are under development: VAP-1-neutralizing mAbs suitable for therapeutic use appear to be well tolerated in preclinical models. Small molecule inhibitors of VAP-1 enzymatic activity, such as haloallylamines and alkylhydrazino compounds, have been described but advances in the design of these compounds have been hampered by lack of selectivity, poor pharmacokinetics, and class-dependent safety concerns such as the presence of hydrazine groups. Recently, PXS-4681A and PXS-4728A, two selective and irreversible SSAO/VAP-1 inhibitors, showed potent anti-

inflammatory and anti-fibrotic activity in preclinical models of CKD[109] and PXS-4681A is scheduled for a phase IIa RCT in NASH.

Targeting common fibrogenic pathways to reverse hepatic and renal fibrosis

Fibrosis is the pathological hallmark that most strongly correlates with the progression of hepatic and kidney disease and accordingly, is targeted by various pharmacological strategies to improve NASH and CKD.

Galectin-3 inhibitors

Galectin-3 is a lectin that is broadly expressed by immune and epithelial cells[26,27], where it regulates cell proliferation, apoptosis, cell adhesion and affinity for AGEs, exerting multiple and sometimes contrasting effects according to its cellular location, cell type and mechanism(s) of injury. Extracellular galectin-3 interacts with the β-galactoside units of ECM and cell surface glycoproteins: at cellular surface and forms multimers driven by increasing concentrations of glycoprotein ligands. These multimers trigger cell signaling and regulate cell adhesion and proliferation by interacting with cell surface adhesion molecules like integrins and with receptors of numerous growth factors[26,27].

In NASH, hepatic extracellular galectin-3 is overexpressed and exerts a proinflammatory effect, by promoting recruitment of circulating immune cells and AGEs uptake by Kupffer and endothelial cells[110] and a profibrotic effect by stimulating myofibroblast and HSC proliferation and hepatic progenitor cell expansion and differentiation[111].

In CKD glomerular galectin-3 expression correlates with renal function impairment[112], while tubular galectin-3 expression promoted tubulo-interstitial fibrosis[113]. Consistently, elevated circulating galectin-3 predicts incidence and progression of CKD[114,115]. Consistently, competitive small carbohydrate molecule galectin-3 inhibitors like GR-MD-02 (galactoarabino-rhamnogalaturonan), GM-CT-01 (galactomannan), and N-acetyllactosamine, prevented hypertensive nephropathy[116] and diet-induced NASH[117] and reversed established cirrhosis[118]. GR-MD-02 was safe and well tolerated and improved fibrosis markers in NASH patients with advanced fibrosis(www.clinicaltrials.gov.ID:

NCT01899859)[119] , while the galectin-3 antagonist GCS-100 is being evaluated in CKD (www.clinicaltrials.gov.ID**:**: NCT01843790)**.**

Several issues remain, however: galectin-3 deficiency exacerbated systemic inflammation and kidney injury, along with upregulation of the receptor for advanced glycation end products (RAGE), in response to overnutrition[120]. To explain this apparent paradox, it has been suggested that inhibition of AGE uptake by the liver, which clears >90% of these end-products from the circulation, promotes their systemic accumulation and uptake by RAGE by other tissues, thereby enhancing extrahepatic toxicity. Beside 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 and opposite role of extracellular and intracellular galectin-3.

Rho-associated protein kinase (ROCK) inhibitors

Rho-associated kinases (ROCKs) are serine/threonine kinases originally identified as downstream effectors of the small GTPase RhoA. ROCKs contain the serine/threonine kinase domain, highly related to the human myotonic dystrophy kinase, followed by a coiled-coil region containing the Rho-binding domain (RBD) and by a pleckstrin homology domain which reduces the kinase activity of ROCKs by an autoinhibitory intramolecular fold if RhoA-GTP is not present[121]. ROCKs consist of two isoforms with only partially overlapping functions and distinct tissue localization: ROCK1, abundantly expressed in the liver, kidney, lung, spleen and immune cells, and ROCK2, mainly found in the muscle, heart and brain. Various signals converge on and activate ROCKs by releasing these proteins from the autoinhibitory conformation, including Rho protein binding to RBD, lipid (arachidonic acid, phosphatidylinositol-phosphates) interaction with the pleckstrin homology domain, and enzymatic removal of the C terminus by caspase-3 or granzyme B[122]. While ROCKs play an important role in various fundamental cellular functions, including contraction, motility, proliferation, and apoptosis, inappropriate ROCK upregulation has been involved in proinflammatory, profibrotic and proatherosclerotic responses of epithelial and endothelial cells to tissue injury, and pharmacologic

ROCK inhibition emerged as an attractive therapeutic tool to prevent or reverse established fibrosis[122, 123] (**supplementary Table 6**). Consistently, fasudil, a nonselective dural ROCK1/2 inhibitor approved for the treatment of cerebral vasospasm, improved inflammation and established fibrosis in diet-induced NASH[122,123] and CKD[124,125]. Fasudil has low potency and selectivity as it inhibits also other kinases than ROCKs and more potent and selective ROCK inhibitors are under development: a phase 1 RCT with the ROCK1/2 inhibitor SAR407899 in patients with CKD has been completed, but resulta are not available (www.clinicaltrials.gov ID: NCT01485900).

A key issue to be elucidated for the future development of this pharmacological class will be the relative contribution of ROCK1 vs. ROCK2 isoform to the development of NASH and CKD, as all experimentally used agents were dual ROCK1/2 inhibitors: as an example, ROCK2 activation has been found to shift the balance between pro-inflammatory T-helper 17 cells and regulatory T-cell (Treg) toward a pro-inflammatory phenotype[126], but also to shift macrophage polarization towards a pro-resolving M2 phenotype[127](**supplementary Table 6**). The selective ROCK2 inhibitor KD025 is scheduled for a phase 2 RCT in NASH.

Epidermal Growth Factor (EGF) inhibitors

EGF belongs to the receptor tyrosine kinase (RTK) family, a group of single-transmembrane proteins comprising an extracellular ligand-binding domain and a linked cytoplasmic catalytic domain, that are activated by dimerisation of two RTK monomers or by autophosphorylation of the intracellular phosphatase domain[128]. EGF signaling pathway activation plays a key profibrogenic role in diverse chronic liver diseases, including NASH: in diverse rodent models of cirrhosis, the expression of EGFR significantly increases during liver fibrosis progression and promoted HSC activation, differentiation, proliferation and migration through activation of the extracellular signal-regulated kinases1/2 (ERK1/2), STAT, and the phosphatidylinositol- 3 kinase(PI3K)/Akt pathways[128,129].

Upregulated renal tubulo-interstitial EGFR expression has been observed in nearly all experimental models of CKD[130], correlates with tubulointerstitial fibrosis and predicts CKD progression[131].

On this basis, EGFR inhibitors are being evaluated for the treatment of fibrotic liver and kidney diseases. Erlotinib, a potent small-molecule EGF receptor (EGFR) inhibitor, currently approved for lung and pancreatic cancer, prevented cirrhosis progression, reversed fibrosis and attenuated HCC development in diverse rodent models of liver fibrosis[129] and ameliorated experimental glomerulonephritis[132]. An undergoing trial is evaluating the effects of erlotinib on fibrogenesis inhibition and HCC recurrence prevention in cirrhotic patients(www.clinicaltrials.gov ID: NCT02273362). Plumbagin, an extract from the roots of traditional medicinal plant Plumbago zeylanica, ameliorated experimental CCl_4-induced hepatic fibrosis via the EGFR signaling pathway inhibition[133].

Conclusions and future perspectives

Growing epidemiological evidence suggests NASH and CKD share common pathogenic pathways and mechanisms of progression, which are only marginally affected by current therapeutic approaches, including SLKT4, resulting in unacceptably high morbidity and public heatlhcare costs. A low awareness of the striking pathophysiological analogy between these 2 conditions may delay the accomplishment of an affective therapeutic stategy to slow progression of both disease conditions.

We found a wealth of cellular pathways and mechanisms involved in both liver and kidney injury and whose pharmacological modulation gave promising results in late preclinical or early clinical stadies. Remarbaly, few of these pharmacological options are at the same stage of development in NASH and CKD, reflecting a low awareness of the shared pathogenic similarities between these 2 condition.

The knowledge of pathogenic and therapeutic analogies between NASH and CKD may boost clinical research to find an effective treatment for liver and kidney disease in NASH-related CKD, which may retard progression of both disease conditions, whose prevalence and heatlhcare costa are increasingly exponentially

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Author Contributions.

Giovanni Musso: conceived and designed the article, undertook literature search and acquired data, critically analyzed the results, drafted the article, gave final approval

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ONLINE-ONLY SUPPLEMENTARY APPENDIX

Table 1. Mechanisms connecting ACE2-Angiotensin((1-7)-Mas receptor axis **modulation to liver and renal disease in NASH and CKD**

ACE2-Angiotensin((1-7)-Mas receptor axis	
Cellular mechanism	**Biological effect**
Hepatocyte: **↓SREBP-1c activation→↓ *de novo* lipogenesis** **↑ ROS production by mitochondria and NOX** **↓ NF-κB axis activation, TNF-α, resistin and IL-6 secretion** **↓ TLR4 expression→↓ sensitivity to circulating LPS and FFA** **↓ MAPK activation→↑ insulin signaling** ↑Akt/PI3K/IRS-1/JNK insulin signaling **Hepatic stellate cell:** ↓ ERK-1/2 phosphorylation→↓ **TGF-β production →↓ HSC activation**	↓ oxidative stress ↓ steatosis ↓ inflammation ↓ fibrosis ↑ insulin sensitivity
Adipocyte **↓ NF-κB axis activation, TNF-α, IL-6 and resistin secretion** ↑ GLUT4 expression ↑ SIRT1-AMPK/FOXO1 axis activation→↑ fat oxidation, ↓ SREBP-1c-mediated lipogenesis	↓adipose tissue inflammation ↑ insulin sensitivity ↓fat mass
Kidney: **Glomerular arterioles, mesangial cells, podocytes:** **↑NO synthesis→Efferent artery vasodilation→↓ glomerular hypertension→ ↓endothelial cell injury** **↓ NOX activation→↓ ROS production** **↓ mesangial expansion→↓ glomerulosclerosis** **Proximal tubule cell:** **↓ Na reabsorption** **↓NOX activation→↑ ROS production** ↓ NF-κB axis activation→↓ TNF-α, IL-1β, MCP-1 and CCL5 secretion→↓ monocyte recruitment	**↓ proteinuria** ↓ oxidative stress ↓renal inflammation ↓ tubulo-interstitial fibrosis

↓ ERK-1/2 phosphorylation→↓ **TGF-β production →↓ fibrogenesis** ↓TGF-β/Smad2/3 signaling→↓ **fibrogenesis**	

Abbreviations: ACEIs: angiotensin converting enzyme inhibitors; ACO-1: acyl-CoA oxidase-1; AdipoR: adiponectin receptor; AMPK: adenosine-monophosphate kinase; **ARBs: angiotensin receptor blockers;** ATP: adenosine triphosphate; CCL5: Chemokine (C-C motif) ligand 5; CPT-1: carnitine palmitoyltransferase-I**;** EGFR: epithelial growth factor receptor; EMT: epithelial-to-mesenchymal transition; ERK: extracellular signal-regulated kinase;; ERK: extracellular signal-regulated kinase; -FFA: free fatty acids; HSC: hepatic stellate cell; IL-6: interleukin-6; IRS-1: insulin receptor substrate-1; JAK: Janus kinase; LPL: lipoptorein lipase; LPS: lipopolysaccharide; MCP-1: monocyte chemotactic protein-1; NO: nitric oxide; NOX: NADPH oxidase; NPY: neuropeptide Y; PAI-1: plasminogen activator inhibitor-1; PI3-K: phosphoinositide 3-kinase; PKC: protein kinase C; POMC: proopiomelanocortin; PPAR: peroxisome proliferators-activated receptor; ROS: reactive oxygen species; SREBP: sterol-responsive element binding protein; STAT3: signal transducer and activator of transcription-3; Tg: triacylglycerol; TGF-β: transforming growth factor- β.

Table 2. Role of nuclear transcription factors in the pathogeness of NASH and CKD

PPAR-α		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatocyte, Proximal tubule cells ↑ mitochondrial and peroxisomal FFA ß-oxidation ↑FFA ω-oxidation ↑ ketogenesis ↑ FATP/CD36/L-FABP activity ↑LPL activity and ↓ apoC-III synthesis ↑apo-AI/apo-AII synthesis	↑ FFA oxidation ↑ FFA uptake ↑ VLDL lipolysis ↑ HDL synthesis	↓ **hepatic steatosis and insulin resistance** ↓ **plasma Tg** ↑ HDL-C levels
Hepatocytes ↑catalase activity ↓ NF-κB pathway activation ↓ ICAM-1/VCAM-1 expression, ↑ CREBH-mediated FGF21 expression	↑H_2O_2 detoxification ↓ proinflammatory cytokine secretion ↓ endothelial dysfunction ↑ metabolic effects of FGF21	↓ **inflammation and ahterosclerosis**
Mesangial cells ↓ **TGF-ß1 and TGF-ß1 receptor II expression**	↓ **matrix deposition**	↓ **glomerulosclerosis**
PPAR-β(δ)		
Hepatocyte, proximal tubule cell, mesangium ↑ mitochondrial β-oxidation ↑ABCA1 expression	↑ FFA oxidation ↑ cholesterol efflux	↓ hepatic steatosis ↑ HDL-C levels
Macrophage, Kupffer cell, tubule cell Switch to a pro-resolving M2 phenotype ↓NF-κB pathway activation and TGF-ß1 secretion ↓NLRP3 inflammasome activation	↓ proinflammatory cell recruitment ↓ fibrogenesis	↓ **inflammation and fibrosis**
Adipocyte, miocyte ↑ PGC-1α-mediated mitochondrial biogenesis, ß-oxidation and UCP-1/3 expression	↑fat oxidation and EE	↓ body fat and plasma Tg

↑ LPL expression	↑ VLDL lipolysis	
Mesangial cell ↓ p38 MAPK pathway activation	↓ **matrix deposition**	↓ **glomerulo-sclerosis**
Podocyte ↑ nephrin expression and podocyte integrity ↓ RAGE expression	↑ **glomerular barrier integrity**	↓ **albuminuria**
PPAR-γ		
Adipocyte ↑**s**ubcutaneous preadipocyte differentiation ↑apoptosis of visceral adipocytes ↑insulin signalling and adiponectin secretion ↑GLUT1/GLUT4 translocation ↓release of toxic FFAs and pro-inflammatory cytokines	↑insulin sensitivity, glucose disposal and FFA oxidation ↓ inflammation	subcutaneous fat storage ↓ hepatic steatosis
Skeletal miocyte, hepatocyte ↑GLUT1/GLUT4 translocation ↓gluconeogenesis	↑insulin sensitivity, glucose disposal and FFA oxidation	
Macrophage Kupffer cell, proximal tubule cell Switch to an anti-inflammatory M2 phenotype ↓NF-*κ*B pathway activation	↑ release of pro-inflammatory cytokines	↓ inflammation
HSC, proximal tubule cell, renal fibroblast ↓TGFβ-1/Smad3 signaling pathway activation Induction of cell apoptosis	↓ fibrogenesis	↓ fibrosis
Glomerular podocyte and endothelial cell ↓ RAGE expression→↓ apoptosis ↓ mitochondrial dysfunction ↓iNOS, COX-2 and PAI-1 expression	↑ **glomerular barrier integrity**	↓ **albuminuria**
Mesangial cells ↓ RAGE expression	↓ **matrix deposition**	↓ **glomerulosclerosis**
Farnesoid X receptor (FXR)		
Hepatocyte, mesangial and tubule cells ↓ SREBP-1c-mediated lipogenesis	↓ lipid accumulation	↓ **ectopic fat deposition**

↑ PPAR-α-mediated FFA β-oxidation		
Hepatocyte, glomerular endothelial cell, proximal tubule cell ↑DDAH-1 and CAT-1 expression→↑ADMA catabolism	↑eNOS activity ↓ER stress ↓ **endothelial dysfunction and insulin resistance**	↓ hepatic and renal necrosis and fibrogenesis ↓Glomerular/tubule-interstitial ischemia
Hepatocyte, renal glomerular endothelial and proximal tubule cell ↓ eNO synthase →↓ NO production→ ↑ endothelial dysfunction ER stress→↑ cellular apoptosis, TGF-β production	Hepatic steatosis and necroinflammation Insulin resistance	hepatic and renal necrosis and fibrogenesis
Hepatocyte ↓ hepatic gluconeogenesis ↑ IRS-1 phosphorylation ↓ ApoC-III and apoA-1 synthesis ↑ ApoC-II synthesis ↓CYP7A1 ↑ ABCG5/G810	↓ synthesis of HDL-C ↑ clearance of VLDL ↓ bile acid synthesis ↑ cholesterol excretion into bile	↓ **insulin resistance** ↓ **plasma HDL-C** ↓ **plasma triglyceride**
Renal macrophage, Kupffer cell ↓ IκB-α phpsphorylation→↓ NF-κB activation ↓ monocyte chemotatic protein(MCP)1 ↑ KLF-2/KLF-4 expression ↓ TGF-ß secretion	↓ proinflammatory cytokine secretion ↓ fibrogenesis	↓ inflammation and fibrosis
hepatic stellate cell,, renal myofibroblast ↓ expression of TGF-ß-receptor	↓ fibrogenesis	
Podocyte ↓ apoptosis ↑podocin and synaptopodin expression	↑ glomerular barrier function	↓ proteinuria
Mesangial cells ↓ NADPH oxidase(Nox)	↓ oxidative stress ↓ glomerulosclerosis	↓ glomerular

↑MnSOD expression ↓ mesangial matrix deposition		hypeetrohy
Adipocyte ↑PPAR-γ expression ↑ AdipoQ expression ↓ TNF-α secretion	↓ adipose tissue dysfunction	↓ proteinuria

Abbreviations:

ABCA1: ATP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase; ADMA asymmetric dimethylarginine;: **CAT:** cationic amino acid transporter; AP-1: activation protein-1**; CA: cholic acid; CDCA.** chenodeoxycholic acid; CD36: cluster of differentiation-36; CHOP: C/EBP homologous protein; DDAH: dimethylarginine dimethylaminohydrolases ; EMT: epithelial-to-mesenchimal transition; HSC: hepatic stellate cell; **IRS: insulin receptor substrate; JNK:** c-Jun N-terminal kinase; **MAPK: mitogen-activated protein kinase;** PDCD4: programmed cell death protein 4; RECK. Reversion-inducing cysteine-rich protein with Kazal Motifs; **Smad:** Mothers against decapentaplegic homolog ;Trx: thioredoxin; VLDLR: VLDL receptor.

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; 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; NLRP3: NOD-like receptor family, pyrin domain containing 3; NO: nitric oxide; NOX: NADPH oxidase; OCA: obeticholic acid; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1 α; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SCD-1: stearoyl-CoA desaturase-1; SR-A1: scavenger receptor-A1; SR-B1: scavenger receptor-B1; SREBP: sterol-responsive element binding protein; 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;

Table 3. Role of miRNAs and Heat Shock Proteins(HSPs) in the pathogeness of NASH and CKD

miRNA-103/107		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatocyte, adipocyte ↓ caveolin-1 expression→↓ stability of caveolae ↓ mitochondrial β-oxidation enzyme HADHA ↑ ER stress	↓ stability of insulin receptor ↓ FFA oxidation Inflammation	Insulin resistance fat accumulation Adipose tissue dysfunction
miRNA-21		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Tubular cell, podocyte, mesangial cell, hepatocyte, Macrophage, HSC **↓PPAR-α, ↑ SREBP-1c activation** ↓ mitochondrial Mpv17l expression→↑ ROS generation →↓ PDCD4/AP-1 →↓ metalloproteinase inhibitor RECK→ ↑ inflammatory cell migration, invasion and angiogenesis →↓**Smad-7 expression**→↑ NF-κB pathway →↓**PTEN activity**→↑**PI3K and Akt activity** **Adipocyte** ↑ **adipogenic differentiation of mesenchimal stem cells**	**Lipid accumulation** **Oxidative stress** **Podocyte apoptosis** **Mesangial cell proliferation** **Inflammation** **EMT transition,** **HSC activation**	**Albuminuria, tubulointersititial fibrosis, hepatic steatosis, inflammation and fibrosis**
Heat shock protein 90(HSP90)		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**

Hepatic stellate cells, renal tubulointerstitial cells, mesangium: ↓TGF-βRII degradation by ubiquitin-proteasome system GR binding→liberation and nuclear translocation of NF-κB Aκt activation→NF-κB nuclear translocation STAT activation→↑ **proinflammatory cytokine/chemokine TNF-α/CCL2/CCL5 secretion**	**↓ apoptosis** **↑ activation and collagen deposition** **EMT** **↑ inflammatory cell ativation and recruitment**	**Inflammation** **fibrosis**
Heat shock protein 47(HSP47)		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatic stellate cell, Myofibroblast, renal tubule cells ↑ collagen maturation, biosynthesis and secretion	**↑ fibrogenesis**	**fibrosis**

Abbreviations:

ABCA1: ATP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase; ADMA asymmetric dimethylarginine;: **CAT:** cationic amino acid transporter; AP-1: activation protein-1**; CA: cholic acid; CDCA.** chenodeoxycholic acid; CD36: cluster of differentiation-36; CHOP: C/EBP homologous protein; DDAH: dimethylarginine dimethylaminohydrolases ; EMT: epithelial-to-mesenchimal transition; GR: glucocorticoid receptor; HSC: hepatic stellate cell; **IRS: insulin receptor substrate; JNK:** c-Jun N-terminal kinase; **MAPK: mitogen-activated protein kinase;** PDCD4: programmed cell death protein 4; RECK. Reversion-inducing cysteine-rich protein with Kazal Motifs; **Smad:** Mothers against decapentaplegic homolog ;Trx: thioredoxin; VLDLR: VLDL receptor.

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; 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; NLRP3: NOD-like receptor family, pyrin domain containing 3; NO:

nitric oxide; NOX: NADPH oxidase; OCA: obeticholic acid; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1 α; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SCD-1: stearoyl-CoA desaturase-1; SR-A1: scavenger receptor-A1; SR-B1: scavenger receptor-B1; SREBP: sterol-responsive element binding protein; STAT: signal transducers and activators of transcription;TGF-β: transforming growth factor- β; TGFβRII: TGF-β type II receptor; TLR: toll-like receptor; TNF: tumor necrosis factor; TZD: thiazolidinediones; VLDL: very low density lipoprotein; VSCMs: vascular smooth muscle cells;

Table 4. Role of effectors of redox regulation in the pathogenesis of NAFLD and CKD

NADPH Oxidase (NOX)1/4		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatocyte: **↑ JNK activation→apoptosis, secretion of proinflammatory mediators** **Kupffer cells:** **↑ secretion of proinflammatory mediators and of TGF-ß** **Hepatic stellate cells:** **↑ proliferation and collagen deposition**	**Hepatocyte death** **Inflammatory infiltration** **fibrogenesis**	**Necroinflammation** **fibrosis**
Podocyte, mesangium: ↑ podocyte apoptosis and foot process effacement ↓ nephrin expression ↑ MCP-1 and PKC-α expression **↑fibronectin expression** **↓mitochondrial respiratory chain function→ROS generation** **Endothelium** **eNOS uncoupling→↓NO availability, ROS generation** **Proximal tubule cells, interstitial fibroblasts:** ↑ p38MAPK→EMT Fibroblast activation to myofibroblasts	**Endothelial dysfunction** **Matrix deposition** **fibrogenesis**	**Proteinuria** **Glomerulosclerosis** **Tubulointerstitial fibrosis**

Apoptosis Signal-Regulating Kinase 1(ASK1)		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatocyte, mesangium, tubular cells: **ROS→ASK1 dissociation from Trx and activation→activation of MAP2Ks (**MKK4/7 and MKK3/6)→activation of MAPKs JNK and p38	proinflammatory cytokines/chemokine secretion, ECM remodeling cell death(apoptotic and non-apoptotic), IRS-1 phosphorylation	**Necrosis** **Inflammation and fibrosis** **Insulin resistance**
Nuclear erythroid 2-related factor 2 (Nrf2)		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatocyte, macrophage, glomerular endothelial cells, tubule cells: ↑ Antioxidant proteins: Glt-R, Glt-Px, TXN-R, Cat, ↑ Phase I oxidation, reduction and hydrolysis enzymes: ALDH3A1, EPHX1, NQO1 ↑ Phase II detoxifying enzymes: GST, MGST: UGT, PSMB5 ↑NADPH-generating Enzymes: G6PD: ↑ Heme metabolism HO-1 ↑Protein degradation: UbC, PSMB5 ↑ autophagy ↓ IκB-α phpsphorylation→↓ NF-κB activation ↓ iNOS and COX-2 ↑ FGF21 secretion by hepatocyte	↓ oxidative stress ↓ xenobiotic toxicity ↓ inflammation ↓ podocyte loss ↓ EMT of tubule cells ↓ endothelial dysfunction	↓ ectopic fat deposition ↓ hepatic infoammation and fibrosis ↓ albuminuria and tubulo-interstitial fibrosis ↓ CVD risk
Mesangial cells, HSCs: ↓ phosphorylation and nuclear translocation of	↓ fibrogenesis and	↓ **hepatic fibrosis**

Smad3 →↓TGF-β signaling pathway activation ↓ PAI-1 expression	glomerulosclerosis	**and glomerular hypertrophy**

Abbreviations:

AP-1: activation protein-1**;** EMT: epithelial-to-mesenchimal transition; HSC: hepatic stellate cell; **IRS: insulin receptor substrate; JNK:** c-Jun N-terminal kinase; **MAPK: mitogen-activated protein kinase;** PDCD4: programmed cell death protein 4; RECK. Reversion-inducing cysteine-rich protein with Kazal Motifs; **Smad:** Mothers against decapentaplegic homolog ;Trx: thioredoxin **ABCA1: A**TP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase; *ALDH3A1* Aldehyde dehydrogenase 3A1*Cat:* Catalase; CDDO: 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oicacid; DMF: dimethylfumarate,Glt-Px: Glutathione peroxidase; Glt-R: Glutathione reductase ; *G6PD:* Glucose-6-phosphate 1-dehydrogenase; HO-1: Heme oxygenase-1; *TXN-R: GST:* Glutathione *S*-transferase, Thioredoxin reductase; *SOD* Superoxide dismutase; *EPHX1* Microsomal epoxide hydrolase 1 *MGST:* Microsomal glutathione *S*-transferase, NK-252: (1-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)-3-(pyridin-2-ylmethyl)urea).*NQO1* NAD(P)H:quinone oxidoreductase; *PSMB5:* Proteasome 26S PSMB5 subunit; tBHQ: tert-Butylhydroquinone; *UbC* Ubiquitin C; : *UGT:* UDP glucuronosyltransferase; AICAR: 5-Aminoimidazole-4-carboxamide-1-β-D- ribonucleoside; AMPK: adenosine-monophosphate kinase; **CA: cholic acid; CDCA.** chenodeoxycholic acid; 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; 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; NLRP3: NOD-like receptor family, pyrin domain containing 3; NO: nitric oxide; NOX: NADPH oxidase; OCA: obeticholic acid; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator-1 α; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SCD-1: stearoyl-CoA desaturase-1; SR-A1: scavenger

receptor-A1; SR-B1: scavenger receptor-B1; SREBP: sterol-responsive element binding protein; 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; VSCMs: vascular smooth muscle cells; PON-1: paraoxonase-1, NOS: NO synthase, LCAT: lecithin cholesterol acyltransferase;

Table 5. Role of Vascular Adhesion Protein-1(VAP-1) in the pathogenesis of NAFLD and CKD

Vascular Adhesion Protein(VAP)-1		
Cell type and molecular pathways	**Cellular effect**	**Biological effect**
Hepatic endothelium, adipocytes: Binding to Siglec-10→↑ adhesion, extravasation and migration of T lymphocytes, NK cells, granulocytes, proinflammatory monocytes **Hepatic entothelium, renal vessels and tubule cells**: Oxidative deamination of methylamine and aminoacetone→production of H2O2, formaldehyde, methylglyoxal, ammonia→ 1)↑ NFκB-dependent chemokine secretion 2)↑ expression of adhesion molecules (E-selectin, ICAM-1, VCAM-1) 3)↑ROS generation following the conversion of H2O2 to hydroxyl free radicals 4)HSC activation 4) ↑ MCP-1 and TGF-ß secretion	**Inflammatory cell recuitment** **fibrogenesis**	hepatic infoammation and fibrosis tubulo-interstitial fibrosis

Abbreviations: ACC, acetyl-CoA carboxylase; DGAT2, diacylglycerol acyltransferase 2; EGFR: epidermal growth factor receptor; FAS, fatty acid synthase; HSC: hepatic stellate cell; HSL: hormone-sensitive lipase; MCP-1, monocyte chemotactic protein-1; SCD-1, stearoyl-CoA desaturase 1; SREBP1c, sterol regulatory elementbinding protein 1c;

Table 6 Role of Rho-associated protein kinase (ROCK)-1 and ROCK-2 in the pathogenesis of NAFLD and CKD.

Rho-associated protein kinase (ROCK)-1	
Cellular mechanism	**Biological effect**
Hepatocyte plasma membrane blebbing→TRAIL-bearing EV release→macrophage activation	**hepatic and renal inflammation and fibrogenesis**
Glomerular podocytes, mesangium, tubular cells ↑ TGF-β-mediated EMT ↑VEGF, VEGFR1, VEGFR2 and fibronectin expression	**glomerulosclerosis** **albuminuria**
Endothelial cells ↓ eNOS activity→↓NO availability→ **endothelial dysfunction**	**atherosclerosis, hypertension**
Adipocyte ↓ IRS1/PIP3/Aκt signaling	**insulin resistance**
Rho-associated protein kinase (ROCK)-2	
Cellular mechanism	**Biological effect**
T cells ↑STAT3/STAT5 phosphorylation and activation→↑Th17/treg balance→↑ proinflammatory IL-17/IL-23 and ↓ antiinflammatory IL-2/IL-10 secretion	↑**T-cell mediated inflammation**
Monocyte ↓ M1/M2 ratio polarization	↓ macrophage-mediated inflammation
	endothelial dysfunction
	hepatic and renal inflammation and fibrogenesis

Abbreviations: AGE: advanced glycation end-products; AMP: adenosine-monophosphate; AMPK: adenosine-monophosphate kinase; **AngII: angiotensin II;** BNP: brain-derived natriuretic peptide; cAMP: cyclic adenosine-monophosphate; DPP-4: dipeptidyl peptidase protein-4; ERK: extracellular

signal-regulated kinase; EV: extracellular vescicles; FFA: free fatty acids; FGF: fibroblast growth factor; GLP-1: glucagon-like peptide-1; IL: interleukin; IRS-1: insulin receptor substrate-1; LPL: lipoptorein lipase; LPS: lipopolysaccharide; MCP-1: monocyte chemotactic protein-1; NHE3: Na+-H+ exchanger 3; NF-κB: nuclear factor-κB; NO: nitric oxide; NOX: NADPH oxidase; NPY: neuropeptide Y; PPAR: peroxisome proliferators-activated receptor; PYY: peptide YY; RAAS: rennin-angiotensin-aldosterone system; ROS: reactive oxygen species; **SDF-1a: stromal-derived Factor;** SIRT: sirtuin; STAT: signal transducer and activator of transcription; TGF-β: transforming growth factor- β; TLR: toll-like receptor; TMA: trimethylamine; TNF: tumor necrosis factor; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; VLDL: very low density lipoprotein.

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