

Recent advances in drug discovery for diabetic kidney disease

Chhanda Charan Danta¹, Andrew N. Boa², Sunil Bhandari³, Thozhukat Sathyapalan⁴, Shang-Zhong Xu¹

¹Centre for Atherothrombosis and Metabolic Disease, Hull York Medical School, University of Hull, Hull HU6 7RX, United Kingdom.

²Department of Chemistry, University of Hull, Hull HU6 7RX, United Kingdom.

³Department of Renal Medicine and Hull York Medical School, Hull Royal Infirmary, Hull University Teaching Hospitals NHS Trust, Hull HU3 2JZ, United Kingdom.

⁴Academic Diabetes, Endocrinology and Metabolism, Hull York Medical School, University of Hull, Hull HU6 7RX, United Kingdom.

Running title: Drug Discovery for Diabetic Kidney Disease

Corresponding author:

Dr C.C. Danta

Centre for Atherothrombosis and Metabolic Disease, Hull York Medical School, University of Hull, Hull HU6 7RX, United Kingdom. E-mail: ccdanta2011@rediffmail.com

Dr S.Z. Xu

Centre for Atherothrombosis and Metabolic Disease, Hull York Medical School, University of Hull, Hull HU6 7RX, United Kingdom. Phone: 01482 465372. E-mail: sam.xu@hyms.ac.uk

ABSTRACT

Introduction: Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease (ESRD), and 40% of patients with diabetes develop DKD. Although some pathophysiological mechanisms and drug targets of DKD have been described, the effectiveness or clinical usefulness of such treatment has not been well validated. Therefore, searching for new targets and potential therapeutic candidates has become an emerging research area.

Areas covered: The pathophysiological mechanisms, new drug targets and potential therapeutic compounds for DKD are addressed in this review.

Expert opinion: Although preclinical and clinical evidence has shown some positive results for controlling DKD progression, treatment regimens have not been well developed to reduce the mortality in patients with DKD globally. Therefore, the discovery of new therapeutic targets and effective target-based drugs to achieve better and safe treatment is urgently required. Preclinical screening and clinical trials for such drugs are needed.

Keywords: diabetic complications, diabetic nephropathy, drug discovery, endothelin receptor blockers, oxidative stress, renin-angiotensin-aldosterone system, SGLT-2 inhibitors

Article highlights.

- Diabetic kidney disease (DKD) is the single most common cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), which is clinically characterized by persistent albuminuria of 30-300mg/day.
- Multiple pathophysiological mechanisms have been proposed; therefore, the treatment could be difficult with single-drug therapy.
- Current treatment of DKD is not able to stop its pathological progression completely and reduce mortality rate. Novel target discovery and therapeutic strategy development are urgently required.
- This article mainly focuses on new potential drug targets and related new compounds for the potential treatment of DKD.

The box summarizes key points contained in the article.

1. Introduction

Diabetic kidney disease (DKD), can be defined as diabetes with persistent microalbuminuria (ratio of urine albumin to creatinine $\geq 30\text{mg/g}$) and impaired glomerular filtration rate ($<60\text{mL/min/1.73m}^2$) leading to the deterioration of renal function and ultimately morbidity and mortality of patients¹⁻³. Albuminuria is the characteristic clinical biomarker of DKD observed in patients with both type 1 and type 2 diabetes mellitus (T1DM and T2DM). In 2013, the Global Burden of Disease Study reported a systematic analysis and estimated that the age-standardized mortality from DKD had increased by 106% compared to the year 1990⁴. However, a recent study has found that DKD prevalence is not so stable and the death rate is increasing at the global level⁵. As the prevalence of both T1DM and T2DM patients is increasing, the incidence of macro- and microvascular complications, such as stroke, coronary heart disease, diabetic retinopathy, ischaemic nephropathy and kidney interstitial fibrosis, is also significantly increased⁶. The pathophysiological consequences of DKD under hyperglycaemia have been described in Figure 1. The current therapeutic strategy for DKD is

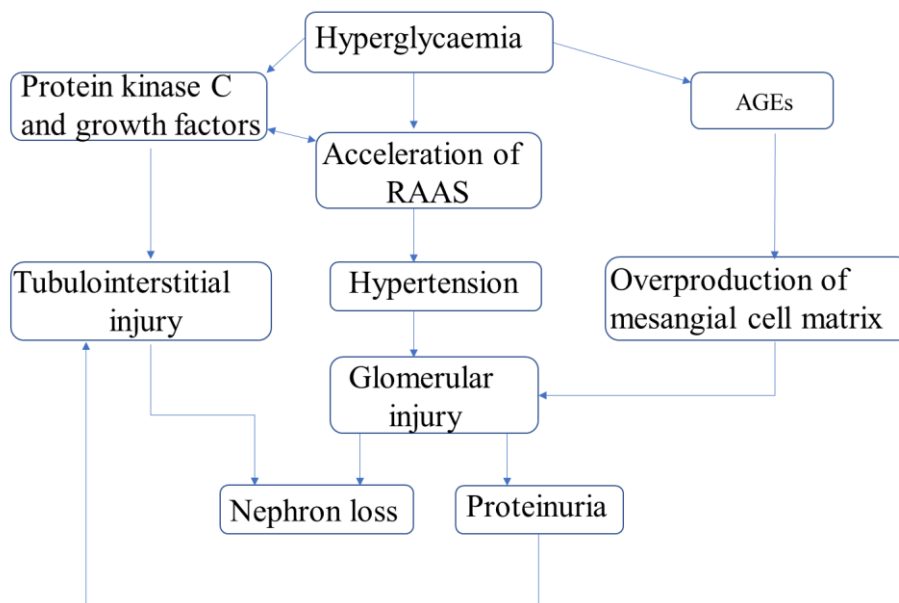


Figure 1. The pathological consequences of hyperglycaemia in DKD. RAAS: renin angiotensin aldosterone system; AGEs: advanced glycation end products.

to control proteinuria, hyperglycaemia, and high blood pressure to reduce cardiovascular and renal failure risk. Inhibition of the renin-angiotensin-aldosterone system (RAAS) and sodium-glucose cotransporter-2 (SGLT-2) has significant clinical outcomes such as reduction in renal progression, all-cause mortality, proteinuria, and cardiovascular events^{7,8}. A clinical trial with empagliflozin (EMPA-KIDNEY (the study of heart and kidney protection with empagliflozin) is ongoing (WWW.ClinicalTrials.gov, Identifier: NCT03594110). These treatments are promising for DKD; however, it is still proving difficult to halt the progression of DKD in patients mainly due to genetic and epigenetic variabilities^{6,9}. Therefore, development of new therapeutic strategies is required to target the underlying pathophysiological mechanisms. Some new drug targets have been recently identified, which could be helpful to develop new drugs to prevent renal and vascular damage in patients with DKD. Development of personalized medicine based on genetic and epigenetic variabilities could also be an approach to improve renal and cardiovascular outcomes in patients with DKD. In this review, we have covered recent advances in the pathophysiological mechanisms, potential new therapeutic targets and candidates for DKD.

2. Pathophysiological mechanisms

Multiple pathophysiological mechanisms are involved in the pathogenesis of DKD, including genetic and epigenetic aspects, abnormal renal haemodynamics, mitochondrial dysfunction and oxidative stress, ischaemia and inflammation, and podocyte injury and autophagy suppression. A schematic diagram for the mechanisms and possible drugs and their targets is shown in Figure 2.

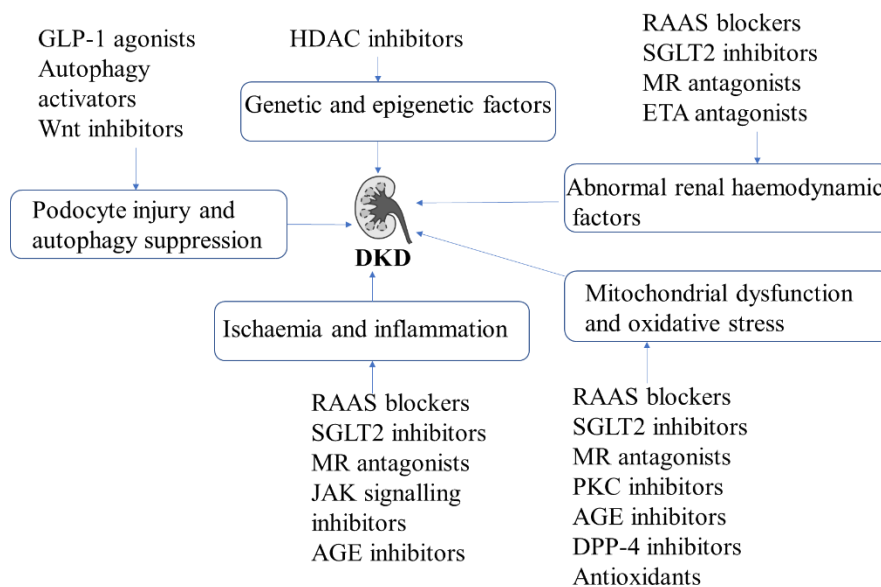


Figure 2. A schematic diagram describing the pathophysiological mechanisms of DKD and the novel therapeutic approach for its treatment. HDAC: histone deacetylases; RAAS: renin angiotensin aldosterone system; SGLT2: sodium glucose cotransporter 2; MR: mineralocorticoid receptor; ETA: endothelin receptor A; PKC: Protein kinase C; AGE: advanced glycation end products; DPP-4: dipeptidyl peptidase-4; JAK: Janus kinase; GLP-1: glucagon-like peptide-1.

2.1. Genetic and epigenetic aspects of DKD development

During the past 20 years, substantial evidence has been collected to understand the genetic basis of DKD in both T1DM and T2DM patients, such as familial clustering of DKD, the heritability and DKD -related traits, gene mapping for DKD in the genome-wide association study (GWAS)^{10,11}. It has been found that not all diabetic patients have developed DKD due to

the difference in genetic susceptibility even though glycemia is not regularly controlled¹². Due to the variation in genetic predisposition, the prevalence of DKD is higher in certain ethnic groups such as in native Americans, African Americans, and Mexican Americans than in other populations like European Americans¹⁰. The genes such as APOL1, RND3/RBM43, SLITRK3, ENPP7, and GNG7 make African American populations more prevalent to DKD^{10, 13}. Identification of such risk genes is a direction for future personal medicine.

The epigenetic modification including histone posttranslational modifications (PTMs), non-coding RNAs and methylation of DNA, may influence the development of DKD. Some pathologic conditions can modify the epigenetic profiles such as hyperglycaemia, hypoxia, inflammation, and release of some inflammatory cytokines^{9, 14, 15}. The importance of DNA methylation on regulating DKD-related genes has been reported¹⁵, and the different degrees of DNA methylation has been demonstrated in T2DM patients with or without DKD¹⁶. The gene, UNC13B, has been reported to involve in the initial pathogenesis of DKD and cause apoptosis of glomerular cells in hyperglycaemic condition^{16, 17}. The gene, RASALI, was found to be involved in hypermethylation, and therefore increased Ras activation in fibroblasts and led to tissue proliferation and fibrosis¹⁸. A case-control association study on blood-derived DNA from T1DM patients has revealed distinct methylation patterns in genes which were related to mitochondrial function and confirmed the influence of DNA methylation in DKD progression¹⁹. Epigenetic modifications in the kidneys of db/db T2DM diabetic mice have been well studied. Hypomethylation was reported for SGLT2, PCK1 and G6PC genes that capable of encoding proteins which involved in glucose metabolism as well as the gene that encodes hepatocyte nuclear factor 4 alpha (HNF4A), a transcription factor, which regulates transporters for reabsorption in the proximal tubule²⁰. The persistence of abnormal DNA methylation pattern was observed in db/db mice when pioglitazone was administered to reduce blood glucose levels and albuminuria, suggesting an important clue of epigenetic metabolic memory in the progression of DKD²⁰. Histone methylation is also involved in the gene expression of transforming growth factor-beta 1 (TGF- β 1) mediated extracellular matrix (ECM)²¹. Applications of histone deacetylase (HDAC) inhibitors such as sodium butyrate, trichostatin, valproate and vorinostat in the streptozotocin (STZ)-induced diabetic rats have shown significant protective effects by decreasing proteinuria, glomerular injury, oxidative stress, fibrosis, and inflammation²²⁻²⁴. Furthermore, microRNAs (miRNAs) are abundantly expressed in the kidney. These small non-coding RNAs are involved in the degradation of mRNA and promote translation repression, which have been shown to affect DKD progressing²⁵. In the early stage of DKD, an increased level of miRNA-192, a key gene regulator, was reported²⁶. The induction of miRNA-192 in DKD was mediated by histone acetylation²⁷. In mesangial cells, miRNA-192 up-regulates collagen type II (COLA2) and collagen type IV alpha 1 (COL4A1), and promotes the autoregulation of TGF- β 1 by regulating other miRNAs and amplifying the response of p53 to TGF- β 1²⁷⁻²⁹. Furthermore, miRNAs and long non-coding RNAs (lncRNAs) have been reported to play an essential role in the regulation of gene expression in DKD, such as the inhibition of lnc-MGC showed reduction of hypertrophy as well as glomerular extracellular matrix accumulation in diabetic mice³⁰. Nuclear factor (erythroid-derived 2)-like 2 (NRF2) is a transcription factor which is reported to have protective effects in diabetes by neutralizing reactive oxygen species (ROS) through the activation of the intracellular antioxidation systems and thus maintaining the balance of redox homeostasis^{31, 32}, the inhibition of TGF- β 1 and downregulation of cyclin-dependent kinase inhibitor 1A, and decrease of ECM production³³. More studies are required to understand the role of non-coding RNAs that could serve as molecular modulators in DKD. Collectively, these studies provide evidence that genetic difference and epigenetic modifications could explain the variability of drug responses and the molecular mechanisms involved in DKD progressing, which could pave the way for the development of personalized medicine.

2.2. Abnormal renal haemodynamics

Hyperfiltration by the glomeruli leads to the occurrence of DKD. Hyperglycaemia causes chronic metabolic and hemodynamic changes by modulating signalling pathways, transcription factors, cytokines, chemokines, and growth factors^{9, 13, 34, 35}. It also leads to afferent arteriolar dilatation by increasing the release of vasoactive mediators, such as insulin-like growth factor 1 (IGF-1), glucagon, nitric oxide (NO), vascular endothelial growth factor (VEGF) and prostaglandins⁹. Due to the haemodynamic changes in diabetic patients, they may predispose DKD through both episodes of acute kidney injury and chronic progression³⁶. Furthermore, upregulation of SGLT-2 in poorly glycaemic controlled diabetic patients was reported, which causes hyperfiltration of glucose as well as increased reabsorption of both glucose and sodium in proximal tubule³⁷. Hence, a small amount of sodium reaches the macula densa, causing constriction of the afferent arteriole via tubuloglomerular feedback. Simultaneously, the efferent arteriole also constricts due to the high levels of angiotensin II (Ang II), causing glomerular hyperfiltration and hypertension³⁸. Recently, it has been reported that Ang-II mediates MYH9 (a gene expressed in podocytes and involved in glomerular pathophysiology) downregulation that leads to podocyte structural and functional damage in DKD². Ang-II also mediates transient receptor potential cation channel, subfamily C, member 6 (TRPC6) activation and reduces MYH9 expression². Therefore, the Ang-II-mediated MYH9 depletion in DKD increases the filtration barrier permeability by inducing apoptosis of podocyte via TRPC6 calcium channels and Ca²⁺ influx². Hence, MYH9 could be regarded as a potential target for DKD. High oral intake of sodium also activates the RAAS system and causes blunting of the antihypertensive effects of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin-II receptor blockers (ARB)^{2, 39-42}. Aldosterone is also reported to be involved in the pathophysiology of DKD through plasminogen activator inhibitor 1 (PAI-1) and TGF- β promoting macrophage infiltration and renal tissue fibrosis⁴³.

Moreover, vascular homeostasis is regulated by endothelin-1 (ET-1), a potent vasoactive peptide produced by endothelial cells, via the activation of endothelin receptor. Compensatory high insulinaemia seen in T2DM increases ET-1 secretion resulting in vasoconstriction and vascular dysfunction^{44, 45}.

2.3. Mitochondrial dysfunction and oxidative stress

Hyperglycaemia is thought to play an essential role in mitochondrial dysfunction through overloading the electron transport chain system that causes reduction of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity, stimulating excess ROS production, and causes oxidative injury to podocytes and consequently their apoptosis^{46, 47}. Excess ROS leads to deleterious effects on DNA, lipids and proteins and hence results in cell death, fibrosis, and eventual kidney dysfunction. Also, there is impairment of glycolytic metabolism that leads to accumulation of upstream glycolytic intermediates which shift the metabolic flux to other metabolic pathways (those are involved in kidney injury) such as polyol pathway, hexosamine pathway, activation of PKC and production of advanced glycation end products (AGEs) precursors relating to cellular dysfunction, DNA damage, vascular leakage, thrombosis, fibrosis, angiogenesis and inflammation⁴⁶⁻⁴⁸. It has been well investigated that hyperglycaemia leads to upregulation of the polyol pathway. The glucose converted to sorbitol via the enzyme, aldose reductase and then the sorbitol is oxidised to fructose. The increase level of sorbitol leads to reduction of intracellular antioxidant, i.e. glutathione (GSH) which is thought to accelerate the intracellular oxidative stress and apoptosis. The fructose is the end product of the polyol pathway and increase of fructose concentration leads to inhibition of glycolysis. Fructose is also considered as a potential nephrotoxin. The endogenous production of fructose via the polyol pathway has led to the increased level of proteinuria, reduction of GFR, and increased of glomerular and proximal tubular injuries which have been investigated in a diabetic murine mice model. Furthermore, these mice also reported to express high levels of

superoxide and inflammatory cytokine like NF- κ B⁶. On the other hand, it has been found that the DKD kidney contains fewer mitochondria, reduced mitochondrial superoxide formation, and therefore has reduced activity of electron chain complexes⁴⁹. Stimulation of inflammatory processes via nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and vascular dysfunction was also identified due to the decreased mitochondrial superoxides that reduced the endothelial nitric oxide synthase (eNOS) phosphorylation process and AMP-activated protein kinase (AMPK) activity⁴⁹. Hyperglycaemia and AGEs activate the RAAS system and induce renin and angiotensin expression in renal cells through kidney-specific G protein-coupled metabolic receptor GPR91 and ROS, causing deterioration of DKD. Ang-II stimulates the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, chronic hypoxia, ROS production, inflammation, renal tissue fibrosis and renal tubular dysfunction and subsequent atrophy, and decrease of the number of small vessels^{2, 39, 41}. Also, endothelial dysfunction due to the high glucose, insulinaemia and insulin resistance through intracellular mechanisms, relating to activation of PKC, increased release of ROS, and AGEs-induced proinflammatory signalling⁴⁴. Activation of endothelin receptor A is also involved with podocyte injury, inflammation, oxidative stress, and fibrosis in the kidney^{44, 45}.

2.4. Ischaemia and inflammation

Renal ischaemia and inflammation contribute to the pathophysiological progress of DKD. In developed DKD, the susceptibility of renal ischaemia is increased and this aggravates the renal inflammation by infiltration of neutrophils and macrophages in the kidney⁵⁰. Renal medulla hypoxia occurs as the supply of oxygen is reduced due to glomerular and vascular lesions in DKD. Therefore, more ROS are generated and hypoxia-inducible factors (HIFs) are activated⁵¹. Along with this, intrarenal NO production is reduced⁵². Thus, the overproduction of ROS and hyperglycaemia lead to renal injury^{51, 52}. Smoking also contributes to the progression of DKD in both T1DM and T2DM patients through upregulation of growth factors, escalating oxidative stress, and endothelial dysfunction⁵³.

In DKD, NF- κ B is activated by phosphorylation and participates in the regulation of inflammatory factors. The activation of NF- κ B is due to the increased levels of ROS and tumour necrosis factor (TNF). NF- κ B is also involved in proteinuria and infiltration of renal interstitial inflammatory cells⁵⁴. It has been reported that the immune system is also involved in the development of DKD^{55, 56}. Diabetic kidney cells are prone to produce pro-inflammatory factors such as danger-associated molecular patterns (DAMPs), chemokines and cell adhesion molecules (CAMs) to facilitate innate immune responses. Hyperglycaemia increases the oxidized lipoproteins content, cellular stress, and activates macrophages, which disturb the functions of mitochondria and endoplasmic reticulum. Also, hyperglycaemia leads to abnormal activation of intracellular signalling pathways that are involved in the formation of immune complexes. Deposition of these immune complexes in the kidney cells amplifies the innate immune response through the activation of complement and immunoglobulin Fc γ receptors^{9, 57}. It has further been reported that Janus-kinase signal transducer and activator of transcription (JAK-STAT) pathway is involved in the progression of DKD through angiotensin receptors, cytokines and chemokines with assist innate immune responses⁵⁸. Recently, the involvement of gut microbiome in chronic inflammation with T2DM and CKD has been identified because of the release of toxin products such as indoxyl sulfate, cresyl sulfate and beta-2 microglobulin, by them into the blood which can trigger the activation of innate immune responses and cause systemic inflammation, which is thought to be associated with the pathophysiology of DKD⁵⁹.

2.5. Podocyte injury and autophagy suppression

Podocyte injury plays an important role in the pathogenesis of DKD because it maintains the glomerular filtration barrier. The level of albuminuria is an early biomarker of the severity of

DKD. There is evidence that a balance between the activation and repression of the WNT/ β -catenin (CTNNB1) pathway is essential for podocyte health. The increased expression of CTNNB1 pathway components such as transcripts and proteins in podocytes has been reported in both humans and mice with DKD^{60, 61}. In addition, some potential mechanisms such as RAAS (including ACE1 and ACE2), peroxisome proliferator-activated receptors (PPAR), prorenin and its receptor, AGEs, and their receptors (RAGE), ROS, prostanoids, adiponectin, and miRNAs have been reported for the irreversible podocyte injury and loss due to the contribution of diabetic milieu. Furthermore, some biomarkers that include podocyte-released microparticles and expression of podocyte-specific markers noticed in the urine of early podocyte injury have been identified^{62, 63}, such as nephrin, a key structural and signalling molecule in the podocyte slit diaphragm^{64, 65}. Recently, the involvement of autophagy in diabetic podocyte injuries has been reported, such as the two proteins, β -arrestins and ubiquitin carboxy-terminal hydrolase 1 (UCH-L1), through the inhibition and upregulation of autophagy respectively⁶⁶⁻⁶⁹. Activated podocyte autophagy has protective effects on DKD through mammalian target of rapamycin (mTOR) regulation and autophagy-related (Atg) protein conjugation system; however, activation of mTOR suppresses autophagy⁶⁹. Podocyte injury and autophagy are important targets to limit DKD progression because it has been identified that modulation of autophagic pathways in podocytes has shown promising results in the treatment of DKD patients. Autophagy activators such as metformin, resveratrol, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AMPK activators), rapamycin and sirtuin-1 (mTORC1 inhibitors) have been reported as showing significant protective effects on renal structural integrity and function, and decrease albuminuria in experimental DKD models⁷⁰. Similarly, selective apoptosis signal-regulating kinase (ASK1) inhibitor such as selonsertib has shown to reduce the rate of eGFR decline by 71% in phase 2 clinical trial in adult T2DM patients with moderate-to-advanced DKD. Selonsertib at oral daily doses of 2, 6, or 18 mg was found to be safe with no dose-dependent adverse effects and considered to be able to slow DKD progression⁷¹. Recently, resveratrol (a non-flavonoid polyphenol) has shown protective effects against podocyte apoptosis by stimulating autophagy, and astragaloside IV (the active component in a traditional Chinese herbal remedy) has also been shown to promote autophagy in podocyte and to attenuate endoplasmic reticulum (ER) stress in diabetic mice models with DKD^{72, 73}. These data will allow researchers to expand the discovery of targets susceptible to pharmacological manipulation and compound screening via targeting podocyte injury and activation of autophagy.

3. Therapeutic candidates and drug development for DKD

Current clinically used therapeutic drugs have shown some therapeutic efficacy for DKD, but they are unable to halt progression of the disease and stop death of patients¹³. Hence, many potential therapeutic targets have been explored in order to search alternate safe and effective therapeutic candidates. Several drug discovery programmes, such as preclinical small molecule screening (both natural and synthetic compounds) using cellular and animal models, and clinical trials on existing drugs, have been developed. The therapeutic target-based drugs used clinically (**Table 1**), and list of compounds in preclinical development (**Table 2**) for the treatment of DKD are summarized.

3.1. RAAS inhibitors

Over the last two decades, RAAS inhibitors have been the first-line treatment choice due to their successful clinical results¹³. Clinical trials on ACE inhibitors, such as captopril, and ARBs, including losartan and irbesartan, have demonstrated positive outcomes in decreasing albuminuria in DKD patients⁷⁴⁻⁷⁷. However, it has been reported that in clinical trials paricalcitol fails to show a renoprotective effect⁷⁸. RAAS inhibitors have been shown to merely slow the progression of DKD, and some diabetic patients have still progressed to CKD and

ESRD¹³. Inclusion of a mineralocorticoid receptor (MR) antagonist with ACE or ARB inhibitor regimen is beneficial in decreasing albuminuria, inflammation, and kidney fibrosis in patients with DKD. The risk of hyperkalaemia due to MR antagonists can be lowered using novel non-steroidal MR antagonist such as finerenone, which is an MR antagonist. Recent trials with finerenone showed reductions in proteinuria, kidney failure and DKD progression⁷⁹. In another clinical trial, finerenone also showed to reduce cardiovascular mortality and morbidity in DKD patients⁸⁰. Some other MR antagonist such as spironolactone has been tested in combination with antidiabetic/ renoprotective/antihypertensive drugs which showed positive results in reducing DKD progression and lowering proteinuria⁸¹. The finerenone is still under phase 3 clinical trial with T2DM patients to evaluate its efficacy in long-term for DKD⁸². Therefore, further investigation is still required to find better therapeutic candidates for this class of drug.

3.2. SGLT-2 inhibitors

SGLT-2 inhibitors are currently considered as the best therapeutic option for the treatment of DKD as this class of drugs have been shown to slow the progression of DKD by preventing glucose reabsorption at the proximal tubule of the kidney, and therefore lower the blood glucose levels in diabetic patients³⁸. SGLT-2 inhibitors, such as canagliflozin, dapagliflozin and empagliflozin, have shown renoprotective effects in clinical trials⁸³. The mechanisms for renoprotection in T1DM and T2DM patients for SGLT-2 inhibitors could be due to the reduction of hyperfiltration and post-glomerular vasodilation^{83, 84}. In addition, SGLT-2 inhibitors have been shown to exhibit antioxidant and anti-inflammatory effects, and downregulate RAGE expression in DKD patients⁸⁴. Canagliflozin has been shown to reduce the levels of matrix metalloproteinase-7, fibronectin-1, tumour necrosis factor receptor 1 (TNFR1) and interleukin-6 (IL-6) to protect the kidney⁸⁵. Dapagliflozin also had protection against proteinuria, podocyte loss and glomerular injury in DKD⁸⁶. The renoprotective effects of lusogliflozin in an ischemia-reperfusion injury model was thought to be due to lowering hypoxia by increasing the VEGF-A level and decreasing capillary rarefaction⁸⁷. The renoprotective effects of SGLT-2 inhibitors is also considered to be due to the increase of erythropoietin production and reduction of diuretic requirement, as a result by lowering hypertension, increasing renal perfusion and oxygenation⁸⁷. Hence, the role of SGLT-2 inhibitors as renoprotectors can provide critical mechanistic insights into the use of these class of drugs for treating DKD. Recent clinical trials on SGLT-2 inhibitors in the treatment of DKD have shown exciting results: 1) Empagliflozin has been shown to induce a 44% decrease in the risk of incidence or to worsen of nephropathy along with beneficial cardiovascular effects in DKD patients; 2) Canagliflozin (100 mg daily) treatment in T2DM patients has been shown to decrease 30% risk of ESRD; 3) Dapagliflozin has been shown to reduce 46% in sustained eGFR decline and to reduce ESRD risk or renal death, and shows cardiovascular protection⁸⁸⁻⁹⁰. Therefore, the clinical use of SGLT-2 inhibitors is currently a good option for the treatment of DKD.

3.3. Endothelin receptor blockers

Selective endothelin receptor A (ETA) blockers have shown some promising results by decreasing albuminuria, hypertension, fibrosis, structural injury, and the maintenance of podocyte integrity in experimental models of DKD, and therefore, blockage of ETA has become an attractive line of approach for the treatment of DKD. Currently, atrasentan, a selective ETA antagonist, is in phase III clinical trials. Atrasentan has an antialbuminuric effect which may be due to the possible mechanism of restoring glomerular endothelial glycocalyx barrier and improving endothelial function, which were confirmed by the increase of NO concentrations, the decrease of glomerular heparinase expression and inflammation⁹¹. However, despite a

40% decrease in albuminuria, clinical studies on avosentan in combination with RAAS blockers for DKD has been terminated early due to high rates of cardiovascular adverse effects. These included edema, heart failure and cardiovascular deaths, which was thought to be due to high dose of avosentan that blocks both ETA and the endothelin receptor B (ETB)⁹². Therefore, it may be assumed that selective inhibitors of ETA could be a potential approach for DKD treatment.

3.4. New targets and drug development

3.4.1. PKC inhibitors

Protein kinase C (PKC) inhibitors have shown some promising results in DKD treatment^{93, 94}. Ruboxistaurin, a selective PKC- β inhibitor, decreases albuminuria and improves renal function, and this was thought to be due to the prevention of TGF- β signalling upregulation in the STZ-induced DKD model⁹³. In clinical trial results, ruboxistaurin decreased albuminuria and increased eGFR in patients with DKD⁹⁴. Moreover, the use of recombinant human GLP-1 has been reported showing protective effects in diabetic rats, which were thought to be mediated by partial PKC inhibition and PKA activation⁹⁵. Therefore, development of novel PKC inhibitors could be a potential way to treat DKD.

3.4.2. WNT signalling pathway inhibitors

The wingless-type MMTV integration site (WNT) pathway involves in multiple physiological and pathological processes, such as angiogenesis, inflammation, and fibrosis. This signalling pathway also plays an important role in the pathogenesis of DN⁹⁶. It has been shown that WNT pathway is activated in the kidneys of both T1DM and T2DM mice models. WNT signalling pathway inhibitors have been shown to decrease renal inflammation, fibrosis, and proteinuria in an Akita diabetic mice model. The blockade on the WNT pathway with the monoclonal antibody 2F1, which binds the ligand-binding domain of low density lipoprotein-receptor-related protein 6, has been shown to decrease proteinuria and renal fibrosis in both in db/db mice and STZ-induced T1DM rats⁹⁶. Therefore, the WNT signalling pathway could be a potential therapeutic target for DKD and inhibitors of the WNT pathway could provide useful new drug candidates.

3.4.3. GLP-1 receptor agonists and DPP-4 inhibitors

Two incretin hormones, glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), are secreted from the intestine in response to food intake. This process can stimulate the secretion of insulin and maintains the homeostasis of glucose. Both peptides act on the GLP-1 receptor and decrease the concentration of glucose. However, dipeptidyl peptidase-4 (DPP-4), a proteolytic enzyme, rapidly degrades these peptides, so they exhibit a very short half-life (<2 mins). In T2DM patients, low GLP-1 levels have been reported, but patients with DKD have upregulated expression of DPP-4 in the glomeruli^{97, 98}. Therefore, both GLP-1 receptor agonists and DPP-4 inhibitors have been developed to control glycemia. These classes of compounds are also reported to show nephroprotective effects and to maintain renal functions. Exedin-4, a GLP-1 analogue, is reported to decrease albuminuria, glomerular hypertrophy, mesangial expansion, collagen accumulation, inflammatory infiltration, and the number of apoptotic cells in the glomeruli in type 2 diabetic mouse model with DKD⁹⁹. Liraglutide has also been reported to alter the proteome of diabetic kidney and increases the levels of the Na⁺-H⁺ antiporter 3 regulatory cofactor-2 protein and normalizes the peroxiredoxin-2 levels¹⁰⁰. Linagliptin, a DPP-4 inhibitor has been reported to decrease the kidney fibrosis in STZ-induced diabetic mouse model¹⁰¹. Similarly, sitagliptin has been shown to attenuate DKD and inhibited the TGF- β 1 expression¹⁰². More studies are required on the two classes of compounds for DKD treatment.

3.4.4. RAGE blockers

Some RAGE blockers, such as OPB-9195, LR-90, ALT-946 and pyridoxamine, have been tested in DKD models and have shown some beneficial effects both in animal models and clinical trials. The results include: 1) Pyridoxamine has been reported to improve the creatinine levels in DKD patients¹³; 2) RAGE-aptamer has beneficial effects in animal models of DKD¹⁰³; 3) Pimagedine did not show any significant benefit in DKD with T1DM patients as it decreased the glomerular filtration rate very slowly, and at a higher dose (>300mg) caused glomerulonephritis^{103, 104}. Therefore, more study is needed to evaluate the safety and potential therapeutic value for RAGE blockers.

3.4.5. MCP-1, JAK and phosphodiesterase inhibitors targeting inflammation

Inhibitors of the monocyte chemotactic protein-1 (MCP-1, also named CCL2) that can either neutralize the antibodies or antagonize the receptor (CCR2) have been shown to reduce albuminuria, kidney injury and inflammation in rodent models of DKD¹⁰⁵. In phase II clinical trial, emapticap pegol, a CCL2 inhibitor, caused a 29% reduction in albuminuria¹⁰⁶. Similarly, the Janus kinase 1 and 2 (JAK1 and JAK2) inhibitor baricitinib, in phase II randomized control trial on T2DM patients, has been shown to reduce albuminuria by 40% relative to placebo¹⁰⁷.

Patients with DKD have a high serum level of TNF- α , an inflammatory mediator, which causes progression of the disease through inflammatory pathways. As a result, pentoxifylline, a phosphodiesterase inhibitor, was selected for a clinical trial on the basis that it has anti-inflammatory properties including the ability to lower TNF- α levels. Pentoxifylline was also reported to have ability to downregulate the effect of other inflammatory mediators, such as interferon-gamma, interleukin-1, IL-6, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1¹⁰⁸⁻¹¹¹. Additionally, it has been reported that pentoxifylline in combination with angiotensin II receptor blockers had an additive antiproteinuric effect in T2D patients¹¹². Clinical evidence on anti-inflammatory agents belonging to all three being MCP-1, JAK and phosphodiesterase inhibitors class of drugs have shown to be safe and effective in the reduction of albuminuria in the treatment of DKD^{106, 107, 112}. Therefore, new potential selective anti-inflammatory agents could be developed for improved outcomes for DKD patients.

3.4.6. Antioxidants targeting mitochondrial dysfunction

Based on the pathophysiological mechanisms involved in the generation of excess free radicals, and subsequent oxidative stress due to the mitochondrial dysfunction, antioxidants may play an essential role in halting the progression of DKD. This provided a potential rationale to evaluate both natural and synthetic antioxidants in DKD models or clinical trials¹¹³. Clinical evidence revealed that SGLT-2 inhibitors exhibiting antioxidant and anti-inflammatory effects have renoprotective activity in DKD patients¹¹⁴. Similarly, DPP-4 inhibitors have also shown to protect the kidney by stimulating antioxidant defence systems and inhibiting fibrosis¹⁰². Mitoquinone (MitoQ), a well-known antioxidant targeting the mitochondria, has been tested and shown to prevent kidney injury, and to reduce albuminuria in db/db mice with DKD, by improving mitochondrial structural appearance and function as well as facilitating the mitophagy of defective mitochondria¹¹⁵. Genetic, modification or pharmacological inhibition of NADPH subunit Nox4 has shown significant renoprotection in T1DM mouse models¹¹⁶. In diabetic mice, the glutathione peroxidase (GPX) mimic, ebselen, reduced the oxidative stress and attenuated diabetes-associated renal injury¹¹⁷. Several other antioxidants, such as ascorbic acid, resveratrol, and ubiquinone have been tested in the context of DKD, which have shown potential therapeutic benefits in animal models and DKD patients^{72, 118}. Furthermore, the so-called "third gasotransmitter", hydrogen sulfide (H₂S), possessing antioxidant properties, has been tested in several DKD models. Evidence shows that H₂S is produced in large amount in the kidney and the enzymes responsible for its production are expressed in the glomeruli, proximal and distal tubules, and interlobular arteries. On the contrary, it has also been identified that H₂S production is low in DKD patients¹¹⁹. The H₂S supplementation

has been shown to reduce oxidative stress and inflammation in the STZ-induced diabetic rats¹²⁰. It has also been found that H₂S can activate antioxidant defence mechanisms by increasing the SOD activities, the expression of NRF2 transcription factor, and by inhibiting pro-inflammatory and profibrotic pathways¹²¹. Therefore, H₂S donors, such as NaSH (sodium hydrogen sulfide), have been tested in experimental DKD models and reported to show promising results including reduction of albuminuria and oxidative stress, inhibition of RAAS products, and renal fibrosis, and improvement of kidney function and glomerular filtration barrier^{122, 123}. Therefore, H₂S and its analogues can be deployed in new potential drug development. Taken together, the development of new potential antioxidants could be a good approach for treating DKD.

3.4.7. Targeting ORAI and TRPC channels

The underlying molecular mechanism of impaired albumin reabsorption in DKD has been studied¹²⁴⁻¹²⁷. It has been reported that ORAI1-3 are well expressed in proximal tubular epithelial cells (PTECs) and downregulated in the late stage of patients with DKD. Hyperglycaemia or blockade of insulin signalling reduced ORAI1-3 expression. In addition, albumin uptake is impaired due to the inhibition of ORAI channels by both BTP2 ([N-{4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-4-methyl-1,2,3-thiadiazole-5-carboxamide]) and diethylstilbestrol, or due to the silencing of ORAI expression¹²⁴. In a transgenic mouse model, an increase in albuminuria was noticed due to the expression of a dominant-negative ORAI1 mutant (E108Q). The process of albumin endocytosis is Ca²⁺ dependent and accompanied by ORAI1 internalization. Furthermore, it has been shown that amnionless (AMN) was associated with ORAIs and forms STIM/ORAI/AMN complexes after Ca²⁺ store depletion. These mechanisms for protein reabsorption could be potential targets for the treatment of diabetic proteinuria¹²⁴. It has also been found that mibefradil, a T-type Ca²⁺ channel antagonist, blocks ORAI channels in a dose-dependent manner by acting at the extracellular surface (IC₅₀ = 52.6 μM, 14.1 μM and 3.8 μM for ORAI1, ORAI2, and ORAI3, respectively). Application of mibefradil at more than 20 μM concentration inhibited Ca²⁺ release but did not affect cytosolic STIM1 translocation. Mibefradil inhibited cell proliferation, arrested cell cycle progression and induced apoptosis, and these pharmacological actions were due linked to its interaction with ORAI channels¹²⁵. ORAI store-operated channels were also blocked by 4-chloro-3-ethylphenol (4-CEP), an agonist of the ryanodine receptor (RyR), via direct ORAI1-3 channel inhibition. 4-CEP also caused indirect interference with STIM1 subplasmalemmal translocation and clustering after store depletion¹²⁶. To understand the roles of ORAI and STIM in vascular endothelial cells in diabetic conditions, it has been found, using tissue from patients, *in vivo* diabetic mice and *in vitro* cell models, that hyperglycaemia (25 mM D-glucose) enhanced the store-operated Ca²⁺ entry through upregulation of ORAI/STIM via calcineurin-NFAT signalling. It was concluded that the pathway of upregulation of STIM/ORAI through Ca²⁺-calcineurin-NFAT is a novel mechanism to cause abnormal Ca²⁺ homeostasis and endothelial dysfunction in hyperglycaemia¹²⁷. TRPC channels are Ca²⁺ permeable cationic channels and TRPC6 is related to kidney disease. In podocytes, TRPC6 can bind and activate calpains, which are calcium-dependent cysteine proteases that control the podocyte cytoskeleton, cell adhesion, and motility via cleavage of paxillin, focal adhesion kinase, and talin. Therefore, TRPC6 plays a scaffolding role in podocytes and thus affects the glomerular filter¹²⁸. In addition, the TRPC5 channel was found to be activated by extracellular thioredoxin and interacted with TRPC1 to form heteromultimeric channels, and both isoforms were present in the kidney and vasculatures¹²⁹. Hence, both ORAIs and TRPC channels are worthy of further exploration to understand better their roles in DKD, and their potential as new therapeutic targets.

4. Conclusion

It can be concluded that RAAS inhibitors and SGLT-2 inhibitors are beneficial for DKD patients, but are not able to stop DKD progression completely. SGLT-2 inhibitors possess both antioxidative and anti-inflammatory properties, which could be beneficial in terms of pathogenesis of DKD, and further SGLT-2 inhibitors need to be developed. Additionally, SGLT-2 inhibitors such as canagliflozin, dapagliflozin and empagliflozin have shown potential effects at multiple molecular targets or contributors of DKD. Therefore, it is a good indication for the discovery and development of better future therapeutics for the treatment of DKD considering these SGLT-2 inhibitors as standard in experimental models. Furthermore, potential SGLT-2 inhibitors can be designed and synthesized for biological testing. Some new therapeutic targets such as DPP-4, PKC, JAK, endothelin receptor, ORAIs/TRPCs, etc are involved in the pathological progression of DKD, and the development of inhibitors of these targets needs more investigation to validate their efficacy and safety before clinical applications. Furthermore, targeting renal circulation could be an alternative approach for DKD. For example, a combination of sacubitril and valsartan is under clinical trial phase III for DKD¹, Cinaciguat, a soluble guanylate cyclase (sGC) activator has shown promising preclinical results, and rosiglitazone, a PPAR γ activator has been shown to reduce both microalbuminuria and blood pressure in T2DM patients independently of glycaemia¹³⁰. Long-term use of epalrestat, an aldose reductase inhibitor, on T2DM patients has shown some potential usefulness on renal functions¹³¹. Overall, there remains an urgent need for identification and validation of new drug targets and candidates to achieve better treatment of DKD.

5. Expert opinion

RAAS and SGLT-2 inhibitors have been used clinically to treat DKD. These current standard treatments control hyperglycaemia, high blood pressure, lipid profile etc. to manage the disease, but are not sufficient to stop the disease progression. Many new therapies for DKD (DPP-4 inhibitor-ARBs, GLP-1R agonists, DPP-4 inhibitors, paricalcitol, pentoxifylline, pyridoxamine, ruboxistaurin, soludexide, thiazolidinediones, non-steroidal mineralocorticoid receptor antagonists and JAK inhibitors) have or are being preclinically and clinically tested in order to find new effective regimens. Recently, from clinical trials, meta-analysis on efficacy and safety risks of dual blockade of the RAAS in DKD has shown that dual inhibition therapy was superior to monotherapy in controlling blood pressure and reducing proteinuria. However, this analysis did not translate into long-term improvements including reduction of ESRD progression, cardiovascular mortality, and all-cause mortality. This study concluded that a combination of ACEI and ARB or direct renin inhibitor and ACEI/ARB may be a safe and effective therapy for patients with DKD, and a combination therapy may be more suitable for patients with DKD and in controlling hypertension and microalbuminuria. Therefore, this study reveals that use of dual therapy is tolerable in terms of safety risks of drugs and can be better employed in DKD treatment and more investigation is needed for new drug discovery. However, recent reports have also brought into focus the need for identification of more specific biomarkers to better understand the detailed pathophysiological mechanisms of DKD, and hence, to discover potential therapeutic agents for better future treatment and management of DKD. The specific biomarker discovery may identify the pathway (s) involved in the DKD progression and therefore, target based either mono or multi therapy could be prescribed to treat DKD patient subpopulations and can decrease the mortality rate. Due to the multiple pathophysiological mechanisms of DKD, it may be difficult to treat DKD by single-drug therapy, and so different target-based drug discovery approaches are being sought. It may also be thought that DKD might progress to ESRD through nonalbuminuric pathways (can be defined as 'nonalbuminuric renal impairment'), which can be possibly treated by potential antioxidants or anti-inflammatory agents. Considering the importance and potentiality, use of SGLT-2 inhibitors could be a good option. Some recent research investigations have helped to understand the role and importance of Ca²⁺ channels, such as ORAIs and TRPC, and their

association with podocyte injury or renal artery circulation in DKD. This exploration should be continued in order to find new effective drug candidates via high throughput screening. The successful preclinical results of resveratrol and astragaloside IV on podocyte and animal models of DKD may motivate researchers to search for different classes of natural products. In addition, natural and synthetic antioxidants with potential free radical scavenging abilities, including H₂S donor compounds, could also become candidates for DKD therapeutics.

In summary, the genomic and epigenetic diversity and interplay of heterogeneous multiple pathophysiological mechanisms during the disease development makes the disease much more complex. Therefore, for effective treatment of DKD, novel targets need to be validated urgently so that discovery of new therapeutic drug candidates can be identified.

Funding

This work has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115974. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA with JDRF (to S.Z.X.).

Declaration of interest

CC Danta is as a post-doctoral research associate for SZ Xu who received support via grant No 115,974 supported from the Innovative Medicines Initiative 2 Joint Undertaking. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Roelofs JJ, Vogt L. *Diabetic Nephropathy: Pathophysiology and Clinical Aspects*: Springer International Publishing, 2018.
2. Kang JS, Lee SJ, Lee J-H, Kim J-H, Son SS, Cha S-K, et al. Angiotensin II-mediated MYH9 downregulation causes structural and functional podocyte injury in diabetic kidney disease. *Scientific Reports* 2019 2019/05/22;9(1):7679.
3. Sulaiman MK. Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. *Diabetol Metab Syndr* 2019;11:7.
4. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the global burden of disease study 2013. *Lancet* 2015 Jan 10;385(9963):117-71.
5. Thomas B. The Global Burden of Diabetic Kidney Disease: Time Trends and Gender Gaps. *Current Diabetes Reports* 2019 2019/03/02;19(4):18.
6. Toth-Manikowski S, Atta MG. Diabetic Kidney Disease: Pathophysiology and Therapeutic Targets. *J Diabetes Res* 2015;2015:697010.
7. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* 2019 Jun 13;380(24):2295-306.
- **A recent clinical report of canagliflozin on renal outcomes.**
8. Heerspink HJL, Stefansson BV, Chertow GM, Correa-Rotter R, Greene T, Hou FF, et al. Rationale and protocol of the Dapagliflozin And Prevention of Adverse outcomes in Chronic Kidney Disease (DAPA-CKD) randomized controlled trial. *Nephrol Dial Transplant* 2020 Feb 1;35(2):274-82.
9. Lin YC, Chang YH, Yang SY, Wu KD, Chu TS. Update of pathophysiology and management of diabetic kidney disease. *J Formos Med Assoc* 2018 Aug;117(8):662-75.
10. Guan M, Keaton JM, Dimitrov L, Hicks PJ, Xu J, Palmer ND, et al. Genome-wide association study identifies novel loci for type 2 diabetes-attributed end-stage kidney disease in African Americans. *Hum Genomics* 2019 May 15;13(1):21.
11. McKnight AJ, McKay GJ, Maxwell AP. Genetic and epigenetic risk factors for diabetic kidney disease. *Adv Chronic Kidney Dis* 2014 May;21(3):287-96.
12. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primers* 2015 Jul 30;1:15018.
13. Barrera-Chimal J, Jaisser F. Pathophysiologic mechanisms in diabetic kidney disease: A focus on current and future therapeutic targets. *Diabetes Obes Metab* 2020 Apr;22 Suppl 1:16-31.

●● A review describing pathophysiological mechanisms of DKD.

14. Portela A, Esteller M. Epigenetic modifications and human disease. *Nature Biotechnology* 2010 2010/10/01;28(10):1057-68.
15. Horsburgh S, Robson-Ansley P, Adams R, Smith C. Exercise and inflammation-related epigenetic modifications: focus on DNA methylation. *Exerc Immunol Rev* 2015;21:26-41.
16. Bell CG, Teschendorff AE, Rakyan VK, Maxwell AP, Beck S, Savage DA. Genome-wide DNA methylation analysis for diabetic nephropathy in type 1 diabetes mellitus. *BMC Med Genomics* 2010 Aug 5;3:33.
17. Trégouet D-A, Groop P-H, McGinn S, Forsblom C, Hadjadj S, Marre M, et al. G/T Substitution in Intron 1 of the *UNC13B* Gene Is Associated With Increased Risk of Nephropathy in Patients With Type 1 Diabetes. *Diabetes* 2008;57(10):2843-50.
18. Bechtel W, McGoohan S, Zeisberg EM, Müller GA, Kalbacher H, Salant DJ, et al. Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nat Med* 2010 May;16(5):544-50.
19. Swan EJ, Maxwell AP, McKnight AJ. Distinct methylation patterns in genes that affect mitochondrial function are associated with kidney disease in blood-derived DNA from individuals with Type 1 diabetes. *Diabet Med* 2015 Aug;32(8):1110-5.
20. Marumo T, Yagi S, Kawarazaki W, Nishimoto M, Ayuzawa N, Watanabe A, et al. Diabetes Induces Aberrant DNA Methylation in the Proximal Tubules of the Kidney. *J Am Soc Nephrol* 2015 Oct;26(10):2388-97.
21. Sun G, Reddy MA, Yuan H, Lanting L, Kato M, Natarajan R. Epigenetic histone methylation modulates fibrotic gene expression. *J Am Soc Nephrol* 2010 Dec;21(12):2069-80.
22. Sun XY, Qin HJ, Zhang Z, Xu Y, Yang XC, Zhao DM, et al. Valproate attenuates diabetic nephropathy through inhibition of endoplasmic reticulum stress-induced apoptosis. *Mol Med Rep* 2016 Jan;13(1):661-8.
23. Khan S, Jena G. Sodium butyrate, a HDAC inhibitor ameliorates eNOS, iNOS and TGF- β -induced fibrogenesis, apoptosis and DNA damage in the kidney of juvenile diabetic rats. *Food Chem Toxicol* 2014 Nov;73:127-39.
24. Advani A, Huang Q, Thai K, Advani SL, White KE, Kelly DJ, et al. Long-term administration of the histone deacetylase inhibitor vorinostat attenuates renal injury in experimental diabetes through an endothelial nitric oxide synthase-dependent mechanism. *Am J Pathol* 2011 May;178(5):2205-14.
25. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009 Jan 23;136(2):215-33.
26. Kato M, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, et al. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. *Proc Natl Acad Sci U S A* 2007 Feb 27;104(9):3432-7.
27. Kato M, Dang V, Wang M, Park JT, Deshpande S, Kadam S, et al. TGF- β induces acetylation of chromatin and of Ets-1 to alleviate repression of miR-192 in diabetic nephropathy. *Science signaling* 2013;6(278):ra43-ra43.
28. Kato M, Arce L, Wang M, Putta S, Lanting L, Natarajan R. A microRNA circuit mediates transforming growth factor- β 1 autoregulation in renal glomerular mesangial cells. *Kidney Int* 2011 Aug;80(4):358-68.
29. Deshpande SD, Putta S, Wang M, Lai JY, Bitzer M, Nelson RG, et al. Transforming growth factor- β -induced cross talk between p53 and a microRNA in the pathogenesis of diabetic nephropathy. *Diabetes* 2013 Sep;62(9):3151-62.
30. Kato M, Wang M, Chen Z, Bhatt K, Oh HJ, Lanting L, et al. An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy. *Nat Commun* 2016 Sep 30;7:12864.
31. Yoh K, Hirayama A, Ishizaki K, Yamada A, Takeuchi M, Yamagishi S, et al. Hyperglycemia induces oxidative and nitrosative stress and increases renal functional impairment in Nrf2-deficient mice. *Genes Cells* 2008 Nov;13(11):1159-70.
32. Jiang T, Huang Z, Lin Y, Zhang Z, Fang D, Zhang DD. The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. *Diabetes* 2010 Apr;59(4):850-60.
33. Xu X, Luo P, Wang Y, Cui Y, Miao L. Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) is a novel therapeutic target for diabetic complications. *J Int Med Res* 2013 Feb;41(1):13-9.
34. Sharma D, Bhattacharya P, Kalia K, Tiwari V. Diabetic nephropathy: New insights into established therapeutic paradigms and novel molecular targets. *Diabetes Res Clin Pract* 2017 Jun;128:91-108.
35. Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. *Expert Opin Ther Targets* 2019 Jul;23(7):579-91.
36. Yu SM, Bonventre JV. Acute Kidney Injury and Progression of Diabetic Kidney Disease. *Adv Chronic Kidney Dis* 2018 Mar;25(2):166-80.
37. Wang XX, Levi J, Luo Y, Myakala K, Herman-Edelstein M, Qiu L, et al. SGLT2 Protein Expression Is Increased in Human Diabetic Nephropathy: SglT2 protein inhibition decreases renal lipid accumulation, inflammation, and the development of nephropathy in diabetic mice. *J Biol Chem* 2017 Mar 31;292(13):5335-48.
38. Nespoux J, Vallon V. SGLT2 inhibition and kidney protection. *Clin Sci (Lond)* 2018 Jun 29;132(12):1329-39.
39. Sharma R, Sharma M, Reddy S, Savin VJ, Nagaria AM, Wiegmann TB. Chronically increased intrarenal angiotensin II causes nephropathy in an animal model of type 2 diabetes. *Front Biosci* 2006 Jan 1;11:968-76.
40. Lovshin JA, Boulet G, Lytvyn Y, Lovblom LE, Bjornstad P, Farooqi MA, et al. Renin-angiotensin-aldosterone system activation in long-standing type 1 diabetes. *JCI Insight* 2018 Jan 11;3(1).

41. Rincon-Choles H, Kasinath BS, Gorin Y, Abboud HE. Angiotensin II and growth factors in the pathogenesis of diabetic nephropathy. *Kidney Int Suppl* 2002 Dec(82):S8-11.
42. Vegter S, Perna A, Postma MJ, Navis G, Remuzzi G, Ruggenenti P. Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol* 2012 Jan;23(1):165-73.
43. He J, Xu Y, Koya D, Kanasaki K. Role of the endothelial-to-mesenchymal transition in renal fibrosis of chronic kidney disease. *Clin Exp Nephrol* 2013 Aug;17(4):488-97.
44. Potenza MA, Gagliardi S, Nacci C, Carratu MR, Montagnani M. Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets. *Curr Med Chem* 2009;16(1):94-112.
45. de Zeeuw D, Coll B, Andress D, Brennan JJ, Tang H, Houser M, et al. The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy. *J Am Soc Nephrol* 2014 May;25(5):1083-93.
46. King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. *Histochemistry and Cell Biology* 2004 2004/10/01;122(4):333-38.
47. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010 Oct 29;107(9):1058-70.
- A review article on diabetic complications due to oxidative stress.
48. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, et al. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 2003 Oct;112(7):1049-57.
49. Sharma K. Mitochondrial hormesis and diabetic complications. *Diabetes* 2015 Mar;64(3):663-72.
50. Wada J, Makino H. Innate immunity in diabetes and diabetic nephropathy. *Nat Rev Nephrol* 2016 Jan;12(1):13-26.
51. Bernhardt WM, Schmitt R, Rosenberger C, Münchenhagen PM, Gröne HJ, Frei U, et al. Expression of hypoxia-inducible transcription factors in developing human and rat kidneys. *Kidney Int* 2006 Jan;69(1):114-22.
52. Prabhakar SS. Role of nitric oxide in diabetic nephropathy. *Semin Nephrol* 2004 Jul;24(4):333-44.
53. Phisitkul K, Hegazy K, Chuahirun T, Hudson C, Simoni J, Rajab H, et al. Continued smoking exacerbates but cessation ameliorates progression of early type 2 diabetic nephropathy. *Am J Med Sci* 2008 Apr;335(4):284-91.
54. Ziyadeh FN, Wolf G. Pathogenesis of the podocytopathy and proteinuria in diabetic glomerulopathy. *Curr Diabetes Rev* 2008 Feb;4(1):39-45.
55. Wilson PC, Wu H, Kirita Y, Uchimura K, Ledru N, Rennke HG, et al. The single-cell transcriptomic landscape of early human diabetic nephropathy. *Proc Natl Acad Sci U S A* 2019 Sep 24;116(39):19619-25.
56. Park J, Guan Y, Sheng X, Gluck C, Seasock MJ, Hakimi AA, et al. Functional methylome analysis of human diabetic kidney disease. *JCI Insight* 2019 Jun 6;4(11).
57. Flyvbjerg A. The role of the complement system in diabetic nephropathy. *Nat Rev Nephrol* 2017 May;13(5):311-18.
58. Brosius FC, Tuttle KR, Kretzler M. JAK inhibition in the treatment of diabetic kidney disease. *Diabetologia* 2016 Aug;59(8):1624-7.
59. Anders HJ, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int* 2013 Jun;83(6):1010-6.
60. Bose M, Almas S, Prabhakar S. Wnt signaling and podocyte dysfunction in diabetic nephropathy. *J Investig Med* 2017 Dec;65(8):1093-101.
61. Kato H, Gruenwald A, Suh JH, Miner JH, Barisoni-Thomas L, Taketo MM, et al. Wnt/ β -catenin pathway in podocytes integrates cell adhesion, differentiation, and survival. *J Biol Chem* 2011 Jul 22;286(29):26003-15.
62. Stitt-Cavanagh E, MacLeod L, Kennedy C. The Podocyte in Diabetic Kidney Disease. *TheScientificWorldJournal* 2009 10/14;9:1127-39.
63. Kravets I, Mallipattu SK. The Role of Podocytes and Podocyte-Associated Biomarkers in Diagnosis and Treatment of Diabetic Kidney Disease. *J Endocr Soc* 2020 Apr 1;4(4):bvaa029.
64. Lin JS, Susztak K. Podocytes: the Weakest Link in Diabetic Kidney Disease? *Curr Diab Rep* 2016 May;16(5):45.
65. Liu Y, Su H, Ma C, Ji D, Zheng X, Wang P, et al. IQGAP1 mediates podocyte injury in diabetic kidney disease by regulating nephrin endocytosis. *Cellular Signalling* 2019 2019/07/01;59:13-23.
66. Zhang H, Luo W, Sun Y, Qiao Y, Zhang L, Zhao Z, et al. Wnt/ β -Catenin Signaling Mediated-UCH-L1 Expression in Podocytes of Diabetic Nephropathy. *Int J Mol Sci* 2016 Aug 25;17(9).
67. Geng J, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series. *EMBO Rep* 2008 Sep;9(9):859-64.
68. Liu J, Li QX, Wang XJ, Zhang C, Duan YQ, Wang ZY, et al. β -Arrestins promote podocyte injury by inhibition of autophagy in diabetic nephropathy. *Cell Death & Disease* 2016 2016/04/01;7(4):e2183-e83.
69. Kim WY, Nam SA, Song HC, Ko JS, Park SH, Kim HL, et al. The role of autophagy in unilateral ureteral obstruction rat model. *Nephrology (Carlton)* 2012 Feb;17(2):148-59.
70. Yang D, Livingston MJ, Liu Z, Dong G, Zhang M, Chen JK, et al. Autophagy in diabetic kidney disease: regulation, pathological role and therapeutic potential. *Cell Mol Life Sci* 2018 Feb;75(4):669-88.
71. Chertow GM, Pergola PE, Chen F, Kirby BJ, Sundry JS, Patel UD. Effects of Selonsertib in Patients with Diabetic Kidney Disease. *J Am Soc Nephrol* 2019 Oct;30(10):1980-90.
72. Huang SS, Ding DF, Chen S, Dong CL, Ye XL, Yuan YG, et al. Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of diabetic nephropathy. *Sci Rep* 2017 Apr 4;7:45692.

●● **A recent research explaining the mechanisms of resveratrol in podocyte apoptosis and autophagy.**

73. Guo H, Wang Y, Zhang X, Zang Y, Zhang Y, Wang L, et al. Astragaloside IV protects against podocyte injury via SERCA2-dependent ER stress reduction and AMPK α -regulated autophagy induction in streptozotocin-induced diabetic nephropathy. *Sci Rep* 2017 Jul 31;7(1):6852.
74. Feng Y, Huang R, Kavanagh J, Li L, Zeng X, Li Y, et al. Efficacy and Safety of Dual Blockade of the Renin-Angiotensin-Aldosterone System in Diabetic Kidney Disease: A Meta-Analysis. *Am J Cardiovasc Drugs* 2019 Jun;19(3):259-86.
75. Valente M, Bhandari S. Renal function after new treatment with renin-angiotensin system blockers. *Bmj* 2017 Mar 9;356:j1122.
76. Ahmed A, Jorna T, Bhandari S. Should We STOP Angiotensin Converting Enzyme Inhibitors/Angiotensin Receptor Blockers in Advanced Kidney Disease? *Nephron* 2016;133(3):147-58.

●● **This article has discussed the results of clinical applications of both ACE and RAAS inhibitors.**

77. Bhandari S, Ives N, Brettell E, Valente M, Cockwell P, Topham P, et al. Multicentre randomized controlled trial of angiotensin converting enzyme inhibitor/angiotensin receptor blocker withdrawal in advanced renal disease: The STOP-ACEi trial. *Nephrology Dialysis Transplantation* 2015 09/30;31:gv346.
78. Perez-Gomez MV, Sanchez-Niño MD, Sanz AB, Martín-Cleary C, Ruiz-Ortega M, Egido J, et al. Horizon 2020 in Diabetic Kidney Disease: The Clinical Trial Pipeline for Add-On Therapies on Top of Renin Angiotensin System Blockade. *J Clin Med* 2015 Jun 18;4(6):1325-47.
79. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Nowack C, et al. Design and Baseline Characteristics of the Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease Trial. *Am J Nephrol* 2019;50(5):333-44.

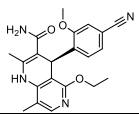
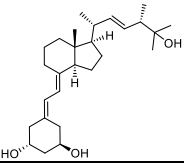
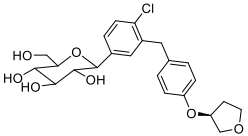
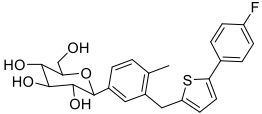
●● **A recent clinical outcome of finerenone on DKD patients.**

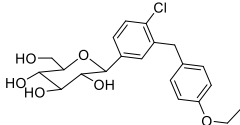
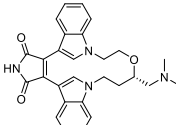
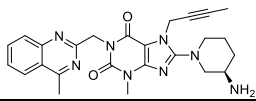
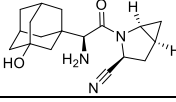
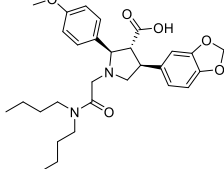
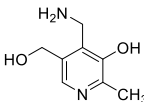
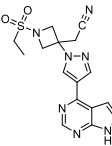
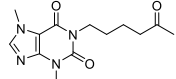
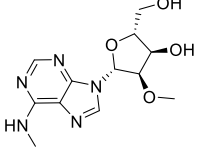
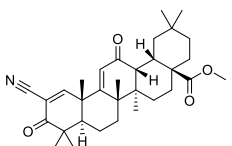
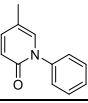
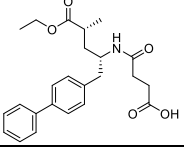
80. Ruilope LM, Agarwal R, Anker SD, Bakris GL, Filippatos G, Nowack C, et al. Design and Baseline Characteristics of the Finerenone in Reducing Cardiovascular Mortality and Morbidity in Diabetic Kidney Disease Trial. *Am J Nephrol* 2019;50(5):345-56.
81. Hou J, Xiong W, Cao L, Wen X, Li A. Spironolactone Add-on for Preventing or Slowing the Progression of Diabetic Nephropathy: A Meta-analysis. *Clin Ther* 2015 Sep;37(9):2086-103.e10.
82. Bakris GL, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H, et al. Effect of Finerenone on Albuminuria in Patients With Diabetic Nephropathy: A Randomized Clinical Trial. *Jama* 2015 Sep 1;314(9):884-94.
83. Cherney DZ, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* 2014 Feb 4;129(5):587-97.
84. van Bommel EJM, Muskiet MHA, van Baar MJB, Tonneijck L, Smits MM, Emanuel AL, et al. The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial. *Kidney Int* 2020 Jan;97(1):202-12.
85. Heerspink HJL, Perco P, Mulder S, Leierer J, Hansen MK, Heinzl A, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. *Diabetologia* 2019 Jul;62(7):1154-66.
86. Cassis P, Locatelli M, Cerullo D, Corna D, Buelli S, Zanchi C, et al. SGLT2 inhibitor dapagliflozin limits podocyte damage in proteinuric nondiabetic nephropathy. *JCI Insight* 2018 Aug 9;3(15).
87. van Raalte DH, Cherney DZI. Sodium glucose cotransporter 2 inhibition and renal ischemia: implications for future clinical trials. *Kidney Int* 2018 Sep;94(3):459-62.
88. Wanner C, Inzucchi SE, Zinman B. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. *N Engl J Med* 2016 Nov 3;375(18):1801-2.
89. Mahaffey KW, Jardine MJ, Bompont S, Cannon CP, Neal B, Heerspink HJL, et al. Canagliflozin and Cardiovascular and Renal Outcomes in Type 2 Diabetes Mellitus and Chronic Kidney Disease in Primary and Secondary Cardiovascular Prevention Groups. *Circulation* 2019 Aug 27;140(9):739-50.
90. Mosenzon O, Wiviott SD, Cahn A, Rozenberg A, Yanuv I, Goodrich EL, et al. Effects of dapagliflozin on development and progression of kidney disease in patients with type 2 diabetes: an analysis from the DECLARE-TIMI 58 randomised trial. *Lancet Diabetes Endocrinol* 2019 Aug;7(8):606-17.
91. Boels MG, Avramut MC, Koudijs A, Dane MJ, Lee DH, van der Vlag J, et al. Atrasentan Reduces Albuminuria by Restoring the Glomerular Endothelial Glycocalyx Barrier in Diabetic Nephropathy. *Diabetes* 2016 Aug;65(8):2429-39.
92. Mann JF, Green D, Jamerson K, Ruilope LM, Kuranoff SJ, Littke T, et al. Avasentan for overt diabetic nephropathy. *J Am Soc Nephrol* 2010 Mar;21(3):527-35.
93. Al-Onazi AS, Al-Rasheed NM, Attia HA, Al-Rasheed NM, Ahmed RM, Al-Amin MA, et al. Ruboxistaurin attenuates diabetic nephropathy via modulation of TGF- β 1/Smad and GRAP pathways. *J Pharm Pharmacol* 2016 Feb;68(2):219-32.
94. Tuttle KR, McGill JB, Bastyr EJ, 3rd, Poi KK, Shahri N, Anderson PW. Effect of ruboxistaurin on albuminuria and estimated GFR in people with diabetic peripheral neuropathy: results from a randomized trial. *Am J Kidney Dis* 2015 Apr;65(4):634-6.

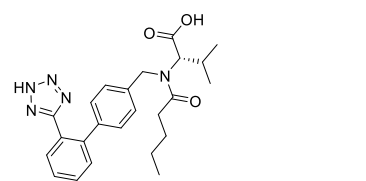
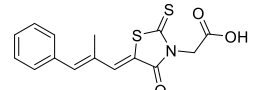
95. Yin W, Jiang Y, Xu S, Wang Z, Peng L, Fang Q, et al. Protein kinase C and protein kinase A are involved in the protection of recombinant human glucagon-like peptide-1 on glomeruli and tubules in diabetic rats. *J Diabetes Investig* 2019 May;10(3):613-25.
96. Zhou T, He X, Cheng R, Zhang B, Zhang RR, Chen Y, et al. Implication of dysregulation of the canonical wingless-type MMTV integration site (WNT) pathway in diabetic nephropathy. *Diabetologia* 2012 2012/01/01;55(1):255-66.
97. Tsimihodimos V, Elisaf M. Effects of incretin-based therapies on renal function. *Eur J Pharmacol* 2018 Jan 5;818:103-09.
98. Sharkovska Y, Reichetzedder C, Alter M, Tsuprykov O, Bachmann S, Secher T, et al. Blood pressure and glucose independent renoprotective effects of dipeptidyl peptidase-4 inhibition in a mouse model of type-2 diabetic nephropathy. *J Hypertens* 2014 Nov;32(11):2211-23; discussion 23.
99. Park CW, Kim HW, Ko SH, Lim JH, Ryu GR, Chung HW, et al. Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol* 2007 Apr;18(4):1227-38.
100. Liljedahl L, Norlin J, McGuire JN, James P. Effects of insulin and the glucagon-like peptide 1 receptor agonist liraglutide on the kidney proteome in db/db mice. *Physiol Rep* 2017 Mar;5(6).
101. Kanasaki K, Shi S, Kanasaki M, He J, Nagai T, Nakamura Y, et al. Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* 2014 Jun;63(6):2120-31.
102. Li L, Lian X, Wang Z, Zheng J, Liu J, Chu Y, et al. The dipeptidyl peptidase-4 inhibitor sitagliptin ameliorates renal injury in type 1 diabetic mice via inhibiting the TGF- β /Smad signal pathway. *Die Pharmazie* 2019 04/01;74:239-42.
103. Matsui T, Higashimoto Y, Nishino Y, Nakamura N, Fukami K, Yamagishi SI. RAGE-Aptamer Blocks the Development and Progression of Experimental Diabetic Nephropathy. *Diabetes* 2017 Jun;66(6):1683-95.
104. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, et al. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004 Jan-Feb;24(1):32-40.
105. Boels MGS, Koudijs A, Avramut MC, Sol W, Wang G, van Oeveren-Rietdijk AM, et al. Systemic Monocyte Chemotactic Protein-1 Inhibition Modifies Renal Macrophages and Restores Glomerular Endothelial Glycocalyx and Barrier Function in Diabetic Nephropathy. *Am J Pathol* 2017 Nov;187(11):2430-40.
106. Menne J, Eulberg D, Beyer D, Baumann M, Saudek F, Valkusz Z, et al. C-C motif-ligand 2 inhibition with emapticap pegol (NOX-E36) in type 2 diabetic patients with albuminuria. *Nephrol Dial Transplant* 2017 Feb 1;32(2):307-15.
107. Tuttle KR, Brosius FC, 3rd, Adler SG, Kretzler M, Mehta RL, Tumlin JA, et al. JAK1/JAK2 inhibition by baricitinib in diabetic kidney disease: results from a Phase 2 randomized controlled clinical trial. *Nephrol Dial Transplant* 2018 Nov 1;33(11):1950-59.
108. Donate-Correa J, Tagua VG, Ferri C, Martín-Núñez E, Hernández-Carballo C, Ureña-Torres P, et al. Pentoxifylline for Renal Protection in Diabetic Kidney Disease. A Model of Old Drugs for New Horizons. *J Clin Med* 2019 Feb 27;8(3).
- **A recent clinical outcomes of pentoxifylline on renoprotective effects in DKD patients.**
109. Goicoechea M, García de Vinuesa S, Quiroga B, Verdalles U, Barraca D, Yuste C, et al. Effects of pentoxifylline on inflammatory parameters in chronic kidney disease patients: a randomized trial. *J Nephrol* 2012 Nov-Dec;25(6):969-75.
110. Leporini C, Pisano A, Russo E, G DA, de Sarro G, Coppolino G, et al. Effect of pentoxifylline on renal outcomes in chronic kidney disease patients: A systematic review and meta-analysis. *Pharmacol Res* 2016 May;107:315-32.
111. Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, Chahin J, Méndez ML, Gallego E, et al. Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial. *J Am Soc Nephrol* 2015 Jan;26(1):220-9.
112. Navarro JF, Mora C, Muros M, García J. Additive antiproteinuric effect of pentoxifylline in patients with type 2 diabetes under angiotensin II receptor blockade: a short-term, randomized, controlled trial. *J Am Soc Nephrol* 2005 Jul;16(7):2119-26.
113. Danta CC, Piplani P. The discovery and development of new potential antioxidant agents for the treatment of neurodegenerative diseases. *Expert Opin Drug Discov* 2014 Oct;9(10):1205-22.
- **A review explaining the mechanisms of antioxidant activity and potential drug development.**
114. Ojima A, Matsui T, Nishino Y, Nakamura N, Yamagishi S. Empagliflozin, an Inhibitor of Sodium-Glucose Cotransporter 2 Exerts Anti-Inflammatory and Antifibrotic Effects on Experimental Diabetic Nephropathy Partly by Suppressing AGES-Receptor Axis. *Horm Metab Res* 2015 Aug;47(9):686-92.
115. Xiao L, Xu X, Zhang F, Wang M, Xu Y, Tang D, et al. The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox Biol* 2017 Apr;11:297-311.
116. Gorin Y, Cavaglieri RC, Khazim K, Lee DY, Bruno F, Thakur S, et al. Targeting NADPH oxidase with a novel dual Nox1/Nox4 inhibitor attenuates renal pathology in type 1 diabetes. *Am J Physiol Renal Physiol* 2015 Jun 1;308(11):F1276-87.
117. Tan SM, Sharma A, Yuen DY, Stefanovic N, Krippner G, Muges G, et al. The modified selenenyl amide, M-hydroxy ebselen, attenuates diabetic nephropathy and diabetes-associated atherosclerosis in ApoE/GPx1 double knockout mice. *PLoS One* 2013;8(7):e69193.

118. Bolignano D, Cernaro V, Gembillo G, Baggetta R, Buemi M, D'Arrigo G. Antioxidant agents for delaying diabetic kidney disease progression: A systematic review and meta-analysis. *PLoS One* 2017;12(6):e0178699.
119. Koning AM, Frenay AR, Leuvenink HG, van Goor H. Hydrogen sulfide in renal physiology, disease and transplantation- the smell of renal protection. *Nitric Oxide* 2015 Apr 30;46:37-49.
120. Zhou X, Feng Y, Zhan Z, Chen J. Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model. *J Biol Chem* 2014 Oct 17;289(42):28827-34.
121. Sun HJ, Wu ZY, Cao L, Zhu MY, Liu TT, Guo L, et al. Hydrogen Sulfide: Recent Progression and Perspectives for the Treatment of Diabetic Nephropathy. *Molecules* 2019 Aug 6;24(15).
- An article describing the role and implications of NaSH in DKD.
122. Li Y, Li L, Zeng O, Liu JM, Yang J. H(2)S improves renal fibrosis in STZ-induced diabetic rats by ameliorating TGF- β 1 expression. *Ren Fail* 2017 Nov;39(1):265-72.
123. Ahmed HH, Taha FM, Omar HS, Elwi HM, Abdelnasser M. Hydrogen sulfide modulates SIRT1 and suppresses oxidative stress in diabetic nephropathy. *Mol Cell Biochem* 2019 Jul;457(1-2):1-9.
124. Zeng B, Chen GL, Garcia-Vaz E, Bhandari S, Daskoulidou N, Berglund LM, et al. ORAI channels are critical for receptor-mediated endocytosis of albumin. *Nat Commun* 2017 Dec 4;8(1):1920.
125. Li P, Rubaiy HN, Chen GL, Hallett T, Zaibi N, Zeng B, et al. Mibefradil, a T-type Ca(2+) channel blocker also blocks Orai channels by action at the extracellular surface. *Br J Pharmacol* 2019 Oct;176(19):3845-56.
126. Zeng B, Chen GL, Daskoulidou N, Xu SZ. The ryanodine receptor agonist 4-chloro-3-ethylphenol blocks ORAI store-operated channels. *Br J Pharmacol* 2014 Mar;171(5):1250-9.
127. Daskoulidou N, Zeng B, Berglund LM, Jiang H, Chen GL, Kotova O, et al. High glucose enhances store-operated calcium entry by upregulating ORAI/STIM via calcineurin-NFAT signalling. *J Mol Med (Berl)* 2015 May;93(5):511-21.
128. Farmer LK, Rollason R, Whitcomb DJ, Ni L, Goodliff A, Lay AC, et al. TRPC6 Binds to and Activates Calpain, Independent of Its Channel Activity, and Regulates Podocyte Cytoskeleton, Cell Adhesion, and Motility. *J Am Soc Nephrol* 2019 Oct;30(10):1910-24.
129. Xu SZ, Sukumar P, Zeng F, Li J, Jairaman A, English A, et al. TRPC channel activation by extracellular thioredoxin. *Nature* 2008 Jan 3;451(7174):69-72.
130. Bakris GL, Ruilope LM, McMorn SO, Weston WM, Heise MA, Freed MI, et al. Rosiglitazone reduces microalbuminuria and blood pressure independently of glycemia in type 2 diabetes patients with microalbuminuria. *J Hypertens* 2006 Oct;24(10):2047-55.
131. Iso K, Tada H, Kuboki K, Inokuchi T. Long-term effect of epalrestat, an aldose reductase inhibitor, on the development of incipient diabetic nephropathy in Type 2 diabetic patients. *J Diabetes Complications* 2001 Sep-Oct;15(5):241-4.

Table 1. List of therapeutic drugs with clinical trials for DKD

Name and References	Drug Chemical Structure	Therapeutic target / mechanism	Clinical Status
Finerenone [79,80,82]		RAAS inhibition	Phase 3 ongoing
Paricalcitol [78]		RAAS inhibition	Clinically failed to show renoprotective effect
Empagliflozin [83,88]		SGLT-2 inhibition	Phase 3 completed in 2015
Canagliflozin [7,83]		SGLT-2 inhibition	Phase 3 ongoing

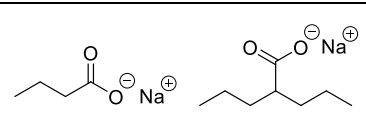
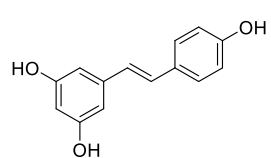
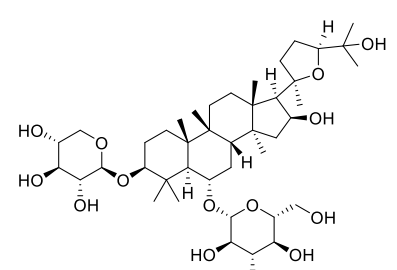
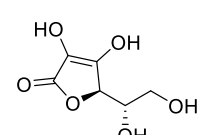
Dapagliflozin [8,83,90]		SGLT-2 inhibition	Phase 3 ongoing
Ruboxistaurin [94]		PKC-β inhibition and oxidative stress reduction	Phase 2 completed
Linagliptin [78]		DPP-4 inhibition	Under clinical trial
Saxagliptin [9]		DPP-4 inhibition	Under clinical trial
Atrasentan [9,44,78]		Selective ETA antagonism	Phase 3 ongoing
Pyridoxamine [9, 13,78]		Blocking AGEs synthesis, and scavenging free radicals and carbonyl products	Phase 3 completed in 2017
Baricitinib [9,78,107]		Selective JAK-1 and JAK-2 inhibition	Phase 2 completed in 2017
Pentoxifylline [108-112]		Phosphodiesterase inhibition	Under clinical trial
Sulodexide [9,78]		Restoration of anionic heparan sulphate charges at the glomerular basement membrane	Phase 4 terminated in 2008
Bardoxolone methyl [9,78]		NRF2 activation and NF-κB pathway inhibition	Phase 2 completed in 2017
Pirfenidone [9,78]		Blocking TGF-β	Phase 1 & 2 completed in 2009
Sacubitril* [1]		NEP inhibition	Phase 3 ongoing

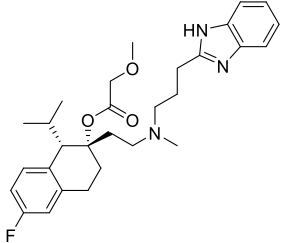
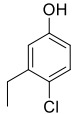
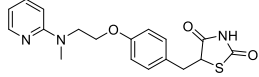
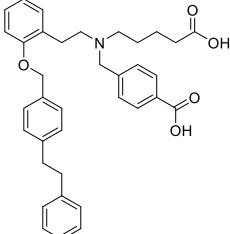
Valsartan* [1]		NEP inhibition	Phase 3 ongoing
Epalrestat [131]		Aldose reductase inhibitor	Under clinical trial

*Both drugs have tested clinically in combination form as LCZ696.

RAAS: renin angiotensin aldosterone system; SGLT2: sodium glucose cotransporter 2; DPP-4: dipeptidyl peptidase-4; AGE: advanced glycation end products; ETA: endothelin receptor A; PKC: Protein kinase C; JAK: Janus kinase; NEP: neprilysin; NRF2: Nuclear factor (erythroid-derived 2)-like 2; NF-κB: nuclear factor κB; TGF: transforming growth factor.

Table 2. List of compounds in preclinical development for the treatment of DKD.

Name and References	Drug Chemical Structure	Therapeutic target / mechanism	Clinical Status
Sodium butyrate and Valproate [21-23]		HDAC inhibition	Preclinical stage
Resveratrol [72]		Autophagy stimulation to protect podocyte apoptosis	Preclinical stage
Astragaloside IV [73]		Attenuation of ER stress and promotion of autophagy in podocyte	Preclinical stage
Ascorbic acid [118]		Antioxidant	Preclinical and clinical stage

<p>Sodium hydrogen sulfide (H₂S donor) [119-121]</p>	<p>NaSH</p>	<p>Antioxidant, reduction of albuminuria, oxidative stress, RAAS products and renal fibrosis</p>	<p>Preclinical stage</p>
<p>Mibefradil [125]</p>		<p>T-type Ca²⁺ channel blocker, ORAI channels inhibitor</p>	<p>Preclinical stage</p>
<p>4-Chloro-3-ethylphenol [126]</p>		<p>Ryanodine receptor agonist, ORAI channels inhibitor</p>	<p>Preclinical stage</p>
<p>Rosiglitazone [9,78, 130]</p>		<p>PPARγ activation</p>	<p>Preclinical stage</p>
<p>Cinaciguat [1,13, 130]</p>		<p>sGC activation</p>	<p>Preclinical/early clinical stage</p>

HDAC: histone deacetylase; ER: endoplasmic reticulum; RAAS: renin angiotensin aldosterone system; PPAR: peroxisome proliferator-activated receptor; sGC: soluble guanylate cyclase.