New updates in diabetic kidney disease

Adenosine receptors as emerging therapeutic targets for diabetic kidney disease
Aims and Scope

Kidney Research and Clinical Practice (KRCP; formerly The Korean Journal of Nephrology; ISSN 1975-9460, launched in 1982), the official journal of the Korean Society of Nephrology, is an international, peer-reviewed journal published in English. Its ISO abbreviation is Kidney Res Clin Pract.

The journal considers articles on all aspects of nephrology and hypertension as well as molecular genetics, anatomy, pathology, physiology, pharmacology, and immunology related to kidney disease. In particular, the journal focuses on translational renal research that helps bridging laboratory discovery with the diagnosis and treatment of human kidney disease. The journal publishes the topics covered basic science with possible clinical applicability and the papers on the pathophysiological basis of the kidney disease. Original studies from areas of diagnostic and interventional nephrology or dialysis access are also welcomed. Major article types considered for publication include original research and reviews on current topics of interest.

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This journal was supported by the Korean Federation of Science and Technology Societies Grant funded by the Korean Government (Ministry of Education).

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Publisher The Korean Society of Nephrology
Editor-in-chief Tae-Hyun Yoo, MD., PhD

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Published on September 30, 2022

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The image on the front cover: Pak et al reported the roles of adenosine receptors as emerging therapeutic targets for diabetic kidney disease. They demonstrated the expression of adenosine receptors in the kidney. They also summarized the roles of various adenosine receptors in diabetic kidney disease. Please see the text for more details (pp. S74-S88).
Diabetic kidney disease, revisited: where do we stand?

Dae Ryong Cha

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Chronic kidney disease (CKD) is rapidly becoming a socio-economic and great public health problem in the world. Moreover, increased life expectancy and the rapid increase in diabetes mellitus lead to a high incidence of diabetic kidney disease (DKD) which is the leading cause of end-stage renal disease and the main disease for renal replacement therapy. DKD is a major cause of morbidity and mortality in diabetic patients worldwide. Since the complexity of mechanisms of DKD and heterogeneous clinical presentation, there are still high medical unmet needs for the management of DKD. However, recent major clinical trials propose some promise in this field. Most remarkable novel treatments that reduce the progression of DKD and improve the risk of cardiovascular events in diabetic patients with CKD are sodium-glucose cotransporter 2 (SGLT-2) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists, and the selective nonsteroidal mineralocorticoid receptor antagonist (MRA).

However, the residual risk for progression of renal disease is still high and the rate of progression is still higher than the rate for the normal aging process. Although there is huge progress in the management of blood pressure and hyperglycemia, there are still controversies about the optimal target of blood pressure and glycemic management, especially in patients with advanced CKD.

In addition, different response to treatment is due to the heterogeneity of DKD, and new strategies to improve the management of DKD suggest novel biomarker that predicts the progression and treatment response.

This special issue of Kidney Research and Clinical Practice contains five different topics for the recent update of DKD. Tong and Adler [1] describe the comprehensive approach to the management of DKD to reduce the progression of renal disease and minimize the risk for cardiovascular events based on the latest published data. Lee et al. [2] gives an overview of the role of NADPH oxidase in DKD, and offer a theoretical basis for the development of various Nox inhibitor as a regulator of reactive oxygen species homeostasis to provide an emerging new therapeutic approach for DKD. Pak et al. [3] present the role of adenosine and adenosine receptor (AR) in the development and progression of DKD. They propose AR modulator as a new therapeutic option to treat DKD based on the mechanistic studies on the pharmacology of AR modulators. Maruno et al. [4] describe the novel therapeutic medications for DKD classified by their pathophysiological targets. Jung and Yoo [5] provide the biomarkers used in current clinical practice and summarize the promising novel biomarkers for DKD based on protein biomarkers, proteomics, metabolomics, and transcriptomics.

Five publications address important issues relevant to DKD. The articles in this special issue demonstrate the high
scientific level in this field and encourage us to continue future clinical and experimental research activities in this field. Based on these considerations, we have assembled leading world experts to prepare this Supplement issue to *Kidney Research and Clinical Practice* by both nephrologists and basic scientists in an attempt to illustrate the current and new diagnostic and therapeutic strategies.

Guest Editor of *Kidney Research and Clinical Practice*
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**References**

Diabetic kidney disease is the leading cause of end-stage kidney disease, and it remains a major challenge. Many factors, such as glomerular hyperfiltration, oxidative stress, inflammation, hypoxia, and epigenetics, are associated with the progression of diabetic kidney disease; however, the whole mechanism is not yet completely understood. No specific treatment for diabetic kidney disease has been established, so new approaches are being explored extensively. Sodium-glucose cotransporter 2 inhibitors have shown renoprotective effects in several human clinical trials. Glucagon-like peptide 1 receptor agonists and mineralocorticoid receptor antagonists have been reported to be effective in diabetic kidney disease, and novel therapeutic candidates are also being examined. In the TSUBAKI trial, a nuclear factor erythroid 2-related factor 2 activator, bardoxolone methyl, improved the glomerular filtration rate of diabetic kidney disease patients. Similarly, new agents that act in the oxidative stress and inflammation pathways are of major interest, such as pentoxifylline, apoptosis signal-regulating kinase-1 inhibitors, C-C chemokine receptor 2 inhibitors, and Janus kinase-1/2 inhibitors. Endothelin-1 receptor A antagonists and soluble guanylate cyclase stimulators are also expected to affect renal hemodynamics. Some preclinical studies suggest that hypoxia-inducible factor prolyl hydroxylase inhibitors, which influence multiple inflammations and oxidative stress pathways, reduce albuminuria in diabetic kidney disease. Advanced glycation end-product inhibitors and treatments related to epigenetics have also shown promise as potential diabetic kidney disease treatments in preclinical studies. The discovery of new targets could provide new therapeutic options for overcoming diabetic kidney disease.

**Keywords:** Bardoxolone methyl, Diabetic nephropathies, Endothelin A receptor antagonists, Chronic kidney failure, Pentoxifylline

**Introduction**

Diabetic kidney disease (DKD) is a major complication of diabetes mellitus that can lead to end-stage kidney disease (ESKD), cardiovascular disease, and high mortality [1]. Conventionally, chronic kidney disease (CKD) caused by diabetes mellitus is diagnosed as diabetic nephropathy. The typical clinical manifestation starts with glomerular hypertrophy and hyperfiltration, followed by microalbuminuria that progresses to macroalbuminuria [2]. Subsequently, kidney function declines gradually, leading to ESKD. DKD is characterized histologically by mesangial matrix expansion, thickening of the glomerular basement membrane, and the presence of nodular lesions [3]. However, in recent years, it has become apparent that some patients with diabetes mellitus present with a low glomerular filtration rate (GFR) without albuminuria [4]. The redefined term ‘DKD’ includes not only the conventional proteinuric nephropathy of diabetes mellitus but also nephropathy without overt albuminuria. In this context, it...
is assumed that the diabetic population is aging, and the prevalence of comorbidities such as hypertension and dyslipidemia, which increase nephrosclerosis, is growing. Until recently, managing blood glucose and blood pressure with renin-angiotensin system (RAS) inhibitors (such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers [ARBs]), lipid control, and weight management have been the standard therapeutic strategy for DKD. However, even that comprehensive treatment cannot entirely arrest the progression of DKD to ESKD (residual risk), and the prevalence of DKD among diabetic patients has not changed significantly during the past decade [4].

The approach to glycemic control in patients with DKD has started to change. Dipeptidyl peptidase-4 inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists, which are both incretin-related drugs, appeared as novel hypoglycemic agents in the 2000s, and they were followed by sodium-glucose cotransporter 2 (SGLT2) inhibitors. Recent clinical trials have revealed that some of those agents not only lower blood glucose levels but also protect renal function.

Additional novel therapeutic agents have been developed and examined in animal models and clinical trials. Here, we review the pathophysiology and recently revealed effects of existing drugs and outline prospective therapeutic candidates for DKD.

**Pathophysiology of diabetic kidney disease**

The pathophysiology of DKD is multifactorial, complex, and not fully understood. Many new agents in use and development focus on specific mechanisms and relevant molecular pathways, as shown in Fig. 1. In this section, we review the pathophysiology of DKD by dividing it into several factors.

**Glomerular hypertension and hyperfiltration**

In the early phase, hyperglycemia causes glomerular hyperfiltration and hyperfiltration of the kidneys. Glomerular hyperfiltration is suspected to be a risk factor for albuminuria and future decreases in the estimated GFR (eGFR) [5]. Increased glomerular capillary pressure heightens the tensile stress on the capillary wall, and the consequent increase

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**Figure 1. Novel therapeutic medications for DKD classified by their pathophysiological targets.**

AGE, advanced glycation end-product; ASK-1, apoptosis signal-regulating kinase 1; CCR2, C-C chemokine receptor 2; DKD, diabetic kidney disease; ET, endothelin; GLP, glucagon-like peptide; HIF-PH, hypoxia-inducible factor prolyl hydroxylase; JAK, Janus kinase; MRA, mineralocorticoid receptor antagonist; sGC, soluble guanylate cyclase; SGLT2, sodium-glucose cotransporter 2.
in ultrafiltrate flow into Bowman’s space elevates the shear stress on the podocyte foot processes and body surface. These mechanical stresses induce an increase in the glomerular basement membrane length, podocyte hypertrophy, and eventually podocyte loss and segmental sclerosis. Hyperfiltration and the consequent increase in proximal tubular flow increase the delivery and reabsorption of small- and large-molecular-weight solute, with ensuing tubulointerstitial inflammation, hypoxia, and fibrosis [6].

The mechanisms of hyperfiltration can be explained in three ways. First, a kidney with DKD becomes large due to an expansion in nephron size caused by various cytokines and growth factors in response to hyperglycemia. The increased kidney size and filtration surface area per glomerulus are thus related to hyperfiltration. Second, the imbalance of vasoactive factors controls efferent and afferent arteriolar constriction and dilatation. For example, angiotensin II and endothelin-1 (ET-1) mediate glomerular hyperfiltration by increasing efferent arteriolar resistance [5]. Third, glomerular hyperfiltration is caused by dysregulated tubuloglomerular feedback. Under hyperglycemic conditions, excessive glucose is filtered at the glomeruli and reabsorbed via SGLTs in the proximal tubules. Sodium is also reabsorbed with glucose at SGLTs, so the amount of sodium that reaches the distal tubular macula densa decreases. To keep the amount of sodium constant, a feedback mechanism is set in motion such that afferent arterioles expand while efferent arterioles contract relatively, resulting in glomerular hypertension and hyperfiltration. These three mechanisms are thought to be the major causes of hyperfiltration.

**Oxidative stress and inflammation**

Oxidative stress and inflammation also play central roles in the progression of DKD. Hyperglycemia activates several intracellular metabolic pathways: the polyol pathway, the advanced glycation end-product (AGE) formation, the activation of protein kinase C (PKC) isoforms, and the hexosamine pathway, all of which lead to the generation of reactive oxygen species (ROS) [7].

The PKC pathway also stimulates nuclear factor kappa B (NF-κB) and releases tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [8]. In addition, PKC activation has various pathogenic consequences by affecting the expression of endothelial nitric oxide synthetase (eNOS), ET-1, vascular endothelial growth factor, transforming growth factor-β (TGF-β), and plasminogen activator inhibitor-1 [7].

Chronic hyperglycemia increases the expression and activity of various nicotinamide adenine dinucleotide phosphate oxidase (NOX) isoforms and generates high levels of ROS. Among the NOX isoforms, NOX4- and NOX5-derived ROS have been suggested to mediate glomerular injury and podocytopathy, leading to albuminuria in DKD [9]. Increased angiotensin II levels induce oxidative stress through NOX activation [10]. PKC-α and PKC-β also play important roles in diabetic renal pathology via NOX activation [9].

Mitochondrial dysfunction is another source of ROS. Under hyperglycemic conditions, more glucose-derived pyruvate is oxidized in the mitochondria, which pushes more electron donors into the electron transport chain and leads to electron leakage, resulting in the overproduction of superoxide [11].

Excessive ROS production leads to renal fibrosis and inflammation and induces tissue damage through different mechanisms, including lipid peroxidation, DNA damage, protein modification, and mitochondrial dysfunction [12].

Hyperglycemia induces AGEs, which are the final products of non-enzymatic glycation and accumulate in CKD patients because of reduced clearance [13]. AGEs activate various pathways, such as the phosphatidylinositol 3-kinase, Janus kinases (JAK), and mitogen-activated protein kinase (MAPK) pathways, by activating AGE receptors (RAGE). RAGE activation also increases oxidative stress and activates NF-κB [14].

Many more pathways interact and are related to oxidative stress and inflammation in ways that affect DKD progression, and new medications targeting those mechanisms have been developed (Table 1).

**Hypoxia**

Renal hypoxia is an important factor in DKD progression and a common final pathway to ESKD, regardless of the cause of CKD. However, this condition is exacerbated in DKD due to the mismatch between oxygen demand and supply. Oxygen demand increases due to glomerular hyperfiltration and elevated activity of SGLT2 under hyperglycemic conditions. Oxidative stress also induces renal fibro-
sis and contributes to renal hypoxia by decreasing oxygen delivery. Oxidative stress increases intracellular oxygen consumption, resulting in a demand/supply mismatch for oxygen and progressive hypoxia.

Epigenetics

Epigenetics is a regulatory mechanism for controlling gene expression without changing DNA sequences. This mechanism includes chromatin histone modifications, DNA methylation, and noncoding RNAs. Metabolic memory is a phenomenon by which DKD-related gene and phenotype expression caused by prior hyperglycemia continue even after blood sugar levels become normal in response to appropriate treatment. Epigenetic mechanisms contribute to metabolic memory by inducing the persistent expression of pathogenic genes, even after normoglycemia has been established [15].

Table 1. Novel anti-inflammatory therapeutic agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Anti-inflammatory effect</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bardoxolone methyl</td>
<td>Activation of Nrf2</td>
<td>Improved GFR</td>
<td>[48]</td>
</tr>
<tr>
<td>Pentoxifyline</td>
<td>Inhibition of PDE and activation of protein kinase A</td>
<td>Slowed decline of eGFR and reduced albuminuria</td>
<td>[58]</td>
</tr>
<tr>
<td>ASK-1 inhibitors</td>
<td>Downregulation of p38 MAPK and JNK signaling</td>
<td>Slowed decline of eGFR except for the first 4 weeks</td>
<td>[62]</td>
</tr>
<tr>
<td>CCR2 inhibitors</td>
<td>Inhibition of CCR2</td>
<td>Reduced albuminuria</td>
<td>[65]</td>
</tr>
<tr>
<td>JAK-1/2 inhibitors</td>
<td>Inhibition of JAK-STAT signaling</td>
<td>Reduced albuminuria</td>
<td>[69]</td>
</tr>
</tbody>
</table>

ASK-1, apoptosis signal-regulating kinase 1; CCR2, C-C chemokine receptor 2; eGFR, estimated GFR; GFR, glomerular filtration rate; JAK, Janus kinase; STAT, signal transducer and activator of transcription; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; Nrf2, nuclear factor erythroid 2-related factor 2; PDE, phosphodiesterase.

Renoprotective effects of sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide 1 receptor agonists, and mineralocorticoid receptor antagonists

To date, RAS inhibitors have been at the center of DKD treatment because they reduce albuminuria and slow the decline in eGFR. RAS inhibitors function mainly by reducing intraglomerular pressure and hyperfiltration and repressing oxidative stress. Their effects in DKD patients have been demonstrated in several randomized clinical trials, such as the collaborative study, RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan), and IDNT (Irbesartan Diabetic Nephropathy Trial) [16–18]. However, RAS inhibitors cannot completely halt DKD progression. Intensive RAS inhibition was examined in combination with ACE inhibitors and ARBs in the ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global End-point Trial), but the combination therapy significantly increased the worsening of kidney function and DKD-related mortality [19]. In the VA NEPHRON-D (Veterans Affairs Nephropathy in Diabetes) trial, another combination therapy of ACE inhibitors and ARBs also showed increased adverse events, hyperkalemia, and acute kidney injury [20].

Novel treatments have been explored for a long time, and the renoprotective effects of existing drugs have recently attracted attention. The effectiveness of SGLT2 inhibitors has been demonstrated, and SGLT2 inhibitors have been approved for CKD treatment. The effects of GLP-1 receptor agonists and mineralocorticoid receptor antagonists (MRAs) have also been explored, and we review those results next.

Sodium-glucose cotransporter 2 inhibitors

SGLT2 is a cotransporter that transports sodium and glucose using a sodium gradient established by the Na+/K+ ATPase pump. SGLT2 inhibitors are hypoglycemic agents that suppress glucose reabsorption in the proximal tubules and stimulate glucose excretion in the urine. SGLT2 inhibitors also suppress the reabsorption of sodium along with glucose, which corrects the hyperglycemia-induced disorder of tubuloglomerular feedback and ameliorates glomerular hyperfiltration in DKD. SGLT2 inhibitors reduce mitochondrial damage, NOX4 expression, and subsequent oxidative stress [21,22]. In the kidneys of diabetic mice, SGLT2 inhibitors normalized glucose metabolism by eliminating accumulated tricarboxylic acid cycle intermediates, which suppressed the increase in oxidative stress [23]. SGLT2 inhibitors also improve renal hypoxia. Oxygen consumption
in the kidney is attributed to Na⁺/K⁺ ATPase, and SGLT2 inhibitors can suppress the increase in Na⁺/K⁺ ATPase activity caused by SGLT2 [24]. SGLT2 inhibitors increase the production of ketone bodies, which can produce more ATP than glucose or fatty acids with a small amount of oxygen. SGLT2 inhibitors were reported to improve anemia [25] and ketone bodies can also correct the hyperactivation of the mechanistic target of rapamycin complex 1 [26], which produces further renoprotective effects.

The renoprotective effects of SGLT2 inhibitors in established DKD were demonstrated in the CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) trial of canagliflozin [27]. Recently, the DAPA-CKD (Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease) trial, a phase 3 clinical trial of dapagliflozin, showed that in CKD patients with or without type 2 diabetes mellitus, dapagliflozin significantly lowered the composite renal risk of a sustained decline in eGFR of at least 50%, ESKD, and death from renal or cardiovascular causes compared with those who received placebo [28]. Based on those results, the U.S. Food and Drug Administration approved dapagliflozin for CKD treatment. This is the first SGLT2 inhibitor to be approved to treat CKD. The EMPA-KIDNEY (the Study of Heart and Kidney Protection With Empagliflozin) trial, a phase 3 study of empagliflozin, is in progress to investigate the effects of empagliflozin on kidney disease progression and cardiovascular death in CKD patients. It is encouraging that SGLT2, originally developed as a hypoglycemic agent, now provides an additional choice for CKD treatment.

Glucagon-like peptide 1 receptor agonists

GLP-1 is an incretin hormone that stimulates insulin secretion and suppresses glucagon secretion in response to food intake. GLP-1 receptor agonists have been used as hypoglycemic agents, and their potential to offer renal protection is of great interest.

GLP-1 receptor agonists inhibit Na⁺/H⁺ exchanger isoform 3 and reduce proximal sodium reabsorption [29]. As with SGLT2 inhibitors, GLP-1 receptor agonists relieve hyperfiltration by correcting abnormal tubuloglomerular feedback. GLP-1 receptor agonist have also been reported to have antioxidative and anti-inflammatory effects in animal models. In a rat model of streptozotocin-induced type 1 diabetes mellitus, activation of the GLP-1 receptor with exendin-4 ameliorated albuminuria, glomerular hyperfiltration, glomerular hypertrophy, and mesangial matrix expansion without changing blood pressure or body weight. Exendin-4 also prevented macrophage infiltration and decreased protein levels of intercellular adhesion molecule-1 (ICAM1) and type IV collagen, as well as decreasing oxidative stress and NF-κB activation in kidney tissue [30].

In the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) and SUSTAIN-6 (Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes) trials, liraglutide and semaglutide, respectively, were shown to reduce the frequency of cardiovascular events in type 2 diabetes mellitus patients with high cardiovascular risk [31,32]. A significant reduction in negative renal outcomes was observed in both trials, but they were mainly due to a decrease in new-onset persistent macroalbuminuria; the effects of those agents on hard renal endpoints remain elusive [32,33]. The AWARD-7 (Dulaglutide Versus Insulin Glargine in Patients With Type 2 Diabetes and Moderate or Severe CKD) trial showed that 52 weeks of once-weekly dulaglutide treatment significantly reduced eGFR decline compared with insulin glargine in patients with type 2 diabetes and moderate-to-severe CKD [34]. The AMPLITUDE-O (Cardiovascular and Renal Outcomes with Efpeglenatide in Type 2 Diabetes) trial demonstrated that weekly subcutaneous injections of efpeglenatide, an exendin-based GLP-1 receptor agonist, lowered the risk of cardiovascular events in patients with type 2 diabetes who had either a history of cardiovascular disease or current kidney disease plus at least one other cardiovascular risk factor [35]. Additionally, efpeglenatide led to a 32% lower risk of composite renal outcomes (a decrease in kidney function or macroalbuminuria) than placebo in that trial. Currently, the FLOW (Effect of Semaglutide Versus Placebo on the Progression of Renal Impairment in Subjects With Type 2 Diabetes and Chronic Kidney Disease) trial is progressing to examine the 5-year use of semaglutide, which might clarify the hard renal outcomes of long-term treatment.

Mineralocorticoid receptor antagonists

MRAs, such as spironolactone and eplerenone, are widely used as antihypertensive drugs and work by suppressing...
Aldosterone activity. Preclinical data showed that aldosterone increases mineralocorticoid receptor-dependent NOX activity and ROS, induces a proinflammatory state, stimulates fibrosis, regulates vascular tone, and affects renal hemodynamics [36]. Addition of spironolactone on the RAS blockade has been shown to decrease albuminuria in DKD [37]. However, because of the risk of hyperkalemia, its use is limited in CKD patients.

Recently, nonsteroidal MRAs, esaxerenone, finerenone, and uppatroone, have been developed. They have higher selectivity and affinity to mineralocorticoid receptors than steroidal MRAs such as spironolactone and eplerenone, which might lower the risk of hyperkalemia.

A phase 3 clinical trial of esaxerenone, ESAX-DN (Esaxerenone [CS-3150] in Patients with Type 2 Diabetes and Microalbuminuria) trial, demonstrated that adding esaxerenone to existing RAS inhibitor therapy in patients with type 2 diabetes and microalbuminuria increased the likelihood that albuminuria would return to normal levels and reduced the progression of albuminuria to higher levels [38]. A phase 3 trial of finerenone, FIDELIO-DKD (Finnenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease) trial, confirmed that in patients with CKD and type 2 diabetes, treatment with finerenone lowered the risk of CKD progression, including kidney failure, a sustained decrease of ≥40% in the eGFR from baseline, and death from renal causes, compared with placebo [39]. Hyperkalemia was observed as an adverse event, but its frequency was markedly lower than in trials of a dual RAS blockade. We need to pay attention to hyperkalemia, but nonsteroidal MRAs could be an additional option for DKD treatment.

Finnerone also reduced the risk of cardiovascular events, and a reduced risk of death from cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, and hospitalization for heart failure was observed in a wide range of CKD patients with type 2 diabetes (FIGARO-DKD [Finerenone in Reducing Cardiovascular Mortality and Morbidity in Diabetic Kidney Disease] trial) [40]. In the FIGARO-DKD trial, there was no significant difference between the finerenone and placebo groups in the incidence of the first secondary composite renal outcome of kidney failure, a sustained decrease from baseline of at least 40% in the eGFR, or death from renal causes. The mean eGFR of enrolled patients was higher in the FIGARO-DKD trial than the FIDELIO-DKD trial, so more time would be needed to achieve the secondary outcome in the FIGARO-DKD trial, which might explain why it could not confirm a significant difference in the incidence of the first secondary composite renal outcome. Nonetheless, the effects of finerenone treatment on the kidney were similar in the FIGARO-DKD and FIDELIO-DKD trials.

**New molecular targets and treatment options**

Many new prospective therapeutic agents have been developed, and some of them have been subjected to clinical trials.

**Bardoxolone methyl**

Treatment of DKD has focused on suppressing the decline in kidney function. Bardoxolone methyl was the first agent to show an improvement in GFR in clinical trials, which has attracted attention.

Bardoxolone methyl is a synthetic triterpenoid that activates the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which has anti-inflammatory and antioxidant effects. Kelch-like ECH-associated protein 1 (Keap1) forms an ubiquitin E3 ligase complex with Cul-3 and polyubiquitinitates Nrf2 in the cytoplasm. Under normal conditions, Nrf2 is constantly degraded. However, under oxidative stress, the reactive cysteine residues of Keap1 are directly modified, and ubiquitin E3 ligase activity decreases, which leads to the stabilization of Nrf2. Nrf2 then translocates into the nucleus and binds to antioxidants response elements with small musculoaponeurotic fibrosarcoma and induces the expression of its target genes [41]. Nrf2 regulates the expression of more than 250 genes, including those encoding antioxidant and phase 2 detoxifying enzymes and related proteins, such as catalase, superoxide dismutase, uridine 5’-diphospho-glucuronosyltransferase, NAD(P)H: quinone oxidoreductase-1, heme oxygenase-1, glutamate cysteine ligase, glutathione S-transferase, glutathione peroxidase, and thioredoxin [42]. Bardoxolone methyl induces conformational changes in Keap1 that enable Nrf2 to translocate into the nucleus and exhibit its anti-inflammatory and antioxidant effects. It was reported that Nrf2 suppressed the macrophage inflammatory response by blocking the transcription of proinflam-
matory cytokines [43]. Bardoxolone methyl also blocks the NF-κB pathway by suppressing the inhibitor of the NF-κB kinase subunit β [44].

Bardoxolone methyl was first developed as an antitumor agent. However, in a clinical trial for malignancy, it was found to increase eGFR, so the study of it expanded to the renal area. Bardoxolone methyl is related to anti-inflammatory and antioxidant factors; therefore, its efficacy in DKD was considered because both factors affect DKD progression. In the phase 2 BEAM (52-Week Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes) clinical trial, bardoxolone methyl significantly increased the eGFR of patients with type 2 diabetes and CKD whose eGFR was 20 to 45 mL per minute per 1.73 m² of body surface area [45]. However, the phase 3 BEACON (Bardoxolone Methyl Evaluation in Patients with CKD and Type 2 Diabetes Mellitus: the Occurrence of Renal Events) clinical trial enrolling patients with type 2 diabetes and stage 4 CKD was terminated prematurely because of the high rate of cardiovascular events in patients receiving bardoxolone methyl [46]. Subsequent analyses revealed that elevated baseline B-type natriuretic peptide and prior hospitalization for heart failure were risk factors for cardiovascular events associated with bardoxolone methyl treatment [47]. In Japan, another phase 2 clinical trial, the TSUBAKI (The Phase 2 Study of Bardoxolone Methyl in Patients with CKD and Type 2 Diabetes) trial, was initiated and enrolled only type 2 diabetes and CKD stage 3–4 patients without previous cardiovascular risk factors [48]. The bardoxolone methyl group in that trial saw significant increases in GFR measured by inulin clearance without any cardiovascular deaths or cases of heart failure. That study suggested that bardoxolone methyl might be a safe and effective medication for DKD patients free of the relevant risk factors. To examine its safety and efficacy in the longer term, a phase 3 trial, the AYAME (A Phase 3 Study of Bardoxolone Methyl in Patients With Diabetic Kidney Disease) trial, is currently in progress.

Some clinical trials have reported that bardoxolone methyl increases albuminuria [49], possibly as a result of the increase in GFR [50]. Decreasing the reabsorption of albumin at the proximal tubules relieves inflammation of the proximal tubules, which might be related to the improvement in GFR [49,50]. However, it is still not well understood whether the increase in GFR and albuminuria protectively affect DKD over a long period. The AYAME trial is expected to clarify these points.

**Endothelin-1 receptor A antagonists**

ET-1 is a vasoconstrictor peptide that is mainly secreted from endothelial cells and acts through two types of receptors: ET-A and ET-B receptors. ET-1 was reported to constrict afferent arterioles more strongly than efferent arterioles via ET-A receptors in a mouse model [51]. ET-A receptor activation also contributes to pro-apoptosis pathways, leading to the loss of podocytes, albuminuria, and the destruction of glomerular capillaries *in vivo* [52]. ET-1 damages the vasculature, induces fibrosis, and promotes inflammatory cell infiltration, and in mesangial cells, ET-1 causes proliferation and extracellular matrix accumulation [53]. Therefore, it is hypothesized that ET-A receptor antagonists might be therapeutic agents for DKD. However, the ASCEND (A Randomised, Double Blind, Placebo Controlled, Parallel Group Study to Assess the Effect of the Endothelin Receptor Antagonist Avosentan on Time to Doubling of Serum Creatinine, End Stage Renal Disease or Death in Patients With Type 2 Diabetes Mellitus and Diabetic Nephropathy) phase 3 clinical trial was terminated prematurely because of an increased incidence of cardiovascular events in the avosentan group, mainly congestive heart failure and fluid overload [54]. A high dose of avosentan might be inadequately selective for the ET-A receptor and block ET-B receptors which inhibit sodium reabsorption at the proximal tubules and collecting ducts. Based on that result, an antagonist with higher efficacy and selectivity for the ET-A receptor, atrasentan, was examined in the short term and found to reduce albuminuria without causing significant fluid retention [55]. After that, the SONAR phase 3 clinical trial of atrasentan was initiated [56]. In the SONAR (Study Of Diabetic Nephropathy With Atrasentan) trial, responders to atrasentan who saw reduced albuminuria without fluid retention during the initial 6-week open-label period were enrolled and randomized. During a median follow-up of 2.2 years, atrasentan significantly lowered the risk of doubling in serum creatinine and ESKD compared with placebo. Even with that precautionary approach, hospital admission for heart failure was higher in the atrasentan group, indicating the necessity for careful monitoring. ET-1 receptor A antagonists require a strategy...
to control fluid retention to enable safe use.

Currently, sparsentan, a dual-acting angiotensin and endothelin receptor antagonist that can inhibit both ET-A receptors and angiotensin II type 1 receptors of RAS, is being investigated in phase 3 clinical trials against focal segmental glomerulosclerosis (DUPLEX [A Randomized, Multicenter, Double-Blind, Parallel, Active-Control Study Of The Effects Of Sparsentan, A Dual Endothelin Receptor And Angiotensin Receptor Blocker, On Renal Outcomes In Patients With Primary Focal Segmental Glomerulosclerosis] trial) and immunoglobulin A nephritis (PROTECT [A Study of the Effect and Safety of Sparsentan in the Treatment of Patients With IgA Nephropathy] trial). When they become available, those results might provide insight into strategies for controlling fluid retention.

Pentoxifylline

Pentoxifylline is a nonspecific phosphodiesterase (PDE) inhibitor used clinically to treat peripheral vascular diseases. Inhibiting PDE activity increases cyclic adenosine-3,5-monophosphate levels and activates protein kinase A, leading to a reduction in proinflammatory cytokine production. Pentoxifylline modulates levels of TNF-α and other proinflammatory cytokines, including IL-1, IL-6, interferon γ, and other molecules such as ICAM1, vascular cell adhesion molecule 1 (VCAM1), and C-reactive protein [57]. Its anti-inflammatory effect could protect against DKD, and many clinical trials have shown that it has therapeutic effects in DKD. The PREDIAN (Pentoxifylline for Renoprotection in Diabetic Nephropathy) trial in patients with type 2 diabetes and CKD G3–4 taking standard RAS inhibitors reported that 2 years of pentoxifylline treatment significantly slowed the decline in eGFR and decreased albuminuria and urinary excretion of TNF-α [58]. Recently, the proinflammatory cytokines TNF-α and tumor necrosis factor-like weak inducer of apoptosis were reported to reduce renal Klotho expression mediated by NF-κB in mice and cell culture [59]. A recent post hoc analysis of the PREDIAN trial also showed a significant increase in serum and urinary Klotho concentrations [60]. This might also be related to the renal effects of pentoxifylline. A larger randomized clinical trial (gov Identifier: NCT03625648) is ongoing to examine the time to ESKD or death.

**Apoptosis signal-regulating kinase-1 inhibitors**

Apolipoprotein signal-regulating kinase (ASK)-1, a serine/threonine kinase, is an upstream signaling kinase of p38 MAPK and c-Jun N-terminal kinase (JNK). ASK-1 is normally bound and repressed by thioredoxin, an antioxidant protein. However, under oxidative stress, oxidized thioredoxin dissociates from ASK-1, activating it. The downstream p38 MAPK and JNK pathways induce apoptotic, inflammatory, and fibrotic signaling. The increased activation of p38 MAPK in DKD has been demonstrated in human renal biopsy analyses [61]. In an eNOS knockout mouse model of DKD, pharmacological ASK-1 inhibition halted the progressive decline in GFR and decreased albuminuria by reducing apoptosis and fibrosis within the glomerular and tubulointerstitial compartments and preserving podocyte density [61]. A phase 2 clinical trial of selonsertib, a selective ASK-1 inhibitor, found no difference in the primary outcome, change from baseline in eGFR at 48 weeks, between the selonsertib and placebo groups [62]. However, an unanticipated acute decline in eGFR was observed in the first 4 weeks in the 18-mg selonsertib group, which might have confounded the primary outcome. The acute effect of selonsertib on creatinine concentration is thought to be a result of inhibiting multidrug and toxin extrusion proteins 1 and 2 K. A post hoc analysis revealed that the rate of eGFR decline between 4 and 48 weeks was reduced by 71% for the 18-mg selonsertib group relative to the placebo group. The effects on the urine albumin-to-creatinine ratio were not confirmed. The MOSAIC (Study Evaluating the Efficacy and Safety of Selonsertib in Participants With Moderate to Advanced Diabetic Kidney Disease) phase 2b clinical trial is ongoing in type 2 diabetes patients with moderate to advanced DKD to evaluate whether selonsertib can slow the decline in kidney function.

**C-C chemokine receptor 2 inhibitors**

Chemokine C-C motif ligand 2 (CCL2), also known as monocyte chemoattractant protein-1, is a chemoattractant for monocytes, memory T cells, and natural killer cells that works by activating C-C chemokine receptor 2 (CCR2) and CCR4. CCL2 has been suggested as a potential marker for DKD [63]. Inhibiting CCL2 and CCR2 by various methods, such as neutralizing antibodies, receptor antagonists, inhibitors, DNA vaccines, mutant genes, and enantiomeric
RNA oligonucleotides, decreases albuminuria, kidney damage, and inflammation in experimental kidney disease models, including DKD [64]. Some of the tested methods have progressed to clinical trials.

CCX140-B, a selective inhibitor of CCR2, was reported to reduce the urine albumin-to-creatinine ratio after 52 weeks of treatment in patients with type 2 diabetes on RAS inhibitors. In a phase 2 clinical trial, 5 mg of CCX140-B reduced the urine albumin-to-creatinine ratio by 16% compared with placebo without major side effects. The change in eGFR was not significant compared with the placebo group [65].

Inhibition of the CCL2/CCR2 pathway could be a promising therapeutic approach for DKD. However, more studies are needed to evaluate whether it can delay or halt the progression of DKD.

Janus kinase-1/2 inhibitors

The JAK-signal transducer and activator of transcription (STAT) pathway is involved in the transmission of signals such as cytokines and chemokines from extracellular ligands directly to the nucleus and induces a variety of cellular responses. The expression of multiple JAK-STAT family members is increased in the kidneys of DKD patients [66]. Moreover, preclinical experiments have shown that podocyte-specific JAK2 overexpression worsens renal injury [67], and a Stat3 knockdown model reduced albuminuria and renal inflammation in diabetic mice [68]. Based on that evidence for JAK-STAT activation in DKD, a phase 2 clinical trial with the JAK1/2 inhibitor baricitinib was conducted, and 24 weeks of baricitinib treatment significantly reduced albuminuria [69]. With baricitinib treatment, inflammatory biomarkers such as urinary C-X-C motif chemokine 10, urinary CCL2, plasma soluble tumor necrosis factor receptors 1 and 2, serum amyloid A, VCAM1, and ICAM1 decreased dose-dependently from baseline. The reduction in local inflammation might ameliorate DKD progression. However, no change in kidney function could be measured by serum creatinine, 24-hour urine creatinine clearance, or cystatin C-based eGFR. Furthermore, at 6 months, the high-dose baricitinib group had a statistically significant decrease in hemoglobin compared with the placebo group, as an important adverse event. Anemia might be attributed to a further decrease in the action of erythropoietin by JAK2 inhibition in those who already have low erythropoietin levels.

Other important regulators of JAK-STAT signaling are suppressors of cytokine signaling (SOCS). Enhancement of SOCS expression inhibits JAK-STAT signaling and could help prevent DKD progression [70].

Soluble guanylate cyclase stimulators and activators

Soluble guanylate cyclase (sGC) forms a heterodimer consisting of two subunits, α and β. Nitric oxide (NO) activates sGC by binding to the heme group present on the β-subunit, leading to increased cyclic guanosine monophosphate (cGMP). Increased cGMP promotes vasodilation and inhibits smooth muscle proliferation, leukocyte recruitment, platelet aggregation, and vascular remodeling [71]. NO/cGMP regulates renal blood flow, and increased cGMP dilates efferent arterioles, which seems to be beneficial for the prevention of glomerular damage and glomerulosclerosis. Increasing NO/sGC/cGMP signaling could inhibit kidney inflammation and fibrosis [72], potentially preventing or slowing the progression of DKD.

sGC stimulators and activators differ depending on the target. Heme in sGC is prone to oxidation under oxidative stress, which produces a heme-free form of sGC. sGC stimulators and sGC activators stimulate heme-containing sGC and heme-free sGC, respectively, in a NO-independent manner. Activating sGC signaling with cinaciguat, an sGC activator, markedly improved GFR, serum creatinine, mesangial expansion, and kidney fibrosis in streptozocin-induced diabetic eNOS knockout mice [73]. sGC activation by cinaciguat restored the glomerular cGMP content and reduced TGF-β1 expression and ERK1/2 phosphorylation, attenuating podocyte injury, proteinuria, glomerular cell proliferation, and apoptosis in a rat model of type-1 diabetes [74]. In a phase 2 trial, the sGC stimulator praliciguat did not significantly reduce albuminuria compared with placebo. On the other hand, praliciguat was associated with a modest reduction in blood pressure, hemoglobin A1c, and cholesterol, which could support further investigation of praliciguat in DKD [75].

Other medications in preclinical studies

Many other medications that target molecules related to DKD pathology are in development, and some of them have
demonstrated promising effects in animal experiments. For example, hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitors reverse the energy metabolism alterations that occur in the early stages of DKD in rats and mice and ameliorate the accumulation of glycolysis and tricarboxylic acid cycle metabolites in diabetic renal cortical tissue, which is thought to affect the progression of DKD [76]. In an obese type 2 diabetic mouse model, HIF-PH inhibitors improved insulin sensitivity, decreased albuminuria, and ameliorated glomerular epithelial and endothelial damage [77].

AGE-RAGE inhibitory modalities have therapeutic potential, but their clinical efficacy awaits confirmation. Similarly, approaches targeting epigenetics, such as histone demethylase inhibitors, DNA methyltransferase inhibitors, and pharmacological silencing of micro RNAs are under investigation, and some of them have shown renoprotective properties in animal experiments.

**Conclusion**

RAS inhibitors have been at the center of DKD treatment for a long time. Recent cardiovascular outcome trials of glucose-lowering agents, such as SGLT2 inhibitors and GLP-1 receptor agonists, have established the renoprotective roles of those agents. The safety and efficacy of nonsteroidal MRAs are being tested in large clinical trials. Uncovering the pathophysiology of DKD could allow the identification of novel therapeutic targets by which to prevent or slow the progression of DKD.

**Conflicts of interest**

Tetsuhiro Tanaka received personal fees from Kyowa Kirin, AstraZeneca, Mitsubishi Tanabe, and Bayer. Masaomi Nangaku has received personal fees from Kyowa Kirin, Boehringer-Ingelheim, Bayer, Astellas, Mitsubishi Tanabe, Takeda, Torii, JT, Novo Nordisk, GSK, Daiichi-Sankyo, Ono, Chugai, and AstraZeneca. All authors have no other conflicts of interest to declare.

**Funding**

This work was supported by a Grant-in-Aid for Scientific Research KAKENHI grant (20K08626 to TT) from the Japan Society for the Promotion of Science.

**Authors’ contributions**

Writing–original draft: SM
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Novel biomarkers for diabetic kidney disease

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Although diabetic kidney disease (DKD) remains one of the leading causes of reduced lifespan in patients with diabetes mellitus; its prevalence has failed to decline over the past 30 years. To identify those at high risk of developing DKD and disease progression at an early stage, extensive research has been ongoing in the search for prognostic and surrogate endpoint biomarkers for DKD. Although biomarkers are not used routinely in clinical practice or prospective clinical trials, many biomarkers have been developed to improve the early identification and prognostication of patients with DKD. Novel biomarkers that capture one specific mechanism of the DKD disease process have been developed, and studies have evaluated the prognostic value of assay-based biomarkers either in small sets or in combinations involving multiple biomarkers. More recently, several studies have assessed the prognostic value of omics-based biomarkers that include proteomics, metabolomics, and transcriptomics. This review will first describe the biomarkers used in current practice and their limitations, and then summarize the current status of novel biomarkers for DKD with respect to assay-based protein biomarkers, proteomics, metabolomics, and transcriptomics.

Keywords: Biomarkers, Diabetic nephropathies, Metabolomics, Pathophysiology, Proteomics, Transcriptome

Introduction

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide [1]. Due to its association with a concomitant increase in cardiovascular morbidity and mortality, it remains one of the leading causes of reduced lifespan in people with diabetes mellitus (DM). Given the high risk of progressive deterioration in kidney function eventually leading to ESKD that requires kidney transplantation or chronic kidney replacement therapy, as well as increased risk of cardiovascular morbidity and mortality, early identification and risk stratification of disease is essential. Although our understanding of the pathophysiology of the disease has improved over the years, the prevalence of DKD has not changed significantly over the past 30 years [2].

To reduce disease prevalence and reverse or slow down disease progression, efforts to develop novel drugs have been ongoing, but many phase 3 clinical trials have failed to show any clinically significant findings [1,3]. For example, as oxidative stress is an important pathophysiological process in DKD, antioxidants have been developed to target the pathways of this disease. Although the antioxidant bardoxolone methyl (Reata Pharmaceuticals, Plano,
TX, USA) showed promising phase 2 study results [4], administration of this agent in a phase 3 study showed an increased risk of early-onset fluid overload in patients with risk factors for heart failure [5]. Sulodexide, a purified mixture of sulfated glycosaminoglycan polysaccharides, can ameliorate DKD in animal experiments [6]. However, larger randomized controlled trials failed to demonstrate any beneficial clinical outcomes with the use of this agent [7,8]. As a result, efforts have been ongoing to develop novel biomarkers of DKD that can detect DKD at very early stages, as well as identify high-risk patients that are highly likely to eventually develop ESKD. Such novel biomarkers have also been developed to allow for risk stratification in clinical trials, as well as the development of drugs that specifically target these biomarkers.

This review will first describe current biomarkers used to predict the risk of DKD and disease progression, and then describe the current status of multiple novel biomarkers with respect to assay-based biomarkers, as well as omics-based biomarkers that include proteomics, metabolomics, and transcriptomics.

**Biomarkers used in current practice**

**Markers of kidney function**

The most commonly used current biomarkers of DKD are albuminuria and estimated glomerular filtration rate (eGFR) [9].

Given that albuminuria is an important component of DKD, as well as the strongest predictor of ESKD and cardiovascular morbidity in patients with type 2 DM [10], it is important to establish the degree of albuminuria in patients with DKD at the time of diagnosis. However, a major limitation of albuminuria as a biomarker of DKD is that not all patients with DKD have albuminuria. For example, approximately 30% of patients with DKD do not have albuminuria [11,12]. There is also a growing body of evidence suggesting that patients with type 1 or 2 DM can progress to ESKD in the absence of albuminuria, even after accounting for renoprotective agents [13–18]. In a recent study of 935 patients with type 1 DM and 1,984 patients with type 2 DM, followed for up to 16 years after the development of CKD stage 3, mean annual declines in eGFR for normo-, micro-, and macroalbuminuria for the first 10 years following the development of CKD stage 3 were 1.9, 2.3, and 3.3 mL/min/1.73 m² in type 1 DM, and 1.9, 2.1, and 3.0 mL/min/1.73 m² in type 2 DM, respectively [18]. In patients with normoalbuminuria, two distinct eGFR patterns were found, with one displaying an accelerated rate of eGFR decline. Of note, patients displaying this accelerated rate of eGFR decline were associated with less use of lipid-lowering treatment, renin-angiotensin system (RAS) blockers, and other antihypertensive treatments. These findings suggest that albuminuria has its limitations as a prognostic marker for DKD.

Current eGFR and past glomerular filtration rate (GFR) trajectory are well-established predictors of the future risk of ESKD [19]. Thus, eGFR is the most common prognostic biomarker used for predicting ESKD in both clinical practice and clinical trials. However, similar to albuminuria, eGFR also has important limitations as a prognostic biomarker of DKD. Limitations of using eGFR to predict DKD progression include different equations used to estimate eGFR, which include the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), the Modification of Diet in Renal Disease (MDRD) equation, and eGFR calculated using cystatin C instead of creatinine [14]. The risk of DKD development or progression may be under or overestimated depending on which eGFR equation is used for risk stratification. For instance, in a study to assess population-based incidence rates of CKD in patients with DM depending on the eGFR equation, CKD incidence rates were higher when the MDRD equation, rather than the CKD-EPI equation, was used [20]. Moreover, considering that GFR is the product of the number of nephrons and the mean single nephron GFR, a reduction in the number of nephrons due to the progression of DKD can be compensated by an increase in the single nephron GFRs of surviving nephrons. Such changes may not be accurately reflected by the eGFR, as eGFR decline may only happen when each nephron exceeds its maximal filtration capacity. This is further confounded because the vasodilation of the afferent arteriole in patients with DKD may increase single nephron GFR even in the absence of nephron loss [21]. Thus, patients without actual functional nephron loss and those with actual functional nephron loss, but with compensation in GFR by remnant nephrons, may have identical eGFRs with vastly different prognoses.

As both albuminuria and eGFR have important limita-
tions as prognostic biomarkers of DKD, the identification of novel diagnostic and prognostic biomarkers for the early risk stratification of DKD is much needed. Potential novel biomarkers for DKD are summarized in Table 1.

**Novel biomarkers of diabetic kidney disease—assay based**

Considering the implications of the delayed identification of DKD progression, many studies have investigated potential predictive and prognostic biomarkers [22]. These biomarkers typically capture one specific aspect of the pathophysiology of the disease process such as tubular damage, inflammation, or oxidative stress [23]. However, given that DM is a heterogeneous disease involving multiple pathophysiological mechanisms, using just a single biomarker for the risk stratification of disease progression has several limitations. Single biomarkers have problems with individual, biological, and analytical variability. For example, novel biomarkers such as tissue necrosis factor receptor (TNFR) 1, TNFR2, fibroblast growth factor-23 (FGF-23), and high-density lipoprotein cholesterol are known to predict kidney outcomes in patients with type 2 DM [24–26]. However, TNFR1 and TNFR2 are not specific to type 2 DM, and measurements of FGF-23 may vary according to the choice of the analytical method [27]. Due to such issues, it is more likely that a panel of biomarkers will be needed to predict disease progression.

**Single biomarkers or small sets of biomarkers—assay based**

Single biomarkers typically capture a specific pathophysiological pathway of the DKD process, such as tubular damage, inflammation, oxidative stress, or endothelial dysfunction, whereas others focus primarily on glomerular features such as glycocalyx abnormalities, podocyte damage, or glomerular fibrosis.

**Markers of tubular damage**

Markers of tubular damage include kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and liver fatty acid-binding protein (L-FABP). Urinary concentrations of KIM-1, which is a protein expressed on the apical membrane of the proximal tubule cells, increase in response to acute kidney injury [28]. In a nested case-control study and a prospective cohort study, plasma KIM-1 levels were independently associated with a higher risk of eGFR decline in persons with early or advanced DKD [29]. Similarly, in a case-cohort study of 894 participants with DKD from the Chronic Renal Insufficiency Cohort (CRIC), higher plasma levels of KIM-1 were associated with an increased risk of DKD progression [30]. Another tubular marker that has been extensively studied is NGAL. NGAL is a 25-kDa protein from the lipocalin superfamily that was initially found in activated neutrophils but is also produced by kidney tubular cells in response to tubular injury. Higher levels of urinary NGAL have been shown to precede microalbuminuria in patients with type 1 DM [31,32]. In another study of 117 patients with type 2 DM, higher values of urinary NGAL have been observed in normoalbuminuric type 2 DM patients, and rose progressively in those with micro- and macroalbuminuria, suggesting that tubular damage may occur even in the very early stages of DKD [33]. Urinary L-FABP levels have also been shown to be associated with DKD progression. In patients with type 1 DM, high levels of urinary L-FABP predicted the initiation and progression of DKD and all-cause mortality, independent of the severity of albuminuria and other established risk factors [34]. In another cross-sectional and longitudinal study of 140 patients with type 2 DM without DKD and 412 healthy control subjects, urinary L-FABP levels accurately reflected the severity of DKD, and these levels were particularly high in those with normalalbuminuria [35]. High urinary L-FABP levels were found to be a strong and independent predictor of DKD progression [36].

**Markers of inflammation**

Biomarkers of inflammation such as tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β) were first associated with DM in diabetic mouse models. Macrophages incubated with glomerular basement membranes produced significantly greater levels of both TNF-α and IL-1β than in nondiabetic mice [37]. This has led to further investigations into the use of TNF-α as a predictive marker of DM. Urinary TNF-α excretion and serum TNF-α levels were both found to be elevated in DKD [38]. Further investigations into the receptors that TNF-α bind to, namely TNFR1 and TNFR2,
Table 1. Summary of studies reporting on novel biomarkers for diabetic kidney disease (DKD)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Study</th>
<th>Sample size</th>
<th>Study population</th>
<th>Main results</th>
<th>Adjustments</th>
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<tbody>
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<td>Assay-based novel biomarkers</td>
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<td>Single biomarkers or small sets of biomarkers</td>
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<tr>
<td>Plasma KIM-1, TNFR-1, TNFR-2, and MCP-1</td>
<td>Schrauben et al. [30]</td>
<td>Case-cohort study (n = 894)</td>
<td>Type 1 and 2 DM</td>
<td>Higher plasma levels of KIM-1, TNFR-1, TNFR-2, and MCP-1 were associated with a risk of DKD progression</td>
<td>Age, sex, race/ethnicity, education, clinical center, systolic and diastolic blood pressure, BMI, hsCRP, HbA1c, antihypertensive medication use, smoking status, baseline eGFR, and UPCR</td>
</tr>
<tr>
<td>Urinary KIM-1 and NGAL</td>
<td>de Carvalho et al. [33]</td>
<td>Cross-sectional study (n = 117)</td>
<td>Type 2 DM</td>
<td>Urinary KIM-1 and NGAL were increased in type 2 DM patients with normal or mildly increased albuminuria</td>
<td>HbA1c, LDL cholesterol, fasting glucose, and medication</td>
</tr>
<tr>
<td>Urinary L-FABP</td>
<td>Nielsen et al. [34]</td>
<td>Prospective cohort study (n = 165)</td>
<td>Type 1 DM</td>
<td>High levels of urinary L-FABP predict the initiation and progression to DKD and all-cause mortality</td>
<td>Age, sex, HbA1c, systolic and diastolic blood pressure, albuminuria, serum creatinine, and smoking</td>
</tr>
<tr>
<td>Urinary L-FABP</td>
<td>Kamijo-Ikemori et al. [35]</td>
<td>Cross-sectional study (n = 552)</td>
<td>Type 2 DM</td>
<td>Urinary L-FABP was increased in advanced DKD and was also high in patients with normoalbuminuria</td>
<td>A high value of L-FABP at entry, albuminuria, systolic and diastolic blood pressure, HbA1c, age, sex, and use of RAS inhibitors</td>
</tr>
<tr>
<td>Urinary L-FABP</td>
<td>Panduru et al. [36]</td>
<td>Prospective cohort study (n = 1,549)</td>
<td>Type 1 DM</td>
<td>High urinary L-FABP levels were found to be a strong and independent predictor of DKD progression</td>
<td>Risk factors of DKD and albuminuria</td>
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<tr>
<td>Markers of inflammation</td>
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<tr>
<td>Plasma TNF-α, TNFR1, TNFR2</td>
<td>Niewczas et al. [24]</td>
<td>Prospective cohort study (n = 410)</td>
<td>Type 2 DM</td>
<td>Elevated circulating TNFR levels are strong predictors of progression to ESKD in subjects with and without proteinuria</td>
<td>Age, HbA1c, albuminuria, and eGFR</td>
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<tr>
<td>Serum TNFR</td>
<td>Skupien et al. [39]</td>
<td>Prospective cohort study (n = 349)</td>
<td>Type 1 DM</td>
<td>Circulating TNFR2 is a major determinant of kidney function decline</td>
<td>No adjustments</td>
</tr>
<tr>
<td>Urinary 8-OHdG</td>
<td>Xu et al. [41]</td>
<td>Cross-sectional study (n = 69)</td>
<td>Type 2 DM</td>
<td>Individuals with type 2 DM have higher levels of 8-OHdG compared to healthy individuals</td>
<td>No adjustments</td>
</tr>
<tr>
<td>Plasma 8-OHdG</td>
<td>Sanchez et al. [42]</td>
<td>Prospective cohort study (n = 704)</td>
<td>Type 1 DM</td>
<td>Higher levels of 8-OHdG were associated with an increased risk of kidney disease</td>
<td>Age, sex, cohort, duration of DM, HbA1c, insulin therapy, systolic blood pressure, use of antihypertensive drugs, RAS inhibitors, diabetic retinopathy stage, lipid-lowering drugs, eGFR, and albuminuria</td>
</tr>
<tr>
<td>Urinary 8-OHdG</td>
<td>Serdar et al. [43]</td>
<td>Cross-sectional study (n = 92)</td>
<td>Type 2 DM</td>
<td>Although urinary 8-OHdG levels increase in diabetic patients, their levels do not improve the prediction of progressive DKD by measuring albuminuria</td>
<td>No adjustments</td>
</tr>
<tr>
<td>Markers of glomerular damage</td>
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<tr>
<td>Urinary transferrin</td>
<td>Kanauchi et al. [44]</td>
<td>Cross-sectional study (n = 45)</td>
<td>Type 2 DM</td>
<td>Increased urinary transferrin levels in microalbuminuria patients significantly correlated with kidney biopsy-proven tubulointerstitial injuries</td>
<td>No adjustments</td>
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<td>Urinary transferrin</td>
<td>Sánchez-Hidalgo et al. [45]</td>
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<td>Urinary ceruloplasmin</td>
<td>Narita et al. [46]</td>
<td>Prospective cohort study (n = 140)</td>
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<td>Urinary ceruloplasmin levels were found to be increased in normoalbuminuric DM patients and were highly predictive of the development of microalbuminuria</td>
<td>Age, sex, BMI, DM duration, HbA1c, systolic and diastolic blood pressure, lipid profile, presence of retinopathy, use of antihypertensive drugs, use of insulin, and urinary lab</td>
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<td>Urinary immunoglobulin G and M</td>
<td>Bakoush et al. [47]</td>
<td>Case-control (n = 72)</td>
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<td>Increased urine excretion of immunoglobulin G and M accompanied albuminuria in patients with type 2 DM</td>
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<td>Multiple biomarkers</td>
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<td>Plasma KIM-1, TNFR-1, and TNFR-2</td>
<td>Coca et al. [29]</td>
<td>Nested case-control study (n = 380)</td>
<td>Type 2 DM</td>
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<td>Treatment arm, baseline eGFR, albuminuria, age, race, systolic and diastolic blood pressure, and medications</td>
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<td>NGAL/Creatinine ratio, FABP/Creatinine ratio</td>
<td>Fufaa et al. [48]</td>
<td>Prospective cohort study (n = 1,156)</td>
<td>Type 2 DM</td>
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<td>Age, sex, duration of DM, hypertension, HbA1c, and study cohort</td>
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<td>17 potential urinary and 7 plasma biomarkers</td>
<td>Agarwal et al. [49]</td>
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<td>Baseline eGFR, log urine albumin/creatinine</td>
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<td>Baseline eGFR, albuminuria, sex, hypertension, HbA1c, smoking, lipid profile, BMI, and RAS inhibitors</td>
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<td>Omics-based novel biomarkers</td>
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<td>Urinary CKD-273</td>
<td>Currie et al. [58]</td>
<td>Prospective cohort study (n = 155)</td>
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<td>CKD-273 was associated with mortality in individuals with type 2 DM and microalbuminuria</td>
<td>Age, sex, systolic blood pressure, smoking status, eGFR, albuminuria, CAC score, and NT-proBNP</td>
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<td>Lindhardt et al. [60]</td>
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<td>CKD-273 predicted the development of albuminuria</td>
<td>Treatment group, age, sex, systolic blood pressure, albuminuria, eGFR, HbA1c, and DM duration</td>
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<tr>
<td>Urinary CKD-273, α1 type 1 collagen chain</td>
<td>Zürbig et al. [62]</td>
<td>Prospective cohort study (n = 35)</td>
<td>Type 1 and type 2 DM</td>
<td>CKD-273 predicted progression to macroalbuminuria 5 years before actual onset</td>
<td>Age, sex, DM type, albuminuria, eGFR, systolic and diastolic blood pressure, HbA1c, and glucose</td>
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<td>Urinary CKD-273</td>
<td>Roscioli et al. [63]</td>
<td>Prospective cohort study (n = 88)</td>
<td>Type 2 DM</td>
<td>CKD-273 predicted the development of albuminuria independent of other kidney biomarkers used to predict DKD development or progression</td>
<td>Albuminuria, eGFR, and use of RAS inhibitors</td>
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<tr>
<td>Urinary CKD-273</td>
<td>Zürbig et al. [67]</td>
<td>Prospective cohort study (n = 1,014)</td>
<td>Type 1 and 2 DM</td>
<td>In patients with type 1 or 2 DM, baseline eGFR ≥ 70 mL/min/1.73 m², and normoalbuminuria, CKD-273 was able to identify progression to eGFR &lt; 60 mL/min/1.73 m² in the absence of albuminuria</td>
<td>Age, baseline eGFR, and systolic and diastolic blood pressure</td>
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<td>Urinary CKD-273</td>
<td>Tofte et al. [68]</td>
<td>Prospective cohort study (n = 1,775)</td>
<td>Type 2 DM</td>
<td>High-risk patients defined by CKD-273 were more likely to develop microalbuminuria</td>
<td>Age, sex, HbA1c, systolic blood pressure, retinopathy, albuminuria, eGFR</td>
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<tr>
<td>Urinary CKD-273</td>
<td>Siwy et al. [69]</td>
<td>Prospective cohort study (n = 360)</td>
<td>Type 2 DM</td>
<td>There was a significant correlation between CKD-273 and baseline eGFR, albuminuria, as well as with eGFR decline</td>
<td>No adjustments</td>
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<td>Urinary type I and III α1 collagen, α2-HS-glycoprotein</td>
<td>Rossing et al. [70]</td>
<td>Prospective cohort study (n = 165)</td>
<td>Type 2 DM</td>
<td>Type I and type III α1 collagen and α2-HS glycoprotein were found to be prominent collagen markers in patients with diabetic renal damage</td>
<td>No adjustments</td>
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<tr>
<td>Urinary uromodulin, progranulin, clusterin, and α1 acid glycoprotein</td>
<td>Schlather et al. [71]</td>
<td>Nested case-control study (n = 465)</td>
<td>Type 1 DM</td>
<td>A panel including uromodulin, progranulin, clusterin, and α1 acid glycoprotein predicted early eGFR decline</td>
<td>Baseline age, DM duration, albuminuria, HbA1c, cystatin C, and uric acid</td>
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<td>Urinary haptoglobin</td>
<td>Bhensdadia et al. [72]</td>
<td>Prospective cohort study (n = 204)</td>
<td>Type 2 DM</td>
<td>The haptoglobin to creatinine ratio may be useful in predicting the risk of DKD before the development of albuminuria or kidney function decline</td>
<td>Treatment arm, use of ACEi</td>
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<tr>
<td>Metabolomics</td>
<td>Han et al. [75]</td>
<td>Cross-sectional study (n = 150)</td>
<td>Type 2 DM</td>
<td>Non-esterified and esterified fatty acid discriminated albuminuria stages</td>
<td>No adjustments</td>
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<tr>
<td>35 serum non-esterified and 32 serum esterified fatty acids</td>
<td>Hirayama et al. [76]</td>
<td>Cross-sectional study (n = 156)</td>
<td>Type 2 DM</td>
<td>Combination of 19 serum metabolites enabled accurate discrimination of DKD</td>
<td>No adjustments</td>
</tr>
<tr>
<td>19 serum metabolites</td>
<td>Zhang et al. [77]</td>
<td>Cross-sectional study (n = 66)</td>
<td>Type 2 DM</td>
<td>Serum metabolite levels of leucine, dihydrosphingosine, and phytosphingosine were significantly different in patients with type 2 DM and healthy controls</td>
<td>No adjustments</td>
</tr>
<tr>
<td>Serum leucine, dihydrosphingosine, phytosphingosine</td>
<td>Colombo et al. [78]</td>
<td>Nested case-control study (n = 840)</td>
<td>Type 2 DM</td>
<td>12 biomarkers showed significant associations with a rapid decline in eGFR, of which serum KIM-1 and B2M showed the most robust association</td>
<td>Age, sex, baseline eGFR, albuminuria, HbA1c, calendar time, and treatment allocation</td>
</tr>
<tr>
<td>207 serum biomarkers</td>
<td>Pena et al. [80]</td>
<td>Prospective cohort study (n = 90)</td>
<td>Type 2 DM</td>
<td>Urine hexose, glutamine, tyrosine, plasma butenylcarnitine, and histidine predicted the development of albuminuria</td>
<td>Albuminuria, eGFR, and RAS inhibitors</td>
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<tr>
<td>Urinary 3-hydroxyisobutyrate, 3-methylcrotonylglycine, acconit acid, citric acid</td>
<td>Kwan et al. [91]</td>
<td>Prospective cohort study (n = 1,001)</td>
<td>Type 1 and 2 DM</td>
<td>3-hydroxyisobutyrate and 3-methylcrotonylglycine had a significant negative association with eGFR slope, while acconit and citric acid showed a positive association</td>
<td>Age, race, sex, smoking, body mass index, HbA1c, mean arterial pressure, albuminuria, and baseline eGFR</td>
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<tr>
<td>Urinary leucine, valine, isoleucine, pseudouridine, threonine, citrate, 2-hydroxy-propionate, pyroglutamate, tyrosine, alanine</td>
<td>Mutter et al. [82]</td>
<td>Prospective cohort study (n = 2,670)</td>
<td>Type 1 DM</td>
<td>7 urinary metabolites that included leucine, valine, isoleucine, pseudouridine, threonine, and citrate were associated with DKD progression. 6 amino acids and pyroglutamate were associated with DKD progression in those with macroalbuminuria</td>
<td>Baseline albuminuria, baseline glycemic control, and CKD stage</td>
</tr>
<tr>
<td>Transcriptomics</td>
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<tr>
<td>Plasma let-7c-5p, miR-29a-3p, let-7b-5p, miR-21-5p, miR-29c-3p</td>
<td>Pezzolesi et al. [83]</td>
<td>Prospective cohort study (n = 116)</td>
<td>Type 1 DM</td>
<td>Baseline miRNA levels of let-7c-5p and miR-29a-3p were independently associated with more than a 50% reduction in the risk of rapid progression to ESKD, while levels of let-7b-5p and miR-21-5p were associated with a higher risk of ESKD</td>
<td>Age, sex, HbA1c, and duration of DM</td>
</tr>
<tr>
<td>Plasma miR-126</td>
<td>Barutta et al. [84]</td>
<td>Nested case-control study (n = 455)</td>
<td>Type 1 DM</td>
<td>miR-126 levels were negatively associated with all DM-related complications. A 25% risk reduction of proliferative diabetic retinopathy was observed even after adjustments for HbA1c and DM duration</td>
<td>HbA1c, DM duration</td>
</tr>
<tr>
<td>Urinary miR-29a, miR-29b</td>
<td>Peng et al. [85]</td>
<td>Cross-sectional study (n = 83)</td>
<td>Type 2 DM</td>
<td>Baseline urinary miR-29a and miR-29b were associated with complications of DM such as albuminuria and carotid intima-media thickness</td>
<td>No adjustments</td>
</tr>
<tr>
<td>miR-130a, miR-145, miR-155, miR-424</td>
<td>Barutta et al. [86]</td>
<td>Cross-sectional study (n = 24)</td>
<td>Type 1 DM</td>
<td>22 of 377 urinary EV-miRNAs were differentially expressed in patients with normoalbuminuria compared to albuminuric patients</td>
<td>No adjustments</td>
</tr>
<tr>
<td>Urinary miR-196a</td>
<td>An et al. [87]</td>
<td>Prospective cohort study (n = 209)</td>
<td>Type 2 DM</td>
<td>Urinary miR-196a levels correlated positively with proteinuria, duration of DM, and systolic blood pressure, whereas baseline eGFR and hemoglobin levels showed a negative correlation with urinary miR-196a</td>
<td>Age, sex, BMI, mean arterial pressure, and HbA1c</td>
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apoA4, apolipoprotein A4; AUC, area under curve; B2M, β2-microglobulin; BMI, body mass index; C1QB, complement C1q subcomponent subunit B; CAC, coronary artery calcium; CDSL, CD5 antigen-like; CFHR2, complement factor H-related protein 2; CKD-273, chronic kidney disease 273; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; EV, extracellular vesicles; FGF-23, fibroblast growth factor-23; HbA1c, hemoglobin A1c; hsCRP, high-sensitivity C-reactive protein; IBP3, insulin-like growth factor-binding protein 3; IL, interleukin; KIM-1, kidney injury molecule 1; L-FABP, liver fatty acid-binding protein; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAI-1, plasminogen activator inhibitor 1; RAS, renin-angiotensin system; TGF-β1, transforming growth factor-beta 1; TNF-α, tissue necrosis factor-α; TNFR-1, tissue necrosis factor receptor 1; TNFR-2, tissue necrosis factor receptor 2; UPCR, urine protein-to-creatinine ratio; VEGF, vascular endothelial growth factor; α2-HS, alpha-2-Heremans-Schmid; 8-OHdG, 8-hydroxydeoxyguanosine.
have also suggested that circulating TNFR levels could also be used as good predictors of DKD [42]. For example, in a cohort consisting of 349 patients with type 1 DM and proteinuria, TNFR2 levels were the strongest determinant of eGFR decline [39].

Another biomarker that captures the oxidative stress characteristics of the DKD pathophysiologic process is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is a product of oxidative DNA damage. It is excreted in the plasma and urine after the repair of DNA by nuclease [40]. This has led to the assessment of 8-OHdG as a biomarker of oxidative stress in patients with DM [41,42]. In two cohorts of patients with type 1 DM, higher plasma concentrations of 8-OHdG were independently associated with increased risk of kidney disease in individuals with type 1 DM, suggesting that this marker may be used to evaluate the progression of DKD [42]. However, in a study of patients with type 2 DM and healthy control subjects, although urine 8-OHdG levels were increased in patients with DM, its ability to predict the development of DKD was inferior to the urine albumin-to-creatinine ratio [43].

Markers of glomerular damage

In contrast to biomarkers that capture specific pathophysiological pathways of the DKD process, some well-known biomarkers, including transferrin, immunoglobulin G (IgG), IgM, and ceruloplasmin, reflect glomerular damage. In theory, as the molecular weight of transferrin is similar to that of albumin, urinary transferrin could also be a biomarker of DKD. In a study of 45 patients with type 2 DM and with normoalbuminuria or microalbuminuria, increased urinary transferrin levels in microalbuminuria patients significantly correlated with kidney biopsy-proven tubulointerstitial injuries, suggesting a potential role of urinary transferrin in the prediction of early tubular damage in patients with DKD [44]. In a more recent study of 60 patients with and without type 2 DM, urine transferrin correlated with subclinical atherogenesis in patients with type 2 DM without kidney dysfunction, suggesting that it could potentially be an early marker of endothelial dysfunction in patients with type 2 DM but without kidney dysfunction [45]. Another marker of glomerular damage that has been investigated is ceruloplasmin. Ceruloplasmin is a copper-carrying metalloenzyme that is more negatively charged than albumin, and thus is more difficult to be filtered by the glomerulus. In a study of 140 patients with type 2 DM with normoalbuminuria, urinary ceruloplasmin levels were found to be elevated in normoalbuminuric patients with DM and were highly predictive of the development of microalbuminuria [46]. Urinary IgM and IgG levels have also been shown to be predictive of DKD. A study of 22 patients with type 1 DM and 20 patients with type 2 DM, all with evidence of DKD, revealed that the increased urine excretion of IgG and IgM accompanied albuminuria in patients with type 2 DM, suggesting a potential role of urinary immunoglobulins in the risk stratification of DKD [47].

Multiple biomarkers—assay based

As DKD is a disease entity that involves multiple pathophysiological pathways, a combination of biomarkers may be required to accurately predict disease progression. For example, most studies that have investigated panels of candidate biomarkers have included TNFR, often in combination with biomarkers of tubular damage such as KIM-1 [29,30]. In a nested case-control of 380 participants, and a prospective cohort study of 1,156 participants with type 2 DM, higher plasma levels of KIM-1, TNFR-1, and TNFR-2 were associated with a higher risk of DKD progression, even after adjustments for age, relevant anthropometric, sociodemographic, and laboratory parameters. Of note, when all three plasma biomarkers were added to the clinical model, the area under the curve (AUC) for DKD progression improved from 0.680 to 0.752 [29]. A case-cohort study consisting of 894 participants with both type 1 and 2 DM reported similar findings, where higher levels of monocyte chemoattractant protein 1 (MCP-1) were also associated with a higher risk of DKD progression [30]. In a study of 260 Pima Indians with type 2 DM, urinary NGAL/creatinine was positively associated with risk of ESKD and mortality, whereas L-FABP/creatinine was inversely associated with ESKD. The addition of NGAL/creatinine and L-FABP/creatinine to models that included albuminuria and eGFR increased the C-statistics for predicting the risk of ESKD [48].

Other studies have explored higher numbers of potential candidate biomarkers to improve the prediction of DKD outcomes. In a study evaluating 17 potential urinary and seven plasma biomarkers in 67 participants with type 2
DM, urinary C-terminal FGF-23 was found to show the strongest association with ESKD, whereas plasma vascular endothelial growth factor (VEGF) was associated with the highest risk of the composite outcome of ESKD and death [49]. Another prospective study followed 83 patients with overt diabetic nephropathy and obtained repeated measurements of proteinuria, IL-1β, IL-6, IL-8, MCP-1, TNF-α, transforming growth factor-beta 1 (TGF-β1), and plasminogen activator inhibitor-1 (PAI-1) [50]. In the study, urinary MCP-1 and TGF-β1 predicted kidney function decline that was independent of albuminuria. In a more recent study involving 345 community-based patients with type 2 DM from the Fremantle Diabetes Study Phase II, eight potential candidate biomarkers were studied after adjustment for clinical parameters. Of these eight biomarkers, apolipoprotein A4 (apoA4), CD5 antigen-like (CD5L), and complement C1q subcomponent subunit B (C1QB) were independently associated with the rapid decline in kidney function, improved predictive performance, fitness, discrimination, and reclassification [51]. However, as evidenced in the SUMMIT (the Surrogate Markers for Micro- and Macro-Vascular Hard Endpoints for Innovative Diabetes Tools) program [52], there are very strong correlations between these biomarkers, and this confounds the interpretation of these biomarkers as predictors of disease progression. Further optimization of a panel of best-reported biomarkers would be needed. Ideally, future studies could explore a panel of biomarkers that show low correlation with each other. Moreover, most studies reported to date are small in sample size, and therefore studies consisting of larger populations would be needed for the validation of the aforementioned biomarkers.

**Novel biomarkers of diabetic kidney disease—omics based**

**The omics platform-based approach**

Over the last decade, the use of approaches that measure large sets of lipids, metabolites, amino acids, peptides, and proteins are increasing [53]. These approaches have been called omics-based tests, and are defined as an assay derived from multiple molecular measurements that allow the quantification of all RNAs, proteins, and metabolites present in biological samples, and interpreted by computational models to produce clinically meaningful results. Omics-based approaches have the advantage of not only measuring a full spectrum of peptides or metabolites in a short amount of time but also producing large sets of unbiased data that can be used for diagnosis, prediction of disease progression, and treatment response. As a result, this omics platform-based approach has emerged as a strong tool in biomarker discovery in recent years [53] (Fig. 1).

**Proteomics**

Proteomics allows the simultaneous quantification of multiple protein markers in a biological sample [54,55]. Although tissue samples provide the most information on protein expression, DKD is often diagnosed clinically, rather than by biopsy, and therefore the number of patients who will undergo kidney biopsy for DKD will not increase. Blood samples can display protein signals that are produced from the kidney and of more generic processes such as fibrosis [56]. However, obtaining large volumes of blood samples in patients is often not feasible. The collection of

![Figure 1. The concept of the omics platform-based approach consists of the genome, transcriptome, proteome, and metabolome.](image-url)
urine samples overcomes the limitations of blood samples. Urine collection is not only available in large volumes, but it is also noninvasive. This led to urinary proteomics gaining more attention as a tool for the identification of diagnostic and prognostic biomarkers in kidney disease [57].

Early studies using proteomics in DKD used urinary samples to improve early diagnosis and the prediction of kidney-related and other outcomes [58–60]. The most studied and well-validated proteomic classifier to date is the capillary electrophoresis-mass spectrometry-based urinary peptide classifier, CKD-273. This mass spectrometry-based method combines the data of 273 urinary peptides into a combined score that has high accuracy in predicting the new onset of albuminuria. Approximately 75% of the peptides are collagen fragments, with uromodulin, clusterin, albumin, β-2 microglobulin, α-1 antitrypsin, and others comprising the remainder. The diagnostic utility of this proteomic classifier was first developed in a cross-sectional study of 3,600 patients with different CKD etiologies, where the classifier showed a sensitivity of 85% and a specificity of 100% for the diagnosis of CKD [61].

This classifier was subsequently validated across several cohorts consisting of patients with type 2 DM, where CKD-273 predicted both the development and progression of albuminuria in patients with DKD. In a prospective study of 35 patients with either type 1 or 2 DM, CKD-273 was able to predict progression to macroalbuminuria 5 years before the actual onset [62]. In another prospective case-control study from the PREVEND (Prevention of Renal and Vascular End-stage Disease) study and from the Steno Diabetes Center (Gentofte, Denmark), the proteomic classifier was independently associated with the transition to micro- or macroalbuminuria. The classifier improved the predictive ability of albuminuria and eGFR in the development and progression of albuminuria [63]. Analyses of both the Effect of Candesartan on Progression of Retinopathy in Type 1 Diabetes (DIRECT-Protect 1) and in Type 2 Diabetes (DIRECT-Protect 2) studies demonstrated that the CKD-273 classifier was able to predict microalbuminuria, independent of treatment, age, sex, systolic blood pressure, albuminuria, eGFR, hemoglobin A1c (HbA1c), and DM duration [60,64–66]. More recently, in a study of 1,014 individuals with type 1 or 2 DM, baseline eGFR of ≥70 mL/min/1.73 m², and normoalbuminuria, CKD-273 was able to identify patients with DM who will progress to eGFR of <60 mL/min/1.73 m² in the absence of albuminuria, independent of age, blood pressure, and baseline eGFR [67]. The concept that CKD-273 may be useful in determining the risk of disease progression and that it may also stratify treatment response to spironolactone was more definitively tested in the recent PRIORITY (Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic nephropathy in Type 2 Diabetic Patients with Normoalbuminuria) trial. In 1,775 participants with type 2 DM and normoalbuminuria over a median follow-up time of 2.5 years, high-risk patients defined by CKD-273 were more likely to develop microalbuminuria, even after adjustments for baseline risk factors such as HbA1c, systolic blood pressure, baseline albuminuria, and eGFR [68]. However, spironolactone did not prevent progression to microalbuminuria in high-risk patients. On the contrary, in an exploratory analysis of the MARLINA-T2D (Efficacy, Safety & Modification of Albuminuria in Type 2 Diabetes Subjects With Renal Disease With LINAgliptin) trial, where participants were randomized to receive either linagliptin or placebo for 24 weeks, there was a significant correlation between CKD-273 and clinical renal parameters as well as eGFR decline. Patient stratification using this classifier found that linagliptin had the potential to slow progressive kidney function decline in high-risk CKD patients [69].

Results from urinary proteomic studies have improved our knowledge of the pathophysiology of DKD. Other proteomic methods include the use of collagen fragments. For example, the α1 type 1 collagen chain is significantly altered in urine 3 to 5 years before the onset of macroalbuminuria [62]. It was found that before urinary albumin excretion starts to increase, urinary collagen fragments decreased. In a multicenter study involving 165 patients with type 2 DM, type I and type III α1 collagen and α2-Heremans-Schmid-glycoprotein were found to be prominent collagen markers. Therefore, it is thought that collagen fragments originate from the kidney, and a decrease in these fragments in patients with DKD may be due to the accumulation of extracellular matrix and increased kidney fibrosis [70].

Other urinary peptides that have been studied include uromodulin, progranulin, clusterin, α1 acid glycoprotein, and haptoglobin. In a nested case-control design, a panel including uromodulin, progranulin, clusterin, and α1 acid
glycoprotein predicted an early decline in eGFR in a cohort of 465 adults with type 1 DM [71]. Moreover, in a study of patients with DKD from the VADT (Veterans Affairs Diabetes Trial), urinary haptoglobin was identified as a candidate biomarker to predict early kidney functional decline [72].

Regardless of which sample is used for proteomic analysis, the use of proteomics to predict DKD development and progression still has several limitations including the absence of well-validated diagnostic criteria. Prospective validation studies are needed before the widespread implementation of proteomics in DKD.

Metabolomics

Metabolomics is the measurement of low-weight intermediates (<500 Da) and small end products of biochemical processes in biological fluids, and they have emerged as another potential tool in the discovery of novel biomarkers for kidney diseases. The metabolome is often regarded as the final downstream integration of biological information that consists of the genome, proteome, transcriptome, and overall enzymatic reactions of an individual [73]. This enables the detection of short- and long-term physiological and pathological changes that occur in chronic diseases. However, their results are often difficult to interpret due to various confounders that include lifestyle, medications, and nutritional status [74].

Metabolites may originate from blood or urine. Plasma nonesterified and esterified fatty acids were found to differentiate albuminuria stages in patients with type 2 DM [75]. In a study of 78 diabetic patients, a combination of serum metabolites with multivariate analyses enabled accurate discrimination of patients with DKD. In the study, the selection of five metabolites that included γ-butyrobetaine, symmetric dimethylarginine, azelaic acid, and two unknowns yielded an AUC value of 0.927 for diagnosing DKD [76]. Another study that included healthy controls and patients with type 2 DM indicated that serum metabolite levels of leucine, dihydrosphingosine, and phytosphingosine were significantly different in these two patient groups [77]. In the SUMMIT study, a combination of biomarkers of tubular damage such as KIM-1 and β2-microglobulin, and metabolite markers were used to predict the rapid progression of DKD in individuals with type 2 DM. A total of 207 serum biomarkers were measured, of which 12 biomarkers showed significant associations with rapid progression, all adjusted for clinical characteristics. A combination of 14 serum biomarkers increased the predictive ability. The addition of biomarkers to clinical data improved baseline AUC from 0.706 to 0.868. Biomarkers in the predictive model included fibroblast growth factor-21, the symmetric to asymmetric dimethylarginine ratio, β2-microglobulin, C16-acylcarnitine, and KIM-1 [78].

Urine metabolites that have been studied to date include octanol, oxalic acid, phosphoric acid, benzamide, creatinine, 3,5-dimethoxymandelic amide, and N-acetylglutamine [79]. One study combined both plasma and urinary metabolites to improve the predictive utility of metabolites. In a study of 90 patients with type 2 DM, urine hexose, glutamine, tyrosine, plasma butenoylcarnitine, and histidine levels predicted the development of albuminuria, independent of baseline albuminuria levels, eGFR, and use of RAS blockers [80]. In a study from the CRIC consisting of 1,001 participants with DM and CKD, after adjustments for clinical variables, urinary 3-hydroxyisobutyrate (3-HIBA) and 3-methylcrotonyglycine levels had a significant negative association with eGFR slope, whereas aconitic and citric acid levels showed a positive association. Levels of 3-HIBA and aconitic acid were each associated with higher and lower risks of ESKD requiring kidney replacement therapy, respectively [81]. Most recently, in 2,670 patients with type 1 DM from the Finnish Diabetic Nephropathy study, seven urinary metabolites, which included leucine, valine, isoleucine, pseudouridine, threonine, and citrate, were associated with DKD progression after adjustment for baseline albuminuria and CKD stage. Moreover, 2-hydroxyisobutyrate was associated with the progression of DKD in individuals with normoalbuminuria, and six amino acids and pyroglutamate were associated with the progression of DKD in those with macroalbuminuria [82].

Although there have been significant advances in the field of metabolomics for patients with DKD, most of the aforementioned studies are cross-sectional. Not only is there a need for more prospective studies to evaluate the predictive utility of these metabolites, but replication of current findings in other cohorts is also needed to convey therapeutic targets and improve the clinical management of DKD.
Transcriptomics

Transcriptomic studies of DKD use micro RNAs (miRNAs), which are small non-coding RNAs that block protein translation and can induce messenger RNA degradation. Thus, miRNAs are regarded as regulators of gene expression. Like metabolites, miRNAs may also originate from plasma, serum, or urine, and their profiling can be performed using either traditional microarray/real-time polymerase chain reaction (RT-PCR) platforms or RNA sequencing.

One of the earliest studies to indicate that certain plasma miRNAs showed an association with the progression of DKD was performed in patients with type 1 DM with albuminuria but normal kidney function. In participants of the Joslin cohort, baseline circulating TGF-β1-regulated miRNA levels were associated with progression to ESKD requiring kidney replacement therapy. Baseline miRNA levels of let-7c-5p and miR-29a-3p were independently associated with more than a 50% reduction in the risk of progression to ESKD, whereas baseline miRNA levels of let-7b-5p and miR-21-5p were associated with a more than 2.5-fold increase in the risk of ESKD [83]. In another cross-sectional nested case-control study from the EURODIAB Prospective Complications Study of 455 patients with type 1 DM, miR-126 levels were negatively associated with all DM-related complications. Although this association was no longer significant after adjustment for both hyperglycemia and duration of DM, a statistically significant 25% risk reduction of proliferative diabetic retinopathy was observed even after adjustments for HbA1c and DM duration [84].

In addition to miRNAs sampled from the plasma or serum, many studies have reported findings from urine samples. One of the earliest studies to report on the association between urinary miRNAs and the risk of DKD progression was conducted with 83 patients with type 2 DM, where baseline urinary miRNA levels of miR-29a and miR-29b were associated with complications of DM. Higher levels of urinary miR-29a were observed in patients with albuminuria compared to those with normoalbuminuria. Urinary miR-29a levels showed a significant correlation with albuminuria and were also correlated with carotid intima-media thickness [85]. Another study assessed the urinary extracellular vesicle (EV)-miRNA profiles of patients with type 1 DM, where 22 of 377 urinary EV-miRNAs were differentially expressed in patients with normoalbuminuria compared to albuminuric patients. Results showed that miR-130a and miR-145 were enriched, whereas miR-155 and miR-424 were reduced in urinary exosomes for patients with albuminuria [86]. More recently, in a study of 209 patients with biopsy-proven DKD, urinary miR-196a levels correlated positively with proteinuria, duration of DM, and systolic blood pressure, whereas baseline eGFR and hemoglobin levels showed a negative correlation with urinary miR-196a. This suggests that increased urinary miR-196a levels were significantly associated with the progression of DKD and could be a noninvasive prognostic marker of kidney fibrosis in patients with DKD [87].

Although several other studies have investigated transcriptomics in patients with DKD, there is no overlap in the specific miRNAs being reported as being relevant to DKD. It is most likely that a combination of miRNAs may be needed for the early detection of DKD rather than a single miRNA [88]. Therefore, the evidence to support a clinically useful role of miRNAs in the early diagnosis and risk stratification of DKD remains uncertain.

Current practice and conclusion

Current treatment of DKD relies on lifestyle modification, and medication that controls hyperglycemia, hypertension, and proteinuria. However, even the optimal implementation of this strategy often fails to prevent progression to ESKD in a substantial proportion of patients. There is hope that novel biomarkers, both assay-based and omics-based, will help to identify patients at the highest risk and guide the treatment of these patients. In reality, only a few trials use biomarkers other than albuminuria or eGFR to enroll and risk stratify study participants [89], and even fewer studies assess the effect of treatments with novel biomarkers [90–92]. In part, this may be because potential novel biomarkers only modestly improve the performance of eGFR and albuminuria, which are the biomarkers currently available. Moreover, considering that the progression of DKD is usually a slow process that may take decades to emerge, the setting of robust clinical endpoints in clinical trials is often not feasible.

Although the novel biomarkers discussed in this review have enormous potential in the field of DKD, future studies should look into using these biomarkers either as enrollment criteria for randomized clinical trials or as surrogates.
of study endpoints. Larger study cohorts with kidney biopsies and both urine and plasma or serum samples from the same patients would also be needed. To obtain comparable and reproducible data, consensus protocols for sample collection, processing, and analysis should be defined across collaborators. Finally, as we gain a deeper understanding of the DKD pathophysiology, an increasing number of potential novel biomarkers will be available. To improve the prognostication of patients with DKD, it will be essential to integrate these novel findings and biomarkers into the design of future clinical trials.

Conflicts of interest

Tae-Hyun Yoo is the Editor-in-Chief of *Kidney Research and Clinical Practice* and was not involved in the review process of this article. All authors have no other conflicts of interest to declare.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (NRF-2020R1F1A1049799).

Acknowledgments

The authors thank the Medical Illustration & Design team of the Medical Research Support Services of Yonsei University College of Medicine for all artistic support related to this work.

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Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease and end-stage kidney disease worldwide, as the obesity epidemic and the burden of diabetes continue to rise globally. In general, guideline management of patients with DKD recommends lifestyle modifications, blood pressure and glycemic control, and dyslipidemia treatment along with other cardiovascular disease risk reduction measures. The inhibition of the renin-angiotensin system (RAS) using an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker remains the foundational therapy for DKD. In type 2 diabetes (T2D), significant advances in therapeutics, including the sodium-glucose cotransporter-2 inhibitors (SGLT2i), the glucagon-like peptide-1 receptor agonists (GLP-1 RA), and the nonsteroidal mineralocorticoid receptor agonist (MRA) finerenone, have dramatically expanded the armamentarium for treating DKD and its cardiovascular complications. Initiating, optimizing, and sustaining evidence-based pharmacological therapy using a therapeutic combination of RAS inhibitor + SGLT2i/GLP-1 RA + nonsteroidal MRA + statin is likely to significantly improve outcomes for T2D with DKD. Research into potential novel therapeutic targets for DKD remains particularly active and brings much anticipation and optimism to this field.

Keywords: Chronic kidney diseases, Diabetic kidney disease, End-stage kidney disease, Glucagon-like peptide-1 receptor, Mineralocorticoid receptor antagonists, Renin-angiotensin-system, Sodium-glucose transporter 2 inhibitors

Introduction

The incidence and prevalence of diabetes mellitus are on the rise in most countries. Globally, approximately one in 10 adults have diabetes, and the International Diabetes Federation has estimated that over 700 million people will have diabetes by 2045 [1]. Patients with type 1 diabetes (T1D) or type 2 diabetes (T2D), as well as diabetes secondary to various metabolic disorders or associated with systemic corticosteroid use, can develop kidney disease, with T2D accounting for the bulk of the disease burden. As many as 25% to 40% of all patients with diabetes will develop kidney problems after 25 years of diabetes, especially those with T2D, thus rendering diabetes the leading cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide [2]. Consequently, in the context of the so-called “diabetes pandemic,” effective therapies for diabetic kidney disease (DKD) and its associated complications will greatly impact health outcomes for many people worldwide.

In recent years, a lower risk of ESKD in patients with T1D has been reported in many countries. Furthermore, when
ESKD does occur in T1D, it appears to occur at an older age [3]. It has been suggested that a combination of treatment strategies including optimized glucose control, blood pressure (BP) management, and the use of renin-angiotensin system (RAS) inhibitors (RASi) likely explain these encouraging data in T1D, a goal not yet achieved in T2D.

This paper aims to present an updated review of DKD and its current therapeutic strategies. Momentous strides in pharmacological therapy have been made in recent years, particularly for T2D patients with DKD, though there remains significant residual cardiovascular (CV) and kidney risks. Important ongoing challenges that clinicians confront in the care of these patients will be highlighted.

**Diagnosis and pathogenesis of diabetic kidney disease**

The term DKD refers to a presumptive diagnosis of CKD caused by diabetes. Diabetic glomerulosclerosis, on the other hand, is a histopathologic descriptor, used to describe the characteristic findings of an increased mesangial substrate, nodular lesions, and tubulointerstitial fibrosis on kidney biopsies.

The pathogenesis of DKD is complex and incompletely understood. Critical metabolic changes, along with genetic and epigenetic factors, glomerular hypertension and hyperfiltration, upregulation of the renin-angiotensin-aldosterone system (RAAS), accumulation of advanced glycation end products (AGEs), oxidative stress, kidney inflammation, and fibrosis, are believed to contribute to the initiation and progression of the disease. Abnormal urinary albumin excretion (UAE) precedes impaired renal function in many, though not in all patients with DKD. Particularly in T2D, it is increasingly recognized that a substantial proportion of patients have a progressive loss of renal function without significant albuminuria [4]. The putative mechanism for the loss of renal function is unclear. Renal vascular disease, hypertension, interstitial inflammation, and fibrosis may all be reasonable contributions to the functional decline of kidneys in diabetes. Therefore, DKD represents a broad term that encompasses a spectrum of vascular, glomerular, and tubulointerstitial components of CKD attributed to diabetes. This heterogeneity in patients recruited in randomized clinical trials (RCT) represents an important limitation in studies of DKD, as the efficacy of a drug will likely vary in patients with different manifestations of the disease.

Most patients with diabetes do not need a kidney biopsy to establish the presumptive diagnosis of DKD, because the diagnosis can usually be made in patients presenting with a classic finding of elevated UAE and diabetes duration greater than 7 to 10 years. However, maintaining suspicion for non-DKD is essential, especially in patients with atypical features. These may include the absence of retinopathy or albuminuria developing <5 years after the onset of diabetes in T1D, the sudden onset of severe proteinuria, rapid kidney function deterioration, active urinary sediment, serologic findings, or other concurrent systemic diseases. An estimated glomerular filtration rate (eGFR) loss of greater than 1 mL/min/mo in a patient with good glycemic and BP control may also suggest an alternate cause of CKD and a need for a kidney biopsy. In general, there is a high prevalence of non-DKD in retrospective studies of biopsies from patients with T2D, although selection bias is inherent in these studies because these patients often have atypical clinical features [5].

Both the level of albuminuria and eGFR have independent predictive importance for progression to ESKD and CV morbidity and mortality, although neither is specific to DKD. Despite an ongoing quest to find novel biomarkers to identify early-stage DKD and to improve risk stratification, none is yet available for clinical application. Patients with a consistent finding of severely elevated albuminuria (urine albumin/creatinine ratio of >300 mg/g) have an elevated risk of rapid progression and should be referred to a nephrologist for evaluation even if eGFR is normal or mildly decreased.

**Extrarenal microvascular and macrovascular disease**

The presence of DKD is often associated with extrarenal manifestations of microvascular disease and macrovascular disease. In T1D, DKD and diabetic retinopathy are highly concordant, whereas only 50% to 60% of T2D patients with DKD have retinopathy [6]. Other diabetic complications, including sensory and autonomic polyneuropathy, gastroparesis, and vascular diseases involving coronary, cerebral, and peripheral vasculature, frequently coexist with DKD.
Although progressing to ESKD is consequential, a greater concern is that patients with DKD are more likely to die from nonrenal causes than to survive long enough to contend with ESKD [7]. As glomerular filtration rate (GFR) declines, both kidney and non-kidney complications develop. Anemia and disorders of bone mineral metabolism develop earlier in patients with DKD compared with patients with nondiabetic CKD and comparable eGFR [8]. Importantly, the risk of death from cardiovascular disease (CVD) or infection is significantly elevated. Therefore, it is critical to consider extrarenal risks in the care of patients with DKD.

Since 2008, the U.S. Food and Drug Administration mandated that new therapies seeking approval for the treatment of diabetes must show CV safety. Greater attention is now focused on clinically pertinent endpoints such as major adverse cardiovascular events (MACE), major adverse renal events (MARE), and all-cause mortality in the development and testing of novel therapeutic agents for diabetes mellitus. Indeed, the novel study designs for sodium-glucose cotransporter-2 inhibitors (SGLT2i), glucagon-like peptide-1 receptor agonists (GLP-1 RA), dipeptidyl peptidase-4 inhibitors (DPP4i), and the nonsteroidal mineralocorticoid receptor antagonist (MRA) finerenone resulting from this mandate have gone far beyond demonstrating lack of CV compromise. For many of these medication classes, studies have affirmed CV protection while providing concomitant kidney protection. Current practices can offer patients with T2D the prospect of a longer life and a higher quality of health.

Pharmacological treatment for diabetic kidney disease

In patients with established DKD, treatment goals include albuminuria regression, preservation of kidney function, and lower CVD-associated morbidity and mortality. It is generally recognized that potentially modifiable factors affecting the rate of DKD onset and progression include hyperglycemia, hypertension, dyslipidemia, smoking, nutrition, weight, and physical activity. For any given patient, successfully targeting all of these factors requires extraordinary motivation and persistence. Accordingly, treatment for DKD requires shared decision-making with a focus on individual risk-benefit assessment within the context of the degree of renal impairment as well as the comorbidities and preferences of the patient.

Glycemic control

Large clinical trials have demonstrated that strict glycemic control prevents microvascular complications including DKD in diabetic patients [9]. In fact, benefits gained from strict glycemic control extend beyond the period of trial treatment intervention. Ongoing beneficial effects on diabetic complications after a period of improved glycemic control, even if followed by a return to less intensive metabolic control has been described as a metabolic memory or legacy effect. For example, after the termination of the UKPDS (United Kingdom Prospective Diabetes Study), which ran for 20 years (1977–1997), patients were observed for a further 10 years. The difference in glycated hemoglobin (HbA1c) was lost within a year, but a lower risk of microvascular disease (~24%) and myocardial infarction (~15%) persisted in the group randomized to tight glycemic control. All-cause mortality was also reduced (~13%). These results underscore the importance of early glycemic control before complications develop.

In patients with DKD, glycemic targets need to be tailored individually. Important trials, including ACCORD (Actions to Control Cardiovascular Risk in Diabetes), VADT (Veterans Affairs Diabetes Trial), and ADVANCE (Action in Diabetes and Vascular Disease, Perindopril and Indapamide Controlled Evaluation), have tested whether strict glycemic control improves clinical outcomes. In all of these studies, no significant decreases in CV events were observed. Hypoglycemia and weight gain were greater in the intensive-therapy group. In the ACCORD trial, very tight glycemic control (HgA1c of <6% vs. 7%–7.9%) was associated with higher mortality (increase in risk by 22%, p = 0.04) [10].

Major guidelines, including Kidney Disease Improving Global Outcomes (KDIGO), recommend lowering HbA1c to a goal ranging from <6.5% to <8.0% [11]. In younger healthier patients, strict glycemic control to <6.5% is beneficial in reducing kidney disease and other microvascular complications. In contrast, for patients with advanced age, long-standing diabetes, preexisting CVD, and other severe comorbidities, or patients particularly susceptible to hypoglycemia, the risk of strict glycemic control likely outweighs
any potential benefit. In these patients, the HbA1c goal may be set closer to 8.0%.

In terms of glycemic monitoring, HbA1c is widely used and should be performed routinely in all patients with diabetes as part of continuing care. The assay has limitations, however, because the result can be influenced by conditions that affect the turnover of red cells, such as anemia states and hemoglobinopathies. Supplementary options for glycemic monitoring, including daily self-monitoring or continuous glucose monitoring, play a greater role in patients with advanced-stage CKD or patients with conditions that can lead to unreliable HbA1c levels. Alternative glycemic biomarkers, such as glycated albumin or fructosamine, are used in some research settings. Experience with these in most clinical settings is limited.

**Specific antihyperglycemic therapies for type 2 diabetes**

For T1D, antihyperglycemic treatment is based on daily insulin injections or the use of an insulin pump. For T2D, glycemic control is best achieved with a combination of lifestyle modifications (e.g., dietary restrictions, physical activity, and weight control) and pharmacologic therapy. Before 1994, selecting an oral agent for T2D was as simple as choosing which sulfonylurea to use. Today, options for antihyperglycemic therapy with unique mechanisms of action have expanded significantly. Many of these confer additional CV and kidney protections beyond their glycemic effects. Consequently, the choice of an antihyperglycemic regimen for T2D requires careful consideration of various factors, including evidence for kidney and CV benefits, risk of medication-associated adverse events, cost, and convenience of therapy. Importantly, the kidney function of patients can impact the efficacy and safety of many antihyperglycemic therapies (Table 1) [12]. Dosing adjustments and careful monitoring for adverse effects may be required for patients with a more advanced-stage kidney disease.

For most patients with T2D and DKD, metformin and an SGLT2i are recommended as the first-line pharmacologic treatment if eGFR is above 30 mL/min/1.73 m². For patients with lower eGFR, a GLP-1 RA with proven kidney and CV benefits should be prioritized, especially in patients at high risk for atherosclerotic CVD. There is robust data on the CV and kidney protective effects of several SGLT2i and GLP-1 RA, independent of their glucose-lowering effect. These agents should be considered the primary choice over other antihyperglycemic therapeutics such as DPP4i or sulfonylureas, especially in patients with T2D with increased risk for CVD or kidney disease. Additional detailed discussions on metformin, SGLT2i, and GLP-1 RA are presented below.

**Metformin**

Metformin remains the preferred initial antihyperglycemic therapy for most patients with T2D due to its low cost, high efficacy, and low risk of hypoglycemia. Additionally, it has weight- and lipid-lowering properties as well as beneficial effects on CV mortality [13]. Metformin is eliminated primarily by the kidney. Despite limited data, the potential for elevated risk of lactic acidosis in patients with lower eGFR has restricted its use to patients with an eGFR of >30 mL/min/1.73 m².

**Sodium-glucose cotransporter-2 inhibitors**

SGLT2i are now widely used antihyperglycemic therapies for T2D. Multiple CV outcome trials, including the EMPA-REG OUTCOME (Empagliflozin Cardiovascular Outcome Event) trial and the CANVAS (Canagliflozin Cardiovascular Assessment Study), have demonstrated that SGLT2i provide significant kidney benefits in addition to CV benefits. For instance, secondary outcome analysis of the EMPA-REG demonstrated an impressive 39% reduction in incident or worsening kidney disease in the empagliflozin group [14]. The CANVAS-Renal trial similarly reported a 40% reduction in the composite kidney outcome (sustained reduction in the rate of eGFR decline, need for kidney replacement therapy, or death from renal causes) [15]. In contrast to these CV outcome trials, the CREDENCE (Canagliflozin and RenalEndpoints in Diabetes with Established Nephropathy Clinical Evaluation) trial was designed to assess SGLT2i canagliflozin primary on kidney outcomes in T2D with albuminuric CKD. Results showed that there was a 34% reduction in kidney-specific composite outcome (ESKD, doubling of creatinine, or kidney-related death) in the canagliflozin group [16]. More recently, in the DAPA-CKD (Dapagliflozin in Patients with Chronic Kidney Disease) study, the kidney and CV benefits of the SGLT2i dapagliflozin was found to extend to patients with CKD (eGFR of 25–75 mL/min/1.73 m²) with or without T2D, thus making an argument for the use of SGLT2i in patients with CKD independent of diabetes status [17].

SGLT2i reduces renal glucose reabsorption, resulting in
Tong and Adler. Diabetic kidney disease treatment: new perspectives

### Table 1. Dosing adjustments of selected antihyperglycemic agents in T2D patients with CKD

<table>
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<th>Antihyperglycemic agent</th>
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The dose and eGFR lower bound for dosing have undergone frequent changes, especially for SGLT2 inhibitors. Please consult the most recent package insert for up-to-date information.

CKD, chronic kidney disease; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; Max, maximum recommended dose; GLP-1, glucagon-like peptide 1; SGLT2, sodium-glucose cotransporter 2; T2D, type 2 diabetes.

Osmotic diuresis and plasma volume contraction. A reversible reduction in eGFR of more than 10% occurs in about a third of patients treated with SGLT2i. Although they are relatively weak glucose-lowering agents, they have the additional benefit of lowering BP and weight and do not cause hypoglycemia. Potential adverse effects include an increased risk for volume depletion, genital and urinary tract infections, perineal necrotizing fasciitis, and euglycemic ketoacidosis. The risk of lower-limb amputation was only seen in one trial with canagliflozin. Nevertheless, patients who have foot ulcers or are at high risk for amputation should be educated on proper foot care and amputation prevention. Importantly, all SGLT2i exhibit a substantial degree of renal excretion and are associated with increased accumulation and toxicity in patients with renal impairment. Specifics vary according to individual medication, but they generally should not be initiated for patients with eGFR of <25–30 mL/min/1.73 m² (Table 1). However, once started, as long as they are tolerated well, they may be continued until the start of dialysis.

**Glucagon-like peptide-1 receptor agonists**

For T2D, the GLP-1 RA represent a newer family of injectable antihyperglycemic therapeutics. Liraglutide, semaglutide, and dulaglutide, in particular, have demonstrated in large CV outcome trials to have significant CV and kidney benefits, particularly in patients with established CVD or those who are at high risk. In the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial, there was a 22% reduction in CV death and 15% reduction in death from any cause with liraglutide compared with a placebo [18]. Long-term
follow-up data in patients with DKD also demonstrated a 22% reduction in the composite renal outcome, primarily due to a lower rate of severely increased albuminuria. Data on semaglutide (SUSTAIN-6 and PIONEER-6 studies) and dulaglutide (REWIND study) similarly showed CV benefits and reduced risk of albuminuria onset and kidney disease progression [19,20]. There is not yet a trial with a primary endpoint of kidney outcomes for any GLP-1 RA. The ongoing FLOW (Effect of Semaglutide Versus Placebo on the Progression of Renal Impairment in Subjects With Type 2 Diabetes and Chronic Kidney Disease) trial should address whether GLP-1 RA can slow the progression of DKD.

A number of GLP-1 RA, including liraglutide, semaglutide, and dulaglutide, have little renal clearance and are safe to use even in patients with advanced-stage DKD (Table 1) [12]. Major guidelines, including the American Diabetes Association (ADA) and the European Association for the Study of Diabetes, now recommend using GLP-1 RA to reduce CVD risk in T2D, independent of glycemic control [21]. In clinical practice, the most common adverse effects of GLP-1 RA include gastrointestinal symptoms, injection site reactions, and an increase in heart rate. GLP-1 RA should also be avoided in patients at risk for medullary thyroid tumors or a history of acute pancreatitis.

**Blood pressure control**

In patients with DKD, hypertension is very prevalent and is associated with renal parenchymal disease, volume expansion, and salt sensitivity. Several studies as well as real-life observations have pointed out that hypertension plays a central role in the pathogenesis and progression of kidney and CV damage. Higher BP is associated with increased albuminuria, more rapid progression, and increased risk of kidney failure [22]. Therefore, adequate BP control, irrespective of the agent(s) used, is an important strategy in the treatment of DKD.

Importantly, the issue of target BP in patients with DKD has not been clearly resolved. Many guidelines published before 2021, including the ADA (2020) and the American College of Cardiology/American Heart Association (ACC/AHA, 2020), recommended a BP target of <130/80 mmHg. Moreover, the National Institute for Health and Care Excellence (NICE, 2019) as well as the European Society of Cardiology/European Society of Hypertension (ESC/ESH, 2018) recommended a clinic BP target of <140/90 mmHg in all patients regardless of diabetes status or renal disease. Individualized consideration with a less stringent goal of <150/90 mmHg in patients aged >80 years old was also suggested. In contrast, the KDIGO 2021 clinical practice guideline for BP management in patients with CKD recommends a systolic BP target of <120 mmHg for individuals with CKD, with or without diabetes. This recommendation is based on standardized office BP measurements and referenced the large SPRINT (Systolic Blood Pressure Intervention Trial), which included mostly nondiabetic patients. Unfortunately, the balance of benefits and harms from intensive BP control remains uncertain in patients with diabetes and advanced-stage CKD. In the ACCORD-BP (Action to Control Cardiovascular Risk in Diabetes-Blood Pressure) trial, which tested a systolic BP less than 120 mmHg vs. less than 140 mmHg in patients with T2D and high CV risk, there was no significant difference between the two BP groups in the primary composite major CV outcomes. Benefits in the secondary outcome of stroke prevention were counterbalanced by an increased risk for hyperkalemia and kidney dysfunction in the intensive BP control group [23].

Consequently, similar to the glycemic target, the optimal BP target in patients with DKD is a shared decision-making process between the patient and the clinician, taking into consideration the patient’s age, CVD and stroke risk, and eGFR. In clinical practice, proper procedures used to measure BP is critical. Increased emphasis is now placed on implementing standardized BP measurements in the office and encouraging home BP monitoring. BP measurement in the office involves preparing the patient and repeated measurements separated by 1 to 2 minutes and using the average of at least two readings obtained on at least two occasions to estimate BP. Patients with DKD often have autonomic neuropathy and orthostasis, and BP measured in the upright position should be taken after a period of rest. Ambulatory BP measurements may be particularly helpful to assess BP control in patients suspected of having “white-coat hypertension” or wide variations in BP throughout the day.

**Renin-angiotensin-aldosterone system blockade**

**Renin-angiotensin system inhibitors**

Until a decade ago, the only drug class specific to the treatment of DKD were the RASi, including angiotensin-con-
converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB). Many historical studies have shown that RAS inhibition with ACEi or ARB reduces proteinuria and provides preferential kidney protection that is independent of BP reduction [24]. For T1D, there is robust data on the kidney protective effects of ACEi but insufficient data is available for ARBs. For T2D, however, several large clinical trials have demonstrated the efficacy of ARB in reducing albuminuria and the progression of nephropathy, including the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan), IDNT (Irbesartan Diabetic Nephropathy Trial), IRMA 2 (Irbesartan in Type 2 Diabetes with Microalbuminuria 2), and MARVAL (Microalbuminuria Reduction With Valsartan) studies, though there are insufficient data for ACEi.

Based on the shared properties of ACEi and ARBs in inhibiting the RAS, both ACEi and ARB are believed to be effective in the treatment of T1D- and T2D-associated DKD. In a small randomized controlled trial of T2D and early nephropathy with 5 years of follow-up, the ARB telmisartan was not inferior to the ACEi enalapril in providing long-term kidney protection. The choice between these two classes of drugs usually depends on factors such as physician and patient preference, cost, availability of generic formulations, and side effect profiles of individual drugs.

Notably, several large trials have failed to show improved clinical outcomes when an ACEi was combined with an ARB, or when an ACEi or ARB was combined with the direct renin inhibitor aliskiren. Combining different RASi engenders hyperkalemia and/or acute kidney injury and should be avoided.

**Aldosterone blockade**

Studies of RAS inhibition do not differentiate between the relative contribution of the RAS vs. aldosterone system blockade. Plasma aldosterone levels are elevated in up to half of the patients on ACEi or ARB therapy after 12 months, known as aldosterone breakthrough. This may partly explain why RAS inhibition does not adequately regress albuminuria in a considerable proportion of patients with DKD. MRA block the effect of aldosterones on the CV and kidney systems. They have anti-inflammatory and antifibrotic effects. They have been shown to reduce proteinuria when they are used alone and have an additive effect on proteinuria when they are used in combination with an ACEi or ARB. However, the use of steroidal MRAs such as spironolactone and eplerenone is frequently limited by the presence of hyperkalemia, especially in patients with reduced eGFR.

Finerenone, which is now approved in the United States, is a nonsteroidal MRA that has greater aldosterone receptor selectivity and affinity compared to steroidal MRA, thus affording a higher potency and a lower risk of hyperkalemia. Two large randomized controlled studies have been published on finerenone in T2D on a background of maximal RAS inhibition therapy. In the FIDELIO-DKD (Finerenone in Reducing Kidney Failure and Disease progression in Diabetic Kidney Disease) study, finerenone reduced the relative risks of the primary composite outcome of CKD progression by 18%, and the secondary composite outcome of CV morbidity and mortality by 14%, in T2D with advanced CKD over a median follow-up of 2.6 years [25]. Insights into the effects of finerenone in patients with T2D and less advanced CKD was assessed by the FIGARO-DKD (Finerenone in Reducing Cardiovascular Mortality and Morbidity in Diabetic Kidney Disease) study. Results showed a 29% risk reduction in hospitalization for heart failure and a 23% risk reduction in the kidney composite outcome of kidney failure, sustained decrease from baseline GFR of >57%, or death from renal causes (2.9% vs. 3.8%) [26]. In both trials, adverse events were similar between the finerenone and placebo groups and hyperkalemia was uncommon.

**Dyslipidemia control**

Dyslipidemia in diabetic patients is believed to substantially contribute to the development of albuminuria and progression of DKD, although the complex pathophysiological link between the two has not been fully clarified [27]. Furthermore, in diabetic patients, the serum lipid profile changes as CKD progresses, with a shift from larger toward smaller low-density lipoprotein cholesterol (LDL-C) and an increase in triglycerides. There is evidence that both the level of proteinuria and kidney functional impairment are independently associated with altered lipid metabolism and accumulation and contribute to the development of atherosclerotic CVD [28]. Thus, dyslipidemia is believed to be partly responsible for the significant residual CV risk seen in patients with DKD, once glucose and BP control are achieved. Consequently, interventions aiming to improve
lipid targets constitute an important aspect of DKD management.

Administration of lipid-lowering drugs, for the primary and secondary prevention of CVD, is an important element of DKD management. Hydroxymethylglutaryl-CoA reductase inhibitor (statin) therapy shows the strongest evidence for reducing atherosclerotic CVD and is generally considered the first choice of hypolipidemic agent. In general, lipid management guidelines emphasize the need to identify a patient’s risk for atherosclerotic CVD and the application of treatment to achieve an LDL-C level that is as low as possible for high-risk patients. Most guidelines of the United States (e.g., ACC/AHA, 2018; ADA, 2021) recommend an LDL-C reduction of ≥50% or an LDL-C goal of ≤70 mg/dL, while the European Society of Cardiology and European Atherosclerosis Society (ESC/EAS, 2019) recommend an even more stringent LDL-C goal of <55 mg/dL for those at very high risk. The addition of non-statin hypolipidemic agents (e.g., ezetimibe, fenofibrate, bempedoic acid, proprotein convertase subtilisin/kexin type 9 inhibitors [PCSK9i], or inclisiran, the newer small interfering RNA therapy) with or, in the case of PCSK9i, in place of maximally tolerated statin intensity, may be considered in high-risk patients who have not achieved lipid reduction goals. It remains unclear at this time whether any combination of dyslipidemia therapy would improve overall kidney or CV outcomes in patients with DKD.

Furthermore, there is controversy regarding the efficacy of lipid-lowering therapy once a patient reaches ESKD and is placed on dialysis. Several RCTs (4D, AURORA, and SHARP) have failed to demonstrate the benefit of statin therapy as a primary prevention of CVD in dialysis patients. In contrast, a recent observational study of 1596 incident dialysis patients in South Korea found that statin initiation was associated with a lower risk of all-cause mortality in statin-naïve ESKD patients [29]. It is unclear whether this represents an influence of race or ethnicity on the efficacy of statin therapy. Currently, there is insufficient data to recommend the use of statin therapy for primary or secondary prevention of CVD in patients on dialysis.

Nonpharmacological treatment for diabetic kidney disease

For all diabetic patients, nutritional counseling, including salt restriction and choice of carbohydrates and fats, constitutes an important component of the patient-directed self-management educational program. In particular, dietary protein restriction has been shown to improve proteinuria and slow the progression of kidney impairment [30]. In patients approaching ESKD, a low-protein diet (<0.8 g/kg/day) may also delay the onset of uremic symptoms. Protein-restricted diets should be supplemented with iron, calcium, and multiple vitamins, and should deliver ~35 kcal/kg per day.

A healthy lifestyle, including smoking cessation, exercise, and weight reduction, has also been shown to significantly reduce the risk of CV events and progression of DKD. Smoking, in particular, is an independent risk factor for CVD and DKD, and smoking cessation has been shown to improve kidney prognosis [31].

Exercise and weight reduction may also improve kidney outcomes. Secondary analysis of the Action for Health in Diabetes (look-AHEAD) randomized controlled trial found that a greater weight loss through intensive diet and exercise interventions (mean 1-year weight loss of 8.6% vs. 0.7%) was associated with a 31% reduced incidence of CKD in overweight or obese T2D patients [32]. Furthermore, there is increasing interest in the role of bariatric surgery in DKD. Results of prospective cohort studies and emerging evidence from RCT have demonstrated that bariatric surgery may prevent or slow the progression of DKD in obese patients with T2D [33].

Multidisciplinary team care

Initiating, optimizing, and sustaining evidence-based pharmacological therapy using combination therapeutics of RASI + SGLT2i/GLP1 RA + nonsteroidal MRA + statin may significantly improve outcomes for patients with DKD. In addition to pharmacological therapeutics, the prevention and treatment of DKD also necessitates dietary restrictions, lifestyle modifications such as smoking cessation and exercise, weight loss, and even bariatric surgery as necessary.

Providing early diagnosis and implementing a comprehensive treatment plan for patients with DKD is best achieved with a multidisciplinary approach, aiming at individualized blood glucose control, BP control, dyslipidemia treatment, and appropriate dietary restrictions and lifestyle modifications (Fig. 1). Even before the discovery of SGLT2i
and GLP-1 RA, intensive multifactorial treatment including both behavioral and pharmacological approaches was demonstrated in the Steno 2 trial to significantly increase the lifespan of patients, lower the risk for CVD, and slow the progression of kidney disease [34].

DKD care teams should ideally consist of physicians (nephrologists and endocrinologists), pharmacists, dieticians, nurses, and ancillary medical staff. They should provide a structured self-management education program in the patient’s preferred language and engage the patients to participate in shared decision-making regarding their treatment plan. The complexity of medical regimens is a major obstacle to achieving adherence. Therefore, the patient’s preference and goals of care must be carefully considered against the anticipated benefits of each medical regimen. In accordance with KDIGO recommendations, such patient education programs should be structured, monitored, individualized, and evaluated regularly by the DKD care team to be most effective [5].

**Future directions**

Despite significant advances in pharmacological therapy for diabetic patients, available DKD treatments can only
slow the decline in GFR, and there remains significant residual CV risk. Currently, research into potential novel therapeutic targets for DKD is particularly active and brings much anticipation and optimism to this field. New targets for therapeutic intervention include drugs that interfere with the formation and action of AGEs or receptors for AGEs, drugs that target oxidative stress, inflammatory cytokines, or fibrosis. The role of micro-RNAs in the pathogenesis of DKD is an emerging field and may also provide additional novel treatment approaches. Cell therapies targeting intrarenal vascular restitution are in early clinical trials. New insights into the molecular mechanisms that underlie the origin and progression of DKD are emerging from large-scale genetic and molecular studies in experimental models and humans.

Conflicts of interest
Sharon G. Adler: AstraZeneca Pharmaceuticals, Bayer Pharmaceuticals, Calladrius Pharmaceuticals. All authors have no other conflicts of interest to declare.

Authors’ contributions
Conceptualization: LLT
Writing–original draft: LLT
Writing–review & editing: All authors
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References


Adenosine receptors as emerging therapeutic targets for diabetic kidney disease

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Diabetic kidney disease (DKD) is now a pandemic worldwide, and novel therapeutic options are urgently required. Adenosine, an adenosine triphosphate metabolite, plays a role in kidney homeostasis through interacting with four types of adenosine receptors (ARs): A₁AR, A₂AAR, A₂BAR, and A₃AR. Increasing evidence highlights the role of adenosine and ARs in the development and progression of DKD: 1) increased adenosine in the plasma and urine of diabetics with kidney injury, 2) increased expression of each of the ARs in diabetic kidneys, 3) the protective effect of coffee, a commonly ingested nonselective AR antagonist, on DKD, and 4) the protective effect of AR modulators in experimental DKD models. We propose AR modulators as a new therapeutic option to treat DKD. Detailed mechanistic studies on the pharmacology of AR modulators will help us to develop effective first-in-class AR modulators against DKD.

Keywords: Adenosine, Purinergic P1 receptors, Purinergic P1 receptor agonists, Purinergic P1 receptor antagonists, Diabetic kidney disease, Fibrosis

Introduction

Diabetic kidney disease (DKD) is a major diabetic complication and the leading cause of chronic kidney disease (CKD) and end-stage kidney disease worldwide [1]. The mainstay treatment of DKD includes glycemic control, blood pressure control, and blockade of renin-angiotensin-aldosterone system [1]. The current prevalence of DKD implies that these strategies may not fully target the underlying pathogenesis of DKD. Sodium-glucose cotransporter 2 (SGLT2) inhibitor has recently been added as a new therapeutic option against DKD [2]. Nevertheless, we need to develop novel therapeutic strategies targeting DKD through understanding its pathogenesis.

Adenosine is an important endogenous extracellular signaling molecule that is intended to keep or restore homeostasis [3]. Adenosine activates P1 purinergic A₁, A₂A, A₂B, and A₃ adenosine receptors (ARs), which are G-protein coupled receptors (GPCR) [4]. All four types of AR are expressed in the kidney, along with metabolically active organs and the immune system [5,6]. Adenosine regulates physiological processes such as glomerular filtration rate (GFR) and homeostasis of water and sodium [7]. Chronically increased adenosine, however, plays a role in various
tissue injury \[8\]. Caffeine, a commonly ingested nonselective AR antagonist, inhibits development of hepatic fibrosis \[9\] and DKD \[10\], suggesting AR antagonists as new therapeutic agents against fibrosis including DKD \[11\]. Increasing evidence highlights the role of adenosine and ARs in the development and progression of DKD \[12–23\].

Since extracellular adenosine is regulated by membrane transporters as well as conversion from adenosine triphosphate (ATP), the role of conversion of extracellular ATP to adenosine \[5\] and agents affecting adenosine transport \[24\] with respect to kidney diseases including DKD have been recently reviewed. In the present review, we provide a background of adenosine and ARs, summarizing the current state of AR modulators in preclinical and clinical evaluation targeting DKD.

**Adenosine and adenosine receptors**

**Adenosine: formation and metabolism**

Adenosine is an endogenous purinergic nucleoside. Intracellular adenosine is synthesized either by the dephosphorylation of adenosine monophosphate (AMP) via intracellular 5’-nucleotidase (5’NTD) or by the hydrolysis of S-adenosylhomocysteine (Fig. 1). Extracellular adenosine is produced by dephosphorylation of ATP via a two-step enzymatic reaction sequence. First, CD39 converts ATP or adenosine diphosphate into AMP. In the second step, CD73 converts AMP into adenosine. Adenosine deaminase (ADA) catabolizes adenosine into inosine, and equilibrative nucleoside transporters (ENTs) carry adenosine across the cell membrane in either direction (Fig. 1). Extracellular adenosine is low under normal conditions (20–300 nM) but rises dramatically upon tissue injury, such as hypoxia and inflammation, due to an increased demand for energy supplied by ATP \[25\]. Increased adenosine up to a certain level provides protection through increased blood flow via vasodilation and through anti-inflammatory/immune-modulatory cascade \[26\]. However, a chronically increased level of adenosine may play a role in tissue injuries such as hepatic steatosis, asthma, and fibrosis \[8\]. In fact, persistent and excessive adenosine exposure under ADA deficiency...

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**Figure 1. Adenosine: formation and metabolism.** Intracellular adenosine is generated from SAH hydrolase or 5’NTD and is degraded by ADA and AK. Extracellular adenosine is generated by CD73 and converted to inosine by ADA. ENTs allow adenosine flux through the cell membrane depending on gradient concentration.

ADA, adenosine deaminase; AK, adenosine kinase; AMP, adenosine monophosphate; ATP, adenosine triphosphate; ENT, equilibrative nucleoside transporter; SAH, S-adenosylhomocysteine; 5’NTD, 5’-nucleotidase.
leads to kidney fibrosis [27]. In addition, adenosine uptake is reduced in kidney proximal tubular cells under diabetic stress resulting from reduced ENT, and ENT deficiency leads to kidney fibrosis [19].

Adenosine receptors: classification and signal transduction

Each AR exhibits differences in affinity for adenosine, G protein coupling, and subsequent intracellular signal transduction (Fig. 2).

Molecular structure of adenosine receptors

All four ARs belong to GPCR, which contains a core domain crossing the plasma membrane seven times (seven transmembrane domains, 7TM), an extracellular N-terminus, and an intracellular C-terminus. The 7TM receptors consist of three intracellular and three extracellular loops with different lengths. Cysteine amino acids forming disulfide

**Figure 2.** Signaling cascade of each AR. AR, adenosine receptor; cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element-binding protein; DAG, diacylglycerol; Epac, exchange proteins activated by cAMP; ERK, extracellular signal-regulated protein kinase; GSK-3β, glycogen synthase kinase 3 beta; IP₃, inositol trisphosphate; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PI3K, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D.
bonds in the extracellular loop 2 are important not only in ligand binding but also in receptor stability and function. The N-terminus possesses glycosylation sites, while the C-terminus possesses phosphorylation and palmitoylation sites. These modifications are all important for structural maturation, ligand binding, and downstream signaling [4]. Accordingly, diabetes-induced posttranslational modification, such as glycosylation and palmitoylation, should be considered as a factor that can modify the characteristics of ARs in diabetic kidneys.

All four ARs have been cloned from various species, including rat, mouse, and human. There is a close similarity between species of the same subtype, at least among mammals. However, A3AR shows the largest variability as follows: human vs. rat 73%, human vs. mouse 72%, and rat vs. mouse 91% [4]. Considering rats and mice are the most widely employed animals in preclinical studies, more attention is needed to translate the data from animal studies on A3AR modulators to clinical trials [28].

**Classification of adenosine receptors: affinity, G protein coupling, and signal transduction (Fig. 2)**

1) A1AR
Adenosine interacts with A1AR with an EC50 in the range of 10 nM to 1 μM. A1AR is coupled to the inhibitory G protein (Gi), which inhibits adenylyl cyclase (AC) activity and subsequent cyclic AMP (cAMP) production [29]. This leads to the inhibition of cAMP-dependent protein kinase A (PKA) activation and subsequent cAMP-responsive element-binding protein (CREB) transcriptional activation by reducing CREB phosphorylation. A1AR also induces phospholipase C (PLC) activation by coupling to the Gq, thus leading to increases in inositol 1,4,5-trisphosphate (IP3) and intracellular Ca2+ concentrations, which activate calcium-dependent protein kinases (PKC). In addition, the βγ subunit of Gi/o stimulates PLC. A1AR directly activates K+ channels and inhibits voltage-gated Ca2+ channels [25,29]. The involvement of A1AR and the cascade of mitogen-activated protein kinase (MAPK) family members, including extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), has also been demonstrated in human A1AR-overexpressed CHO cells [30].

2) A2A AR
Adenosine interacts with A2AAR with an EC50 in the range of 10 nM to 1 μM. A2AAR is coupled to the stimulatory G protein (Gs), which stimulates AC and thereby increases cAMP levels with consequent activation of PKA, CREB phosphorylation, exchange protein activated by cAMP (Epac) signaling, and MAPK family [30]. A2AAR stimulates PLC leading to activation of PKC, ERK, JNK, and AKT [31].

3) A2B AR
Adenosine interacts with A2BAR with an EC50 in the range higher than 10 μM. A2BAR is coupled to Gi and Gq [29]. Gs activates AC, causing phosphorylation of PKA and activation of Epac [32]. Gq activates PLC, leading to increase of Ca2+ concentration [29]. A2BAR also activates MAPK and AKT pathways [30].

4) A3AR
Adenosine interacts with A3AR with an EC50 in the range of 10 nM to 1 μM. A3AR is coupled to Gi and Gq [29]. Gi inhibits AC, causing reduced cAMP and PKA leading to activation of glycogen synthase kinase-3β (GSK-3β) and inhibition of beta-catenin [33,34], while Gq stimulates PLC leading to increase of diacylglycerol and intracellular Ca2+ concentrations, cytosolic Ca2+ activates nitric oxide synthase (NOS) or PKC [29]. In addition, A3AR also stimulates RhoA, phospholipase D, ERK, PI3K, and AKT pathways [30].

**The role of adenosine and adenosine receptors in the kidney**

The kidney is a fundamental organ that maintains the homeostasis by glomerular filtration, reabsorption, and excretion of solutes, ions, and water. The kidney releases diverse hormones that regulate blood pressure, produce red blood cells, and control calcium metabolism [35]. Adenosine, a kidney hormone, participates in such kidney-mediated fundamental physiological functions in homeostasis via regulating kidney blood flow, GFR, tubuloglomerular feedback (TGF), and secretion of renin [7]. However, overproduction of adenosine alters their signaling via the ARs and plays a key role in chronic inflammation, fibrosis, and kidney damage [8,36].
Distribution of adenosine receptors in the kidney

Data from functional studies, immunohistochemistry, and transgenic AR mice show all four ARs are expressed in the kidney [19,36–39]. As summarized in Fig. 3A, all four ARs are expressed in whole kidney homogenates [38], the glomeruli [19,37,39], the proximal tubules [19,40], and the afferent/effferent arterioles [41]. The loop of Henle, distal convoluted tubules, and collecting duct express A1AR/A2AR [37,38], A1AR/A2BAR [37,38], and A1AR [37], respectively. A recent single-cell transcriptomic analysis study [42] provided a comprehensive atlas of AR expression in the healthy mouse kidney (Fig. 3B).

Physiological role of kidney adenosine receptors

Adenosine directly acts on the coronary arteries to increase blood flow by four times compared to that of at rest [43]. Interestingly, exogenous adenosine induces a marked but transient reduction in the kidney blood flow [44]. Blockade of endogenous adenosine signaling by theophylline-mediated AR antagonism does not alter basal kidney function; however, the exogenous adenosine-mediated reduction of GFR is inhibited by theophylline by 31.4% [45]. All AR subtypes are expressed in the kidney vasculature, yet levels of A1AR and A3AR are high in the preglomerular microcirculation, whereas A2BAR and A2AR levels are low [46,47]. Adenosine is also involved in the regulation of the TGF of the nephron and renin release from juxtaglomerular cells [48,49].

Vasoconstriction and vasodilation

Pharmacological blockade of the renin-angiotensin system diminishes the kidney vasoconstrictive action of adenosine [45,50], and activation of the renin-angiotensin system augments adenosine-induced vasoconstriction and lowering of GFR [7,50,51]. Selective A1AR antagonists inhibit the

Figure 3. Distribution of ARs in the kidney. (A) Summary of each AR expressed in the nephron. (B) Expression of each AR in healthy mouse kidney delineated by single-cell transcriptomic analysis (https://susztaklab.com).

A-IC, alpha intercalated cell; ALOH, ascending loop of Henle; AR, adenosine receptor; B-IC, beta intercalated cell; CD-PC, collecting duct principal cell; CD-Trans, CD transient cell; CNT, connecting tubule; DC 11b+, CD11b+ dendritic cell; DCT, distal convoluted tubule; DLOH, descending loop of Henle; Endo, endothelial; Fib, fibroblast; GEC, glomerular endothelial cells; Granul, granulocyte; LOH, loop of Henle; Macro, macrophage; Neutro, neutrophil; NK, natural killer cell; pDC, plasmacytoid DC; Podo, podocyte; PT, proximal tubule; Tgd, gamma delta T cell; Treg, regulatory T cell.
Adenosine-mediated vasoconstriction, which is absent in A<sub>1</sub>AR knockout mice [45,52,53]. A<sub>1</sub>AR-mediated renal vasoconstriction is also supported by the persistent reduction in both kidney blood flow and GFR induced by the infusion of the A<sub>1</sub>AR agonist [54]. In isolated perfused afferent arterioles, adenosine induces a 30% reduction of vessel diameter in proximal parts of the arteriole [55]. In contrast to A<sub>1</sub>AR-mediated persistent vasoconstriction in the afferent arterioles, the elevation of kidney adenosine may result in vasorelaxation via A<sub>2B</sub>AR-mediated generation of nitric oxide [53]. A<sub>2B</sub>AR agonists reduce renal vascular constriction without altering urine volume, sodium excretion, or renin release [56]. A<sub>2B</sub>AR signaling plays a role in increasing medullary blood flow [57,58]. A<sub>2B</sub>AR agonists do not display vasodilatory influence on isolated afferent arterioles but induce vasodilation in A<sub>1</sub>AR-induced constricted isolated afferent arterioles [47], suggesting the counterbalancing effects between A<sub>1</sub>AR and A<sub>2B</sub>AR on afferent arterioles.

Vasoconstriction induced by adenosine or A<sub>1</sub>AR agonists is blocked by pretreatment with pertussis toxin, indicating that Gi/o protein is involved. The PLC but not the AC signaling pathway plays a role in A<sub>1</sub>AR-mediated vasoconstriction in the afferent arterioles, since 1) adenylyl cyclase inhibitor or PKA inhibitor does not induce vasoconstriction and 2) PLC inhibitor blocks adenosine-induced vasoconstriction in perfused afferent arterioles [59]. Adenosine increases intracellular calcium concentration leading to activation of NOS in afferent arterioles [60]. A<sub>1</sub>AR-mediated renal vasoconstriction is, in fact, aggravated by NOS inhibitor, suggesting that adenosine activates NOS to counteract A<sub>1</sub>AR-mediated vasoconstriction [61].

**Tubuloglomerular feedback**

GFR, defined as the total volume of fluid filtered from the kidney glomeruli in a given period of time, is considered the optimal index for kidney function. TGF is a mechanism that helps to regulate single nephron GFR with the tubular transport activity or capacity. Adenosine signaling triggered by an increased sodium chloride concentration in distal tubules has been suggested to mediate TGF [62].

An intact TGF mechanism requires local adenosine responding to the sodium chloride concentration in the tubular fluid at the macula densa [63]. Sodium levels in urine are sensed by the Na-K-2Cl cotransporter (NKCC2) in the macula densa. Activated NKCC2 leads to synthesis of adenosine and elevated adenosine levels in glomerular capillaries. Adenosine induces the contraction of afferent arterioles and dilation of efferent arterioles through activation of adenosine A<sub>1</sub>AR and A<sub>2B</sub>AR, respectively, as described above; subsequently, single nephron GFR and the filtration pressure are suppressed. Regarding this, A<sub>1</sub>AR knockout mice lack the TGF mechanism [64,65]. A<sub>2B</sub>AR in efferent arterioles induces vasodilation and leads to reducing GFR [66]. Administration of an A<sub>2B</sub>AR antagonist increases GFR without a change in renal blood flow, suggesting a tonic influence of endogenous adenosine on efferent arterioles [14].

**Secretion of renin**

Adenosine inhibits secretion of renin in the kidney [7,67]. A<sub>1</sub>AR antagonists increase plasma renin level, indicating a tonic inhibition of renin secretion by A<sub>1</sub>AR activation [68]. Consistently, A<sub>1</sub>AR knockout mice show increased renin expression and content in the kidney [69]. A<sub>1</sub>AR activation, however, increases renin secretion [70].

**The role of adenosine and adenosine receptors in the diabetic kidney disease**

Plasma levels of adenosine and its catabolic product, inosine, are increased in patients with DKD [21,22]. Interestingly, plasma adenosine is not different between healthy and diabetic patients without kidney disease [21]. Our urinary analysis of diabetic patients showed proportional increases in urinary adenosine excretion according to the stage of DKD (Fig. 4A). In addition, urinary excretion of adenosine shows positive correlation with proteinuria or microalbuminuria and negative correlation with creatinine clearance (data not shown). Levels of adenosine in kidney tissue and urine are increased in streptozotocin (STZ)-induced diabetic mice and rats [16,71]. The isolated glomeruli from diabetic rats contains six-fold higher adenosine compared to controls [72]. Adenosine in renal venous plasma but not in renal arterial plasma is significantly increased in diabetic rats compared to controls [23], further suggesting increased synthesis and release of adenosine in diabetic kidneys. Additionally, kidney expression of CD73 transcription and protein are increased in STZ-induced diabetic mice and rats [16,71]. The expression of kidney adenosine kinase (AK) is decreased in diabetic rats, whereas administration of insulin to diabetic rats restores the...
kidney AK expression [73,74]. The administration of ADA in diabetic rats decreases the urinary excretion of proinflammatory cytokines and increases the anti-inflammatory cytokine [20], suggesting a pathogenic role of increased adenosine in DKD. The ENT, which modulates adenosine uptake, is reduced in diabetic kidneys, contributing to kidney fibrosis [19].

In contrast to the above studies, administration of adenosine, inhibition of cellular uptake of adenosine, and blockade of AK have all been shown to reduce kidney injury in diabetes [75-77]. In addition, CD73 knockout diabetic mice exhibited more severe kidney injury than wild-type diabetic mice [16]. The exact reason for these seemingly contradictory findings is not clear but may be due to experimental conditions such as duration of diabetes and severity of DKD.

We confirmed that expression of each AR is significantly increased in both STZ-induced type-1 diabetes (T1D) and db/db type-2 diabetes (T2D) mouse kidney (Fig. 5A, B). In addition, analysis of the Nephroseq database of human diabetic kidney biopsy samples showed increased AR expression (Fig. 5C). Our immunohistochemical analysis shows that A2AAR expression is increased in minor glomerulopathy, minimal change disease, and DKD. Interestingly, A1AR expression is significantly increased only in DKD, and not in minor glomerulopathy or minimal change disease (Fig. 4B).

The involvement of adenosine and ARs in DKD has been also studied using genetically engineered models. Lack of A1AR in diabetic mice aggravates both hyperfiltration and glomerular injuries [78]. Both A2AR or A3AR knockout diabetic mice exhibited more severe kidney injury, as indicated by increased albumin excretion compared to wild-type diabetic mice [13,16]. The severity of DKD in A3AR knockout mice has not been reported, while A2AR knockout mice are protected against ischemia- or myoglobinuria-induced AKI [79]. Considering the ubiquitous distribution and diverse effects of each AR, future studies with spatiotemporal regulation of AR during the progression of DKD are necessary to understand their exact roles in diabetic kidneys.

The role of NKCC2/adenosine/AR in the renoprotective effect of SGLT2 inhibitors was particularly interesting. SGLT2 inhibitors have recently been shown to prevent the progression of DKD [80,81]. SGLT2 inhibitors reduce proximal reabsorption of sodium chloride and increase sodium chloride concentrations at the macula densa, fa-
cilitating NKCC2 transporter which subsequently leads to production of adenosine, activates the TGF mechanism, and reduces hyperfiltration (summarized in section of Tubuloglomerular feedback) associated with DKD [82]. It is speculated that upregulated SGLT2, even in the presence of hyperfiltration in diabetic kidney, will reduce sodium chloride delivered to macular densa, inactivate NKCC2, decrease the adenosine/AR axis in TGF, and maintain hyperfiltration. This speculation is supported by the fact that SGLT2 inhibition in T1D patients increases urinary adenosine excretion [83]. Restoration of TGF as a result of increased extracellular adenosine (due to inhibition of ENT) also has been suggested as a renoprotective mechanism of dipyridamole in STZ-induced diabetic rats [84].

**Therapeutic applications of adenosine receptor modulators against diabetic kidney disease**

The consumption of more than two cups per day of coffee, which contains the nonselective adenosine antagonist caffeine, for 2 weeks improved eGFR in DKD patients [10], and there is an inverse relationship between caffeine consumption and mortality in CKD patients [85]. In addition, a nonselective AR antagonist, 8-(p-sulfophenyl) theophylline, effectively decreases kidney fibrosis and improves kidney function [86]. These data suggest that well-designed AR antagonists may become novel protective agents against DKD.

With respect to specific AR subtype, A1AR antagonists consistently prevent experimental DKD [18–20] as summarized in Table 1 and Fig. 6. LJ2698 and MRS1220, the A2AR antagonists, attenuate fibrosis, oxidative stress, and inflammation in db/db mice [18] and STZ diabetic rats [19,20], respectively. Our previous study showed that LJ1888, a prototype compound of LJ2698, prevents fibrosis in obstructed kidney as well [87]. Inhibition of transforming growth factor-β1 (TGF-β1)-induced extracellular matrix (ECM) up-regulation via A2AR-specific small interfering RNA in renal proximal epithelial cells [87] confirmed the fibrotic effects of A2AR in the kidney. We have shown that LJ1888 reduced obstruction- or TGF-β1-induced upregulation of lysyl oxidase, which induces cross-linking of ECM, suggesting that A2AR antagonists may also regulate ECM accumulation via posttranslational regulation [87]. Yet, more studies are needed to confirm the therapeutic effect of A2AR antagonists against DKD, because decreased proinflammatory cytokines in response to A2AR agonists and increased proinflammatory cytokines in A1AR knockout mice under sepsis-induced kidney injury have been reported [88].

Inflammation plays a key role in the development of DKD [1], and A1AR acts as a strong anti-inflammatory effector responding to extracellular adenosine [89]. In agreement with this, the A1AR agonists ATL146 or CGS21680 attenuate kidney injury including inflammation as well as fibrosis in STZ-induced diabetic rats [13–15]. A2AR activation also reduces cytokine and chemokine expression.
Table 1. Structure and efficacy of AR modulators against DKD

<table>
<thead>
<tr>
<th>Name</th>
<th>Ligand type</th>
<th>Structure</th>
<th>Model of DKD</th>
<th>Effects</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ATL146</td>
<td>A$_{2A}$AR agonist</td>
<td>[Image]</td>
<td>STZ-induced diabetic rats</td>
<td>↓ BG</td>
<td>[13]</td>
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<td>↓ UAE and P$_{cr}$</td>
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<td>↓ Urinary MCP-1, IFN-γ, TNF-α</td>
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<td>↓ Fibronectin and collagen mRNA expression in kidney</td>
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<tr>
<td>CGS21680</td>
<td>A$_{2A}$AR agonist</td>
<td>[Image]</td>
<td>STZ-induced diabetic rats</td>
<td>↓ Hyperfiltration</td>
<td>[14]</td>
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<td>↓ Proteinuria and urinary TNF-α</td>
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<td>↓ ED-1 expression and glomerular hypertrophy in kidney</td>
<td>[15]</td>
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<tr>
<td>Bay60-6583</td>
<td>A$_{2B}$AR agonist</td>
<td>[Image]</td>
<td>STZ-induced diabetic mice</td>
<td>↓ GFR and UAE</td>
<td>[16]</td>
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<td>↓ Urinary MCP-1</td>
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<td>↓ VEGF and nephrin mRNA expression</td>
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<tr>
<td>MRS1754</td>
<td>A$_{2B}$AR antagonist</td>
<td>[Image]</td>
<td>STZ-induced diabetic rats</td>
<td>↓ BG</td>
<td>[12]</td>
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<td></td>
<td></td>
<td>↓ Proteinuria</td>
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<td>↓ VEGF and a-SMA expression</td>
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<td>↑ Nephrin expression in kidney</td>
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<td>↓ VEGF expression</td>
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<td>Glomeruli exposed to high glucose</td>
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<td>STZ-induced diabetic mice</td>
<td>↓ Serum creatinine, BUN, UAE</td>
<td>[17]</td>
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<td>↓ VEGF and collagen mRNA expression in kidney</td>
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<td>↓ UAE and urinary Kim-1</td>
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<td>↓ Profibrotic, proinflammatory, and oxidative stress markers</td>
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<td>↓ Lipid accumulation</td>
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<td></td>
<td></td>
<td>↑ PGC-1α, cytochrome B, TFAM, and NRF1 expression in kidney</td>
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<tr>
<td>LJ2698</td>
<td>A$_{3}$AR antagonist</td>
<td>[Image]</td>
<td>db/db mice</td>
<td>↓ Fibronectin and a-SMA expression</td>
<td>[18]</td>
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<td>↓ a-SMA expression in kidney</td>
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<td>↓ Urinary inflammatory cytokines</td>
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<td>↓ NF-κB, cleaved caspase1, and cleaved caspase 3 activity</td>
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<td>↓ a-SMA expression in kidney</td>
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<tr>
<td>MRS1220</td>
<td>A$_{3}$AR antagonist</td>
<td>[Image]</td>
<td>HK2 cells exposed to high glucose and TGF-β</td>
<td>↓ Fibronectin and a-SMA expression</td>
<td>[19]</td>
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<td>STZ-induced diabetic rats</td>
<td>↓ a-SMA expression in kidney</td>
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<td>↓ Urinary inflammatory cytokines</td>
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<td>↓ NF-κB, cleaved caspase1, and cleaved caspase 3 activity</td>
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<td>↓ a-SMA expression in kidney</td>
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</table>

AR, adenosine receptor; a-SMA, α-smooth muscle actin; BG, blood glucose; BUN, blood urea nitrogen; DKD, diabetic kidney disease; GFR, glomerular filtration rate; IFN-γ, interferon gamma; Kim-1, kidney injury molecule; MCP-1, monocyte chemoattracted protein-1; mRNA, messenger RNA; NRF1, nuclear respiratory factor 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; P$_{cr}$, plasma creatinine; PGC-1α, proliferator-activated receptor-gamma coactivator-1; STZ, streptozotocin; TFAM, mitochondrial transcription factor A; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; UAE, urinary albumin excretion; VEGF, vascular endothelial growth factor.
in the kidney tubular epithelial cells under hypoxia and inhibits the macrophage infiltration after ischemia-reperfusion [90,91]. A2A AR activation, in fact, attenuates kidney fibrosis and suppresses the epithelial-mesenchymal transition in various kidney fibrosis models [92,93].

Since A3 AR antagonists or A2A AR agonists protect against DKD, it is reasonable to develop AR modulators with dual action as an A3 AR antagonist and an A2A AR agonist in order to have better protective effects against DKD. We showed for the first time that LJ4459, a newly synthesized dual-acting ligand, prevents the progression of tubulointerstitial fibrosis in obstructed kidneys [94]. It will be interesting to determine whether this dual-acting ligand has better therapeutic efficacy against DKD than an A3 AR antagonist or an A2A AR agonist.

Opposing pro- and anti-therapeutic roles of A2B AR in DKD have been reported. The A2B AR agonist Bay60-6583 attenuates diabetic kidney fibrosis [16]. On the contrary, 1) Bay60-6583 promotes profibrotic activation of kidney fibroblasts [95] and 2) MRS1754, the A2B AR antagonist, attenuates profibrotic effects along with reduced vascular endothelial growth factor expression in diabetic rats and mice [12,17]. An anti-inflammatory role of A1 AR in ischemia/reperfusion kidney injury has been reported [96-98], but the role of A1 AR modulators in DKD remains to be studied.

Oxidative stress plays a key role in the development and progression of DKD [99]. Diabetic stress including high glucose and lipids increases reactive oxygen species (ROS), and mitochondrial dysfunction and nicotinamide adenine dinucleotide phosphate oxidase (NOX) play key, if not all, roles in increased ROS under diabetic conditions [100]. A1 AR antagonists reduce adriamycin-induced NOS up-regulation in the kidney [101], and A1 AR or A3 AR knockout reduces NOX-derived oxidative stress in mice [102,103].

The involvement of adenosine on metabolism should be considered, since hyperglycemia is a key player in DKD [1]. The adenosine system has been suggested to play a role in glucose homeostasis, contributing to the pathophysiology of T1D and T2D [3]. Caffeine reduces plasma glucose and increases pancreatic insulin in STZ diabetic rats [104]. Pharmacological inhibition of A1 AR or knockout of A1 AR in mice enhances basal insulin secretion [105,106]. On the other hand, A2A AR activation increases insulin secretion and improves beta-cell function by inhibiting the inflammatory response [107,108]. The absence of A2 AR in mice improved a metabolic phenotype including reduced glucose clearance and increased insulin compared to wild-type mice [103] (Fig. 6). Although further mechanistic studies are required, activation of A2A AR and inhibition of A1 AR or A3 AR improves insulin resistance and hyperglycemia, which may play a role in preventing DKD.

While various AR agonists or antagonists are under clin-
ical trials, according to clinicaltrials.gov, targeting inflammatory, cancer, and cardiovascular diseases, none of the AR modulators are U.S. Food and Drug Administration-approved or under clinical trials against DKD at present.

**Conclusion**

The severity of DKD and limited therapeutic modality underscores the urgency to develop new therapeutic strategies based on pathogenesis of DKD. In this review, we summarized the (patho)physiological role of adenosine and ARs in diabetic kidneys and updated AR modulators showing a protective effect in experimental DKD. There has been no AR modulator in clinical trials against DKD. Considering 1) the protective effect of caffeine on kidney injury in diabetic patients, 2) increased plasma and urinary excretion of adenosine under diabetic stress, 3) upregulation of expression of each AR in diabetic kidneys, and 4) the protective effect of AR modulators in experimental DKD models, we propose AR modulators as a new therapeutic option to treat DKD. Detailed mechanistic studies on the pharmacology of AR modulators, including the role of diabetes-induced posttranslational modification such as glycosylation and palmitoylation, in AR function will help us to develop effective first-in-class AR modulator agents for DKD.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

The preparation of this manuscript was supported by a National Research Foundation grant (No. 2020R1A6A3A13076183), Republic of Korea, and by Ewha Womans University (No. 1-2021-1301-001-1).

**Authors’ contributions**

Conceptualization, Funding acquisition: ESP, HH
Supervision: HH
Writing–original draft: ESP
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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NADPH oxidase inhibitor development for diabetic nephropathy through water tank model

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Oxidative stress can cause generation of uncontrolled reactive oxygen species (ROS) and lead to cytotoxic damage to cells and tissues. Recently, it has been shown that transient ROS generation can serve as a secondary messenger in receptor-mediated cell signaling. Although excessive levels of ROS are harmful, moderated levels of ROS are essential for normal physiological function. Therefore, regulating cellular ROS levels should be an important concept for development of novel therapeutics for treating diseases. The overexpression and hyperactivation of NADPH oxidase (Nox) can induce high levels of ROS, which are strongly associated with diabetic nephropathy. This review discusses the theoretical basis for development of the Nox inhibitor as a regulator of ROS homeostasis to provide emerging therapeutic opportunities for diabetic nephropathy.

Keywords: Diabetic nephropathies, Nox inhibitor, NADPH oxidases, Oxidative stress, Reactive oxygen species

Two faces of reactive oxygen species

Reactive oxygen species (ROS) encompassing superoxide anion (O2·−), hydrogen peroxide (H2O2), and hydroxyl radical (OH−) are believed to be simple by-products of aerobic respiration [1–3]. Uncontrolled and high concentrations of ROS result in oxidative stress that can lead to peroxidation of lipids, oxidation of proteins and nucleic acids, and ultimately cause cellular damage [1–3]. Therefore, ROS production should be tightly regulated during physiological events. A growing body of evidence has indicated that ROS can serve as secondary messengers in signaling transduction pathways mediated by various agonists such as growth factors, hormones, and cytokines to regulate cell growth, apoptosis, and differentiation [1–5]. It has been well established that phosphotyrosine of cellular proteins plays an...
essential role in cellular proliferation [6–9]. Stimulation of growth factor can result in increased tyrosine phosphorylation, leading to cell proliferation. Tyrosine phosphorylation is regulated by a balance between protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTPase). The PTPase family contains the conserved sequence Cys-X-X-X-X-X-Arg (Cys-X5-Arg, X indicates any amino acid) in an active center. Conserved cysteine residue in the active center possesses a low pKa through the positive charge of arginine residue and exists as a thiolate anion (−S−), which is easily oxidized by H2O2. Oxidation of thiolate anion in PTPase is converted to sulfenic acid (−SOH), which can then induce the loss of phosphatase activity of PTPase. Oxidation of PTPase can disrupt the balance between PTK and PTPase. PTK activity is then relatively enhanced, resulting in increased tyrosine phosphorylation of target cell signaling-related proteins and cell proliferation [8,9].

**Reactive oxygen species generation from mitochondrial respiratory chains**

It is known that sources of ROS include the electron transport chain (ETC) in mitochondria and metabolic enzymes such as glucose oxidase, xanthine oxidase, cytochrome p450, and NADPH oxidase (Nox) [1–5,10–12]. This suggests that different complexes of cellular events are involved in ROS generation. Metabolic enzymes including cytochrome p450, glucose oxidase, and xanthine oxidase can produce a constant level of ROS generation, indicating that the ROS generation was not correlated with the pathogenesis of diabetic nephropathy (DN). In contrast to metabolic enzyme-generated ROS, mitochondrial ROS are known to account for a large proportion of total cellular ROS [13–15]. The ETC complexes are located in the mitochondrial inner membrane (respiratory complexes I–IV). Electron leakage from the mitochondrial ETC can generate superoxide, which is converted into H2O2 by manganese superoxide dismutase (Mn-SOD) in the mitochondrial matrix [16,17]. It has been reported that complex I and complex III of the mitochondrial ETC are major sites for ROS generation. However, kidney tissues in hyperglycemia can exhibit reduced expression of complex I and complex III of the ETC. An impaired ETC complex in diabetic conditions results in leaked electrons, leading to ROS generation. Many reports have indicated that mitochondrial Mn-SOD expression is decreased in DN. Reduction of mitochondrial Mn-SOD expression can induce the level of mitochondrial superoxide [18]. However, other reports have suggested that Mn-SOD deficiency fails to induce diabetic kidney diseases [19]. More detailed molecular studies between increased mitochondrial ROS and DN must be performed.

**Regulation of NADPH oxidase activation**

Nox is known to contribute to ROS generation in response to various agonists. Since the first discovery of gp91phox/Nox2 in phagocytic cells, an additional six homologues of Nox2 (Nox1, Nox3, Nox4, Nox5, dual oxidase 1 [Duox1], and dual oxidase 2 [Duox2]) have been identified in various nonphagocytic cells [4,5,12,20,21]. All seven Nox isoforms are composed of a single polypeptide. They can be divided into three types based on functional domain: 1) six transmembrane α-helical domains containing tandem heme group homologues to ferric reductase in the NH2-terminal region; 2) flavin adenine dinucleotide (FAD)-binding site in the membrane proximal region; and 3) NADPH-binding site homologues to ferredoxin-NADP+ reductase in the long COOH-terminal region. Electrons from NADPH are transferred to oxygen molecules through FAD and two hemes, leading to the generation of O2− and H2O2. Activations of Nox isoforms are regulated by unique regulatory mechanisms [4,5,22]. The Nox2 protein is required for one integral protein p22phox, three cytosolic proteins p47phox, p40phox, and p67phox, and small G-protein Rac (Fig. 1A). The p47phox serves as a core protein in the complex. The tandem Src homology 3 (SH3) domains of p47phox can interact with the proline-rich region (PRR) in the COOH-terminal region of the p22phox protein, leading to membrane targeting. Meanwhile, the PRR in the COOH-terminal region of p47phox can recruit the SH3 domain of p67phox. Four tetratricopeptide repeats in the NH3 terminal region of p67phox protein provide the binding site for G-protein Rac. The phox and bem1 (PB1) domain between the two SH3 domains of the p67phox protein serves as the site for the molecular interaction of p67phox protein with the PB1 domain of p40phox. Interactions among an integral protein p22phox, three cytosolic proteins p47phox, p40phox, and p67phox; and Rac provide a stable protein complex of Nox2 to allow electron transfer from NADPH to O2 (Fig. 1A).

Nox1 proteins need to form a complex with integral
protein p22\textsubscript{phox} and two cytosolic proteins Nox organizer 1 (NoxO1) as the homologue of p47\textsubscript{phox}, Nox activator 1 (NoxA1) as the homologue of p67\textsubscript{phox}, and small G-protein Rac (Fig. 1B). The activation pattern of Nox1 is similar to that of Nox2. The PRR of p22\textsubscript{phox} with the SH3 domain of NoxO1 can interact with the SH3 of the NoxO1 protein serving as a central scaffolding molecule in Nox1 complex formation. The PRR of NoxO1 provides a binding site for the SH3 domain of NoxA1 protein containing four tetratricopeptide repeat domains in the NH2-terminal region and the SH3 domain in the COOH-terminal region of SH3YL1 can interact with the Nox4-p22\textsubscript{phox} complex to result in Nox4-dependent 
\( \text{H}_2\text{O}_2 \) generation (Fig. 1C). It has been demonstrated that formation of a ternary complex of p22\textsubscript{phox}-SH3YL1-Nox4 leading to \( \text{H}_2\text{O}_2 \) generation induces severe renal failure in a lipopolysaccharide-induced acute kidney injury model. However, the regulatory mechanism by which high glu-
cose or transforming growth factor β1 regulates the ternary complex of p22phox-SH3YL1-Nox4 formation in fibrosis and DN remains to be determined.

**Uncontrolled reactive oxygen species generation is associated with diabetic nephropathy: water tank model**

A water tank has an inlet and outlet. If the amount of water entering from the inlet and exiting to the outlet is constant, a certain amount of water will always remain in the water tank (Fig. 2). ROS homeostasis including ROS generation and elimination exhibits a similar pattern to the water tank model. ROS can be generated from various cellular sources including Nox activation and mitochondrial respiratory chains, whereas ROS elimination is mediated by the action of cellular antioxidants (glutathione, uric acid, ascorbic acid [vitamin C], α-tocopherol [vitamin E], and ubiquinol [coenzyme Q]) and antioxidant enzymes (SOD, catalase, glutathione S-transferase, and peroxiredoxin) [26–30]. In a normal physiological condition, a balance between ROS generation and elimination is well regulated, and a certain amount of remaining ROS serves as secondary messengers in cell signaling. In a pathological stage, Nox isoforms and their regulating proteins are overexpressed, resulting in uncontrolled ROS generation [31–34]. As the balance of ROS homeostasis is disrupted, the level of ROS is gradually increased in the body, which is closely associated with the pathogenesis of DN [35–37]. Therefore, uncontrolled Nox activation should be suppressed by treatment with Nox inhibitors as an emerging therapy for DN (Fig. 2).

Since uncontrolled ROS generation is involved in DN, many studies on antioxidant therapies for DN have been conducted [38,39]. Thirteen independent clinical studies

![Figure 2. Water tank model for ROS homeostasis.](image)

**Figure 2. Water tank model for ROS homeostasis.** ROS homeostasis including ROS generation and elimination has a similar pattern to the water tank model, which has a water inlet and outlet. Over-activation of Nox isozymes leads to uncontrolled ROS generation that can disrupt the balance of ROS homeostasis. Nox activation should be suppressed by treatment with Nox inhibitors as an emerging therapy for diabetic nephropathy.

Nox, NADPH oxidase; ROS, reactive oxygen species.
have shown inconsistent results, strongly indicating that the beneficial effects of antioxidants on DN progression are controversial [40–52]. Why are there so many inconclusive trials on antioxidant therapy? Several points in the clinical studies must be considered. The first point is that the supplement of antioxidants might not be enough to eliminate ROS present in the disease (Fig. 3). Since antioxidants can be distributed to the entire body, they cannot pinpoint pathogenic tissues to eliminate uncontrolled ROS. Therefore, antioxidant therapy is not sufficient for treating chronic kidney disease [38,39]. The second point is that the function of the antioxidant might be nonspecific for scavenging ROS in the entire body. This indicates that non-specific antioxidants might eliminate appropriate ROS that could potentially play a role in the normal physiology of the human body. In contrast to antioxidants, Nox inhibitors might specifically regulate uncontrolled ROS inlets, resulting in decreasing ROS levels in the entire body to support the return of normal ROS balance (Fig. 3).

**Oxidative stress in diabetic nephropathy**

DN is one of the complications of diabetes and is the leading cause of renal failure resulting in end-stage renal disease [53–55]. In the early stages of DN, increasing albuminuria secretion and reduction of the glomerular filtration rate are common. Glomerular basement membrane thickening, mesangial expansion, overexpression of extracellular matrix (ECM) proteins, tubulointerstitial fibrosis, and glomerulosclerosis can contribute to the progression of DN [56–58]. Several lines of evidence suggest that oxidative stress mediated by uncontrolled ROS generation is associated with the pathogenesis of DN [37,53]. Renal oxidative stress is involved in the activation or overexpression of various Nox isozymes [37]. Previous reports have indicated that various Nox isozymes and their accessory proteins such as p22phox, p47phox, and p67phox are expressed in kidney tissues [22]. Mesangial cells express Nox1, Nox2, and Nox4. Podocytes contain Nox2, Nox4, and Nox5. The upregulation of Nox4 and Nox5 plays a role in mesangial cell hypertrophy, tissue expansion, ECM protein accumulation, and apoptosis of podocytes [37]. Blood vessels in kidney tissues are composed of vascular smooth muscle cells and endothelial cells expressing Nox1, Nox4, and Nox5 known to be responsible for ROS generation in response to angiotensin II [59]. Proximal tubule cells express Nox1, Nox4, and Nox5 isozymes [34,60,61]. Most Nox isozymes are expressed in different kidney tissues and cells [34]. Nox4 is predominantly ex-
pressed in all kidney cells [62–65]. Its expression is upregulated in diabetic conditions. Deletion of Nox4 can attenuate mesangial hypertrophy and ECM accumulation in diabetic conditions [66]. Moreover, inflammatory cytokine production and macrophage infiltration are reduced in Nox4 KO mice [31,67]. In contrast to Nox4, the function of Nox1 and Nox2 in DN is controversial [31,68,69]. Although Nox1 is associated with ROS generation and renal oxidation in diabetic conditions, the molecular function of Nox1 in the pathogenesis of DN remains unclear.

**Development of Nox inhibitors for treatment of diabetic nephropathy**

Many studies have demonstrated that oxidative stress plays an important role in DN. Therefore, various clinical trials for therapeutic agents regulating oxidative stress are continuously being conducted (Table 1) [70–89]. Although no effective therapy exists for treatment of DN, clinical trials for therapeutic Nox inhibitors are ongoing [88,89]. A dual Nox1/4 inhibitor GKT137831 (brand name: Setanaxib), a pyrazolopyridine compound, was developed by Genkyotex (Stockholm, Sweden) [90]. In streptozotocin-induced diabetic ApoE−/− mice, GKT137831 attenuated diabetic-induced glomerular damage including ECM accumulation and glomerular structural changes [31]. Moreover, it has been reported that GKT137831 regulates the reduction of mesangial matrix expansion and podocyte loss in a type I diabetes model [33]. GKT137831 is now enrolled in a phase 2 clinical trial for type I DN in Australia.

**Table 1. Therapeutics including Nox inhibitors in diabetic nephropathy**

<table>
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<th>Effect</th>
<th>Target</th>
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<td>GKT137831</td>
<td>Nox1/4 inhibitor</td>
<td>Diabetic nephropathy [31,33,37,38,53,70]</td>
<td>Phase 2 trial completed (NCT0 2010242) [71]</td>
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<td>Atherosclerosis [70]</td>
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<td>Diabetic retinopathy [88]</td>
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<td>APX-115</td>
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<td>Phase 2 trial completed (NCT04534439)</td>
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<td>Plumbagin</td>
<td>Nox4 inhibitor</td>
<td>Diabetic nephropathy [37]</td>
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<td>Diabetic nephropathy [37,74,75]</td>
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<td>XO inhibitor</td>
<td>Diabetic nephropathy [38,89]</td>
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<td>Diabetic nephropathy [38]</td>
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<td>Nrf2 activator</td>
<td>Type 2 diabetes [38,89]</td>
<td>Phase 2 trial completed (NCT0 0811889) [84,85]</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Antioxidant</td>
<td>Diabetic nephropathy [37,86]</td>
<td>Phase 3 trial terminated (NCT0 0220831) [43,87]</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Antioxidant</td>
<td>Type 2 diabetes [37,86]</td>
<td></td>
</tr>
</tbody>
</table>

NHE3, N+/H+ exchanger isoform 3; Nox, NADPH oxidase; PKC, protein kinase C; ROCK, rho-associated coiled-coil-containing protein kinase; SGLT2, sodium glucose cotransporter 2; XO, xanthine oxidase.
compound was first developed for osteoporosis treatment by Joo et al. [91]. The compound was transferred to AptaBio Corp. (Suwon, Korea) and is now referred to as APX-115 (Table 1). In db/db mice as a type II diabetes model, APX-115 suppressed mesangial expansion and urinary albumin excretion [72]. Renal Nox5 expression was highly increased in renal podocyte-specific Nox5 transgenic mice (Nox5 pod+). Moreover, APX-115 inhibited Nox5 expression and levels of urinary albumin and creatinine in Nox5 pod+ mice, indicating APX-115 as a potentially therapeutic agent for treatment of DN through inhibition of macrophage infiltration to the glomerulus, a renal inflammatory signal related to tumor necrosis factor receptor-associated factor and Nox5 expression. APX-115 is now in a phase 2 clinical trial for DN in the European Union. Rho-associated coiled-coil-containing protein kinase (ROCK) and sodium glucose cotransporter (SGLT) 2 are novel treatments for DN [74,75]. Fasudil, a ROCK inhibitor, reduced albuminuria in db/db mice. Canagliflozin, an SGLT inhibitor, is a compound under development by Mitsubishi Tanabe Pharma Corp. and has completed phase III clinical trials in Japan. The novel compound improved renal outcomes in patients with type 2 diabetes [82]. Xanthine oxidase inhibitors and antioxidants regulating oxidative stress are summarized in Table 1.

Conclusion

Homeostasis between ROS generation and elimination plays an important role in normal physiological functions. Uncontrolled ROS generation through over-activation of Nox activity is closely associated with the pathogenesis of DN. Uncontrolled Nox activation should be suppressed by treatment with Nox inhibitors as an emerging therapy for DN. Recently, two Nox inhibitors (GKT137831 from Genkyotex and APX-115 from AptaBio) have been subjected to clinical phase II trials for DN patients. We believe that these Nox inhibitors provide new hope for DN patients.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by the Aging project (2017M3A9D8062955 to YSB) and Bio-SPC (2018M3A9G1075771 to YSB) funded by the National Research Foundation of Korea (NRF) and Ministry of Science and ICT, and by a grant from the Korea Health Technology R&D Project (HI21C0293 to HEL) through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare.
39. Bolignano D, Cernaro V, Gembillo G, Baggetta R, Buemi M, D’Ar-


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These are expected to present major advances and important
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The title page should include article title, each author's first and last names, positions (associate professor, fellow, student, etc.), and ORCID identifiers, and the institutions with which they are affiliated, short running title not exceeding 50 characters, separate word count for abstract and text, and details of the corresponding author (name, address, phone, and e-mail information). Funding sources should be included, and the individual contribution of each co-author must also be detailed (see relevant section 4.3 below).

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