

# Diabetic nephropathy – complications and treatment

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**Abstract:** Diabetic nephropathy is a significant cause of chronic kidney disease and end-stage renal failure globally. Much research has been conducted in both basic science and clinical therapeutics, which has enhanced understanding of the pathophysiology of diabetic nephropathy and expanded the potential therapies available. This review will examine the current concepts of diabetic nephropathy management in the context of some of the basic science and pathophysiology aspects relevant to the approaches taken in novel, investigative treatment strategies.

**Keywords:** diabetes, diabetic nephropathy, albuminuria, kidney disease, inflammation

## Introduction

### Background

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics. Diabetes may be classified as type 1 (autoimmune  $\beta$ -cell destruction and absolute insulin deficiency), type 2 (relative insulin deficiency and resistance), and other types (eg, pancreatic disease).

### Epidemiology

The prevalence of diabetes is phenomenal and the projections are staggering. When one considers the morbidity, mortality, and cost of health care, the burden of the diabetes epidemic becomes apparent. Worldwide, the prevalence of diabetes was estimated at 171 million in 2000, increasing to 382 million in 2013; and is projected to reach 592 million by 2035. This represents 8%–10% of the global population, resulting in at least 548 billion dollars in health expenditure on diabetes care. Type 2 diabetes constitutes about 85%–95% of all diabetes cases.<sup>1</sup> In the US alone for 2011, 25.8 million children and adults have diabetes with another 79 million having a prediabetic state.<sup>2</sup>

The diabetes epidemic has resulted in DN becoming the most frequent cause of end-stage renal disease (ESRD) in most countries. In 2009–2011, diabetes was the primary cause of ESRD in about 60% of patients in Malaysia, Mexico, and Singapore. Countries with an ESRD incidence of 40%–50% include Israel, Korea, Hong Kong, Taiwan, Philippines, Japan, the US, and New Zealand.<sup>2</sup> The incidence of ESRD due to diabetes also rises in the older age group. In 2011, the incident rates of ESRD due to diabetes in the US were 44, 266, and 584 per million for the age groups 20–44, 45–64, and 65–74 years, respectively. A similar finding was noted in the AusDiab study of 11,247 diabetic Australians.<sup>3</sup> Thus, the reason for this boom in diabetes-associated

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ESRD is the increasing prevalence of diabetes and the aging population.

## Risk factors

Not all diabetics develop DN and in those who do, progression is variable. The main modifiable risks are hypertension, glycemic control, and dyslipidemia. Data from the Joslin Diabetes Center, Steno Diabetes Center, and AusDiab studies also strongly implicate smoking as a risk factor for DN.<sup>3–5</sup> The main unmodifiable risks are age, race, and genetic profile. DN is more likely to develop in patients with a family history of DN.<sup>6–8</sup> Certain racial groups are also at higher risk, such as African Americans, Mexican Americans, and Pima Indians.<sup>9,10</sup> One study suggested that males had an increased risk of DN.<sup>11</sup>

A meta-analysis of studies identified 24 genetic variants in 16 genes which are associated with DN. These include: *ACE*, *ALR2*, *APOC1*, *APOE*, *EPO*, *eNOS*, *HSPG2*, *VEGF*, *FRMD3*, *CARS*, *UNC13B*, *CPVL/CHN2*, and *GREM1*. In a subgroup of type 2 diabetic Asians, *ELMO1*, *CCR5*, and *CNDP1* were also relevant.<sup>12</sup> Other meta-analyses implicated polymorphisms of *ADIPOQ*, *PAI-1*, *TGFβ1*, and *PPARγ* in the development of DN. The nature of the polymorphism varies with ethnicity.<sup>13–15</sup> The complexity of genetic studies in DN is discussed in a review by Mooyaart.<sup>16</sup>

## Diagnosis

### Stages and natural history

Incipient nephropathy is the initial presence of low but abnormal amounts of urine albumin, referred to as microalbuminuria (persistent albuminuria at level 30–299 mg/24 hours). Overt nephropathy or macroalbuminuria (persistent albuminuria at level  $\geq$ 300 mg/24 hours) develops after many years in type 1 diabetes but may be present at the time of diagnosis of type 2 diabetes. Patients who progress to macroalbuminuria are more likely to develop ESRD.<sup>11</sup> The natural history depends on the type of diabetes.

In untreated type 1 diabetics, approximately 80% of patients with sustained microalbuminuria increase their albumin excretion by 10%–20% per year until overt nephropathy develops, which normally takes 10–15 years. With the development of overt nephropathy, the GFR declines at a rate of 2–20 mL/minute/year and ESRD develops in 50% within 10 years and in 75% by 20 years.<sup>17</sup> Structural changes can precede albuminuria and reduced GFR, with glomerular basement membrane thickening and mesangial expansion, can be detected as early as 2–8 years after onset of diabetes.<sup>18</sup>

In type 2 diabetics, more patients have DN at the time of diagnosis of diabetes as type 2 diabetes can go unrecognized for years. The AusDiab study of diabetic Australians showed that albuminuria is common among patients with established diabetes, is present before the onset of diabetes, and becomes more prevalent with worsening glucose tolerance.<sup>3</sup> About 20%–40% of type 2 diabetics with microalbuminuria progress to overt nephropathy; and about 20% will develop ESRD after the development of overt nephropathy.<sup>17,19</sup>

## Screening for DN

Most guidelines recommend screening with a spot urine albumin/creatinine ratio (ACR; normal <30 mg/g creatinine), from either first morning (preferred) or random specimens. An abnormal result is repeated once or twice over a few months for consistency. This is coupled with an assessment of renal function, using the Modification of Diet in Renal Disease or Chronic Kidney Disease Epidemiology Collaboration formulas for estimated GFR (eGFR) in order to stage chronic kidney disease (CKD).<sup>20,21</sup> Screening begins at diagnosis of type 2 diabetes and usually 5 years after onset of type 1 diabetes. Timed collections can also be utilized and will average out diurnal variations in albumin excretion (normal <20  $\mu$ g/minute).

## Renal biopsy

The routine use of renal biopsy to confirm DN is much debated. Many nephrologists do not biopsy patients with classic features such as retinopathy, duration of diabetes >10 years, slow decline in GFR, gradual progression of proteinuria, and lack of active urinary sediment. Without standardized criteria, there may be significant variations in epidemiology. An Italian study of 393 type 2 diabetics highlighted this point. In centers with an unrestricted biopsy policy, the rate of finding an underlying glomerulonephritis was lower than those centers with a restricted biopsy policy (33% versus 57%). The unrestricted policy resulted in a greater proportion of patients found to have glomerulonephritis rather than diabetic glomerulosclerosis.<sup>22</sup> The prevalence of specific disease in the population can also affect the biopsy decision. In a Chinese study of 51 type 2 diabetics with >1 g/day proteinuria, one-third of patients had nondiabetic disease, predominantly IgA nephropathy.<sup>23</sup> The largest study to date looked at 620 biopsies from type 1 and 2 diabetics, with a median duration of diabetes of 10 years. Overall, 37% of patients had isolated DN, 36% had isolated nondiabetic disease, and 27% had nondiabetic disease superimposed on DN.

The duration of diabetes >12 years was the best predictor for isolated DN. Interestingly, 43% of biopsies with DN demonstrated superimposed acute tubular necrosis.<sup>24</sup> Thus, a renal biopsy is useful to exclude acute tubular injury and diseases amenable to specific therapy.

## Biomarkers

There are limitations in using albuminuria as a marker of DN as many patients experience GFR loss without deterioration in albuminuria and even normoalbuminuria.<sup>25</sup> In fact, histologically proven advanced diabetic glomerular lesions can develop despite normoalbuminuria.<sup>26</sup> Furthermore, low-grade albuminuria is a lesser predictor of disease progression than macroalbuminuria.<sup>27</sup> Therefore, there is interest in finding biomarkers to detect DN earlier and identify progression risk. There is also interest in urine microRNA profiling but studies are fairly preliminary.<sup>28,29</sup> The most promising biomarker currently is serum TNF- $\alpha$  receptor levels, which may predict progression of CKD and ESRD, in type 1 and type 2 diabetics. In type 2 diabetics, the TNF- $\alpha$  receptor level showed prognostic value in addition to albuminuria.<sup>30,31</sup> Serum uric acid is another biomarker which may also be pathogenic (discussed later). Studies of tubular biomarkers have been conflicting (Table 1). The larger studies have not

shown that these biomarkers offer additional value on top of traditional prediction models. More work is needed to clarify the role of biomarkers in clinical practice.

## Pathogenesis

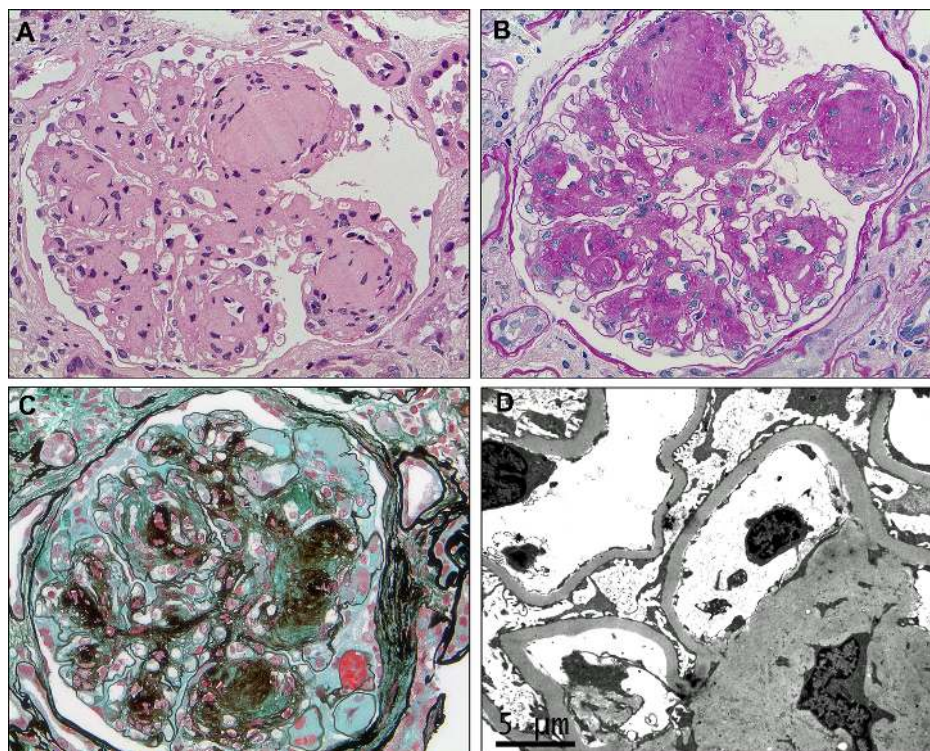
### Pathology and pathophysiology

DN is characterized by structural and functional changes. In glomeruli, there is mesangial expansion, thickening of the basement membrane, and, characteristically, nodular glomerulosclerosis (Kimmelstiel–Wilson nodules). In early DN, tubular hypertrophy is present but eventually interstitial fibrosis with tubular atrophy develops, along with arteriolar hyalinosis. In advanced cases, there is an infiltrate of macrophages and T-lymphocytes. Ultrastructurally, there is podocyte loss and reduced endothelial cell fenestration.<sup>32,33</sup> These characteristic pathological changes are shown in Figure 1. Functionally, there is early glomerular hyperfiltration and increased albumin excretion; and with advancing nephropathy, increasing proteinuria and declining GFR. A brief description of the functional and cellular pathology is provided below. Although it is conceptually easier to describe these pathways individually, these pathways overlap and interact with one another in vivo, and enhance one another's biophysiological effects (Figure 2).

**Table 1** Tubular biomarkers

Biomarker	Source	Cohort (size)	Key points	Reference
KIM-1	Blood	Type 1 (124)	Baseline KIM-1 in proteinuric (>500 mg/day) patients predicted rate of eGFR loss and ESRD during 5–15 years of follow-up.	Sabbiseti et al <sup>208</sup>
	Urine	Type 1 (63)	KIM-1 associated with decline in GFR but not independent of albuminuria.	Nielsen et al <sup>209</sup>
	Urine	Type 2 (978)	Urine KIM-1/Cr associated with faster decline in GFR during 4 years follow-up but offered no additional prognostic information to albumin/Cr ratio.	Conway et al <sup>210</sup>
NGAL	Serum/urine	Type 1 (50)	Elevated before microalbuminuria. Serum NGAL correlated with HbA <sub>1c</sub> and urine NGAL correlated with albuminuria.	Lacquaniti et al <sup>211</sup>
	Urine	Type 1 (63)	NGAL associated with decline in GFR but not independent of albuminuria.	Nielsen et al <sup>209</sup>
L-FABP	Serum/urine	Type 2 (140)	No correlation with eGFR.	Chou et al <sup>212</sup>
	Urine	Type 1 (1,549)	Patients ranged from normoalbuminuria to macroalbuminuria. Urine L-FABP/Cr ratio at baseline predicted progression of DN but adding L-FABP to albumin excretion did not improve prediction model.	Panduru et al <sup>213</sup>
	Urine	Type 1 (277)	Urine L-FABP predicted progression of albuminuria or death.	Nielsen et al <sup>214</sup>
	Urine	Type 1 (63)	L-FABP not related to decline in GFR.	Nielsen et al <sup>209</sup>
	Serum/urine	Type 2 (140)	Serum L-FABP correlated with baseline eGFR but did not predict decline in eGFR.	Chou et al <sup>212</sup>
	Urine	Type 2 (618)	Japanese patients without overt proteinuria and serum creatinine $\leq 1.0$ mg/dL followed for median of 12 years. Urine L-FABP in the highest tertile was associated with 50% decline in eGFR or progression to eGFR < 30 mL/minute/m <sup>2</sup> .	Araki et al <sup>215</sup>
	Urine	Type 2 (140)	High L-FABP associated with progressive albuminuria, ESRD, or hemodialysis.	Kamijo-Ikemori et al <sup>216</sup>
Cystatin C	Urine	Type 2 (237)	Urine cystatin C/Cr ratio associated with decline in eGFR, with the upper tertile of levels associated with progression to stage 3 CKD or higher after 20 months follow-up.	Kim et al <sup>217</sup>

**Abbreviations:** CKD, chronic kidney disease; Cr, creatine; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GFR, global filtration rate; HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>.



**Figure 1** Characteristic histological features of diabetic nephropathy.

**Notes:** In advanced diabetic nephropathy, there is extensive mesangial expansion due to increased extracellular matrix production, with the formation of spherical, eosinophilic nodules with a central hypocellular or acellular area, known as Kimmelstiel–Wilson nodules (**A**) (hematoxylin–eosin,  $\times 400$ ). These nodules are also typically strongly periodic acid–Schiff–positive and may be seen compressing and narrowing the peripheral capillary loops (**B**) (periodic acid–Schiff,  $\times 400$ ). The increased matrix stains dark with silver and the Kimmelstiel–Wilson nodules may demonstrate a lamellated appearance. Capillary microaneurysms can be seen at the periphery on the right (in the 1–5 o'clock position), in association with mesangiolysis (**C**) (Masson's trichrome–methenamine silver,  $\times 400$ ). There is diffuse thickening of the glomerular basement membrane, which is apparent on electron microscopy even if it is difficult to discern by light microscopy in early disease, and often accompanied by some degree of podocyte foot process effacement (**D**) (electron microscopy).

## Hemodynamic factors

There is an imbalance in afferent and efferent arteriolar resistance, resulting in increased glomerular hydrostatic pressure and hyperfiltration. Activation of the renin–angiotensin system (RAS) increases angiotensin II levels, leading to efferent arteriolar vasoconstriction and production of proinflammatory and profibrotic molecules through multiple mechanisms. High angiotensin converting enzyme (ACE) levels are associated with greater albuminuria and nephropathy in diabetic mice and humans.<sup>34,35</sup> Increased levels of endothelin-1 and urotensin II also contribute to vasoconstriction. Various dysregulation of nitric oxide and nitric oxide synthase has been described in DN. Nitric oxide mediates endothelium-dependent vasodilatation, and is formed from L-arginine by endothelial nitric oxide synthase. Diabetic endothelial nitric oxide synthase knockout mice develop more severe glomerular lesions and proteinuria compared to wild-type mice.<sup>36</sup>

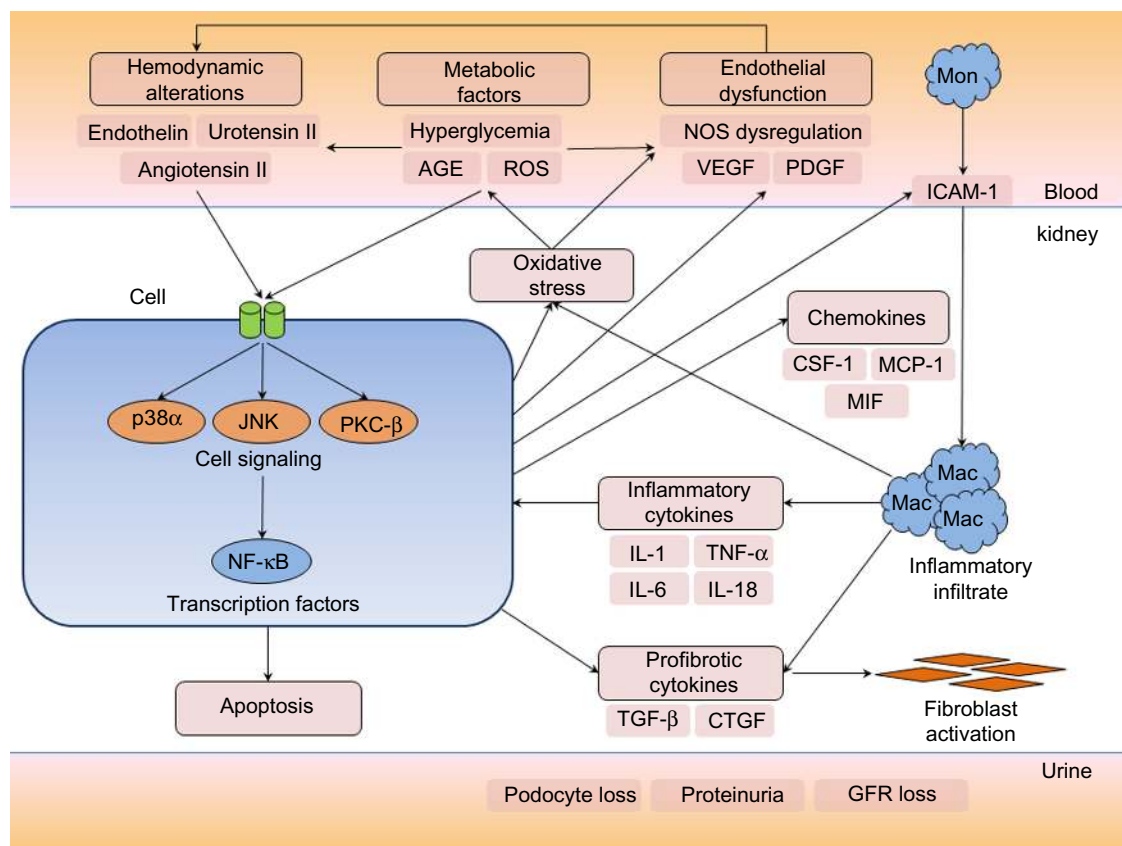
## Metabolic factors

Oxidative stress and generation of reactive oxygen species (ROS) damage DNA and protein, or function as signaling

amplifiers to activate cellular stress pathways such as PKC, MAPK, and NF- $\kappa$ B.<sup>37,38</sup> Activation of the polyol pathway, with aldose reductase converting excess glucose to sorbitol, and subsequent conversion to fructose by sorbitol dehydrogenase contributes to oxidative stress by increasing the NADH/NAD<sup>+</sup> ratio.<sup>39,40</sup> A recently described novel mechanism of injury also involves endogenous fructose production with activation of fructokinase in the proximal tubule.<sup>41</sup> The formation of advanced glycation end-products (AGE) by nonenzymatic binding of glucose to proteins, lipids, and nucleic acids can lead to alteration of protein structure and function, oxidative stress, and expression of proinflammatory cytokines and growth factors.<sup>42</sup>

## Growth factors/cytokines

Activation of TGF- $\beta$  and its downstream cytokine, CTGF, induce extracellular matrix formation and fibrosis. In kidney biopsies, glomerular expression of TGF- $\beta$ 1 and CTGF were higher in diabetics compared to controls, and correlated with albuminuria. PDGF expression is also increased in DN, which can modulate chemotaxis, vascular tone, and



**Figure 2** Overview of the pathological pathways in diabetic nephropathy.

**Notes:** In the diabetic milieu, metabolic derangements and hemodynamic alterations, particularly activation of the renin–angiotensin system, trigger a number of cell signaling cascades, including the MAPKs (p38 and JNK) and PKC- $\beta$ , which mediate a cellular response through activation of key transcription factors such as NF- $\kappa$ B. In response to such signals, renal cells such as tubular epithelial cells, podocytes, and mesangial cells can produce chemokines, growth factors, and profibrotic cytokines. CSF-1 and MCP-1 function as chemotactic molecules and promote the recruitment of monocytes from the circulation. Upregulation of ICAM-1 on endothelial cells – a key leukocyte adhesion molecule – facilitates infiltration of circulating mononuclear cells into the kidney. CSF-1 also promotes monocyte/macrophage differentiation, proliferation, and activation. MIF functions to retain macrophages at sites of inflammation and has counter-regulatory functions against the anti-inflammatory actions of glucocorticoids. Activated macrophages can produce proinflammatory and profibrotic cytokines, reactive oxygen species, and antiangiogenic factors and contribute to a cycle of inflammation, oxidative stress, cellular injury, progressive fibrosis, and loss of glomerular filtration rate. Podocyte loss, endothelial dysfunction, alterations in the GBM, and tubular injury contribute to increasing proteinuria during the development and progression of diabetic nephropathy.

**Abbreviations:** AGE, advanced glycation end-products; GBM, glomerular basement membrane; GFR, glomerular filtration rate; Mac, macrophages; Mon, monocyte; NOS, nitric oxide synthase; ROS, reactive oxygen species.

platelet aggregation. VEGF is crucial in angiogenesis but also mediates vasodilatation and leukocyte trafficking in DN.

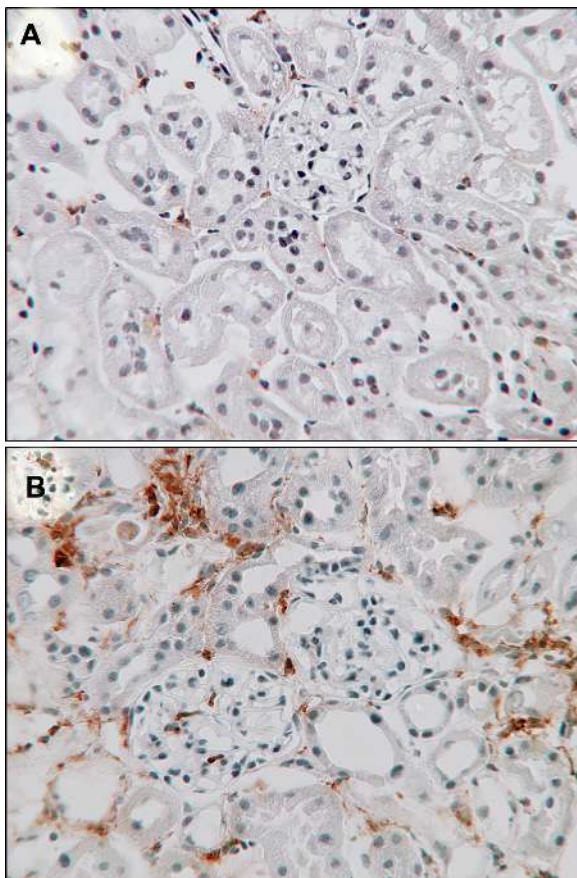
## Cell signaling and transcription factors

Increased renal gene transcription of PKC- $\beta$  showed a strong relationship with glycemic control.<sup>43</sup> PKC activation has wide ranging effects, including enhancing angiotensin II actions, nitric oxide dysregulation, endothelial dysfunction, and activation of MAPK and NF- $\kappa$ B.<sup>44,45</sup> MAPKs are intracellular kinases which integrate cell signaling into cellular responses. MAPKs activate a number of nuclear transcription factors, including NF- $\kappa$ B, which then regulates the gene expression of various cytokines, chemokines, and adhesion molecules. The activation of p38 $\alpha$  isoform of the p38 MAPK pathway is most strongly associated with renal inflammation and DN.<sup>46,47</sup> There may also be a role for toll-like receptors (TLR2, TLR4)

and B7-1 costimulatory signaling in modulating inflammation and injury in DN.<sup>48,49</sup> Finally, transcription factors bind to the promoter regions of genes and modulate transcription of messenger RNA. NF- $\kappa$ B has been the best studied in DN. Activation of NF- $\kappa$ B in both human peripheral blood mononuclear cells and kidney biopsies correlate with severity of proteinuria and glycemic control.<sup>50,51</sup> A review of transcription factors in DN is provided by Sanchez and Sharma.<sup>52</sup>

## Inflammation

In DN, there is recruitment and activation of innate immune cells and elaboration of proinflammatory cytokines.<sup>53</sup> Macrophages and T-lymphocytes are prominent in early diabetic glomeruli while an interstitial infiltrate develops later (Figure 3). Strategies impairing kidney leukocyte recruitment, proliferation, or activation have demonstrated



**Figure 3** Macrophages in diabetic nephropathy.

**Notes:** Immunostaining for a macrophage marker (CD68) in kidney sections shows sparse interstitial macrophages in age-matched, nondiabetic control mice (A) compared to diabetic mice after 20 weeks of diabetes induced by streptozotocin (B) (CD68 brown, counterstained with hematoxylin,  $\times 250$ ).

that macrophages mediate DN.<sup>54,55</sup> In humans, kidney macrophage accumulation is associated with the severity of glomerulosclerosis.<sup>56</sup> Accumulation of interstitial macrophages correlated strongly with proteinuria, interstitial fibrosis, and GFR decline.<sup>57</sup>

The role of lymphocytes is less clear. A higher circulating level of activated T-cells is associated with DN.<sup>58</sup> A kidney T-cell influx is common in early type 1 diabetes, and correlates with renal function and albuminuria.<sup>59</sup> However, absence of lymphocytes did not prevent fibrosis and declining renal function in experimental DN.<sup>60</sup> Recent attention has focused on the subset of regulatory T-cells (Treg), which may play a protective role in DN. Treg numbers are increased in diabetic mice.<sup>60</sup> Treg depletion in diabetic mice exacerbated albuminuria and hyperfiltration, while adoptive transfer of Treg improved DN.<sup>61</sup> In type 2 diabetics, the number of Tregs as determined by flow cytometry showed an inverse correlation with albuminuria, particularly in patients with macroalbuminuria.<sup>62</sup> Treg also demonstrated an anti-inflammatory function, which reduces

the metabolic abnormalities and insulin resistance in a mouse model of type 2 diabetes.<sup>63</sup> The main proinflammatory cytokines implicated in DN are TNF- $\alpha$ , MCP-1, ICAM-1, IL-1, IL-6, and IL-18. These cytokines are increased in diabetic patients and show correlation with albuminuria and glomerular pathology.<sup>53</sup>

## Treatment

Treatment to delay DN progression involves adequate control of metabolic and hemodynamic abnormalities. In practical terms, this means adequate blood glucose lowering and control of hypertension. A description of all glucose lowering agents is beyond the scope of this review but certain agents have theoretical benefits beyond glucose lowering. Certain antihypertensives are also preferred based on studies which have demonstrated reductions in proteinuria or preservation of GFR, or both. The main pharmacological interventions described here are summarized in Table 2. Nonpharmacological approaches and alternative medicine are briefly discussed. There is also interest in novel agents, gene therapy, and stem cell treatment, which may someday find a place in the treatment armamentarium.

## Glycemic control

Good glycemic control is effective in reducing diabetic microvascular complications. DCCT was a trial involving

**Table 2** Summary of pharmacological treatment of diabetic nephropathy

Drug (s)	Antiproteinuric	Preserve GFR	Diabetes type
ACE inhibitor	++	++	Type 1 and 2
ARB	++	++	Type 2
ACE inhibitor plus ARB	+++	–	Type 1 and 2
Aldosterone antagonist	+	?	Type 2
Aldosterone antagonist plus ACE inhibitor or ARB	+++	?	Type 1 and 2
Renin inhibitor	++	?	Type 2
Renin inhibitor plus ACE inhibitor or ARB	+++	–	Type 2
Non-dihydropyridine CCB	+	?	Type 2
Non-dihydropyridine CCB plus ACE inhibitor or ARB	++	?	Type 2
Dihydropyridine CCB	–	–	Type 2
Allopurinol	?	?	?
Statin	+	?	Type 2
Vitamin D	+	?	Type 2

**Notes:** + data exist to indicate benefit; – data exist to indicate lack of benefit or harm; ? insufficient data for conclusion, possible benefit. The number of + indicates a semiquantitative scale of beneficial effect.

**Abbreviations:** ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; GFR, glomerular filtration rate.

1,365 type 1 diabetics and normoalbuminuria. After almost 10 years, patients randomized to intensive glucose control had lower incidences of microalbuminuria and macroalbuminuria.<sup>64</sup> In the UKPDS trial of 3,867 newly diagnosed type 2 diabetics, patients receiving intensive glucose treatment were less likely to develop renal failure.<sup>65</sup> In the ADVANCE trial of 11,140 type 2 diabetics, intensive therapy (mean hemoglobin A<sub>1c</sub> [HbA<sub>1c</sub>] ≤6.5%) also reduced the incidence of nephropathy compared to standard control (mean HbA<sub>1c</sub> 7.3%). Intensive glucose control reduced the risk of ESRD by 65%.<sup>66</sup> In the VADT study of 1,791 type 2 diabetics, intensive glucose control (median HbA<sub>1c</sub> 6.9%) was associated with less worsening of albuminuria and progression to macroalbuminuria but no significant difference in GFR at 6 years.<sup>67</sup> However, intensive glucose control to an HbA<sub>1c</sub> of <6% may confer excess mortality, as demonstrated in the ACCORD trial of type 2 diabetics with cardiovascular disease or cardiovascular risk factors.<sup>68,69</sup> Thus, an HbA<sub>1c</sub> of <6%, particularly if associated with significant hypoglycemic episodes, should be avoided.

Certain drugs may confer beneficial effects independent of glucose lowering. PPAR-γ inhibitors such as pioglitazone and rosiglitazone have demonstrated antifibrotic and anti-inflammatory effects in the kidney of diabetic rats.<sup>70–72</sup> In type 2 diabetics, the addition of rosiglitazone to metformin treatment for 32 weeks reduced albuminuria and blood pressure independent of glycemic control.<sup>73</sup> DPP-4 inhibitors (gliptins) have shown anti-inflammatory and antiapoptotic properties in DN models.<sup>74</sup> In type 2 diabetics, sitagliptin treatment for 6 months reduced albuminuria independent of HbA<sub>1c</sub>.<sup>75</sup> In a study of alogliptin in type 2 diabetics, researchers showed a reduction in oxidative stress but no change in renal function.<sup>76</sup> Lastly, SGLT-2 inhibitors such as empagliflozin may reduce hyperfiltration by their effect on tubuloglomerular feedback.<sup>77</sup> Further trial evidence is needed to determine if these agents should be preferred agents in patients with DN.

## Antihypertensives

### ACE inhibitors

ACE inhibitors have a strong track record in slowing disease progression in type 1 and type 2 diabetics. In the 1990s, captopril demonstrated the ability of ACE inhibitors in reducing the progression of albuminuria and decline in renal function in type 1 diabetics, independent of blood pressure lowering.<sup>78–80</sup> In the Collaborative Study Group trial of 409 type 1 diabetics, captopril treatment reduced the risk of doubling of serum creatinine by 48% and reduced the composite outcome of death, dialysis, and transplantation by

50% compared to placebo.<sup>80</sup> This study also demonstrated that a sustained remission of nephrotic-range proteinuria was possible with ACE inhibitors.<sup>81</sup> This was backed up by a study which showed that patients who achieved remission (albuminuria <600 mg/day) for ≥1 year had better outcomes compared to those who did not, including slower decline in GFR and lower risk of dialysis, transplantation, or death.<sup>82,83</sup>

The perindopril/indapamide combination was studied in the ADVANCE trial of 11,140 type 2 diabetics. After mean follow-up of 4.3 years, perindopril/indapamide treatment reduced new onset microalbuminuria and prevented progression of microalbuminuria to overt nephropathy. However, serum creatinine and ESRD were not affected. It has also been argued that the effect on albuminuria was not independent of blood pressure, given a difference of 5.6/2.2 mmHg between the treatment groups.<sup>84</sup> Finally, the BENEDICT trial also showed that ACE inhibitor treatment could delay onset of microalbuminuria in type 2 diabetics with hypertension and baseline normoalbuminuria.<sup>85</sup>

### Angiotensin receptor blocker (ARB)

In the IDNT trial, 1,715 hypertensive type 2 diabetics with nephropathy were randomly assigned to receive irbesartan, amlodipine, or placebo.<sup>86</sup> Irbesartan reduced the risk of ESRD or doubling of serum creatinine by 20%–23% compared to amlodipine or placebo. In the RENAAL trial, 1,513 type 2 diabetics with nephropathy were randomly assigned to losartan or placebo, in addition to conventional antihypertensives. Losartan reduced the risk of ESRD or doubling of serum creatinine by 25%–28% compared to placebo.<sup>87</sup> These effects were also independent of blood pressure lowering. Much like the early captopril studies in type 1 diabetics, a lower residual level of albuminuria was associated with lower ESRD risk.<sup>88</sup> The ROADMAP trial of 4,447 type 2 diabetics randomized to olmesartan or placebo demonstrated that olmesartan was more effective in delaying the onset of microalbuminuria. However, the olmesartan group had a slightly lower blood pressure (mean difference 3.1/1.9 mmHg) and there appeared to be a higher rate of fatal cardiovascular events in those with preexisting coronary artery disease.<sup>89</sup>

### ACE inhibitor versus ARB

In the DETAIL trial, 250 type 2 diabetics with early DN were randomly assigned to enalapril or telmisartan. This trial indicated that telmisartan was not inferior to enalapril in reducing a decline in GFR over 5 years. However, there was only a relatively small proportion of patients with overt nephropathy in this study.<sup>90</sup> Given the paucity of data for

ARBs in type 1 diabetics, some clinicians prefer initiating treatment with an ACE inhibitor for type 1 DN.

For primary prevention of DN, a recent meta-analysis of eight studies and 11,906 participants found that ACE inhibitors reduced the risk of new onset microalbuminuria, macroalbuminuria, or both when compared to placebo (relative risk 0.71; 95% confidence interval 0.56–0.89). However, similar benefits could not be demonstrated for ARBs.<sup>91</sup> Thus, there is no proven benefit in starting ARB treatment in normotensive, normoalbuminuric type 1 or type 2 diabetics. Neither ACE inhibitor nor ARB is currently recommended in normotensive, normoalbuminuric diabetics for primary prevention of DN.

### ACE inhibitor and ARB

Earlier studies of combination ACE inhibitor and ARB reported superiority of combination therapy for lowering albuminuria and blood pressure versus either alone, in both type 1 and 2 diabetics.<sup>92–94</sup> One study also showed a reduction in urinary TGF- $\beta$  levels as another surrogate marker.<sup>95</sup> Despite the positive effects on these surrogate markers, the impact on preservation of GFR has not been demonstrated. The ONTARGET trial, which combined ramipril and telmisartan in patients with DN, noted no significant difference in the incidence of dialysis or doubling of serum creatinine when compared to single RAS inhibition.<sup>96</sup> In the Veterans Affairs NEPHRON-D study, the addition of lisinopril to losartan treatment did not reduce the composite endpoint of 50% reduction in eGFR, ESRD, or death.<sup>97</sup> Furthermore, combination treatment was associated with higher incidences of acute kidney injury and hyperkalemia in both these trials. Thus, the dual ACE inhibitor/ARB treatment strategy for DN has largely been abandoned.

### Aldosterone antagonists

Aldosterone is the final component of the RAS cascade. Aldosterone promotes fibrosis, inflammation, and generation of ROS, along with endothelial dysfunction, cell growth, and proliferation.<sup>98,99</sup> Spironolactone appears to reduce proteinuria on its own or in combination with ACE inhibitor or ARB, in both type 1 and type 2 diabetics.<sup>100,101</sup> In addition to a blood pressure lowering effect, an anti-inflammatory mechanism is also likely, including reductions in MCP-1, MIF, and macrophage accumulation.<sup>102</sup> In a randomized trial of 268 type 2 diabetics, the addition of eplerenone to an ACE inhibitor reduced albuminuria.<sup>103</sup> However, the combination of aldosterone antagonists and other RAS inhibitors increases the risk of hyperkalemia and there is no long-term data on

loss of renal function with combination blockade. Thus, combination of aldosterone antagonists and ACE inhibitor/ARB is unclear but, if used, careful monitoring of blood potassium is recommended along with dietary limitation of potassium intake.

### Calcium channel blocker (CCB)

The addition of a non-dihydropyridine CCB to RAS inhibition may also be beneficial. Both verapamil and diltiazem have been shown to lower proteinuria in type 2 diabetics.<sup>104</sup> The effects of adding verapamil to lisinopril or trandolapril treatment were additive in reducing albuminuria and a decline in GFR.<sup>105,106</sup> However, the BENEDICT-B study of verapamil in combination with trandolapril did not find an additional benefit in regression of macroalbuminuria in hypertensive type 2 diabetics independent of blood pressure lowering.<sup>107</sup> In the MARVAL study of 332 type 2 diabetics randomized to valsartan or amlodipine (a dihydropyridine CCB) for 24 weeks, valsartan was more effective than amlodipine in reducing albuminuria, including remission to normoalbuminuria.<sup>108</sup> Further evidence from the Nephros and REIN-2 studies in nondiabetic CKD suggests that dihydropyridine CCBs such as felodipine and amlodipine do not have additive value in reducing proteinuria or progression to ESRD when added to ramipril.<sup>109,110</sup> Thus, the non-dihydropyridine CCBs may be considered second- or third-line agents after RAS inhibitors.

### Diuretics

Similar to dietary sodium restriction, thiazide diuretics (eg, hydrochlorothiazide 50 mg) when combined with an ACE inhibitor (lisinopril 40 mg/day) reduced albuminuria in type 2 diabetics. However, the combination is associated with more frequent orthostatic symptoms.<sup>82</sup> For advanced CKD, a loop diuretic may be more appropriate. Diuretics may increase the effectiveness of ACE inhibitors and ARBs.

### Blood pressure target

The current Joint National Committee (JNC 8) guidelines recommend targeting a blood pressure of <140/90 mmHg for diabetic patients, irrespective of CKD.<sup>111</sup> The 2013 European Society of Hypertension/European Society of Cardiology,<sup>112</sup> 2014 Kidney Health Australia Caring for Australians with Renal Impairment,<sup>113</sup> and 2012 Kidney Disease: Improving Global Outcomes guidelines advocate a similar target. However, a lower blood pressure target is recommended by some guidelines for better control of proteinuria. The 2014 Kidney Health Australia Caring for Australians with Renal



Impairment guidelines recommend a lowering of the blood pressure target from <140/90 mmHg to <130/80 mmHg in the presence of macroalbuminuria.<sup>113</sup> The 2012 Kidney Disease: Improving Global Outcomes guidelines suggest that a target of <130/80 would be more beneficial in those with micro- or macroalbuminuria. The National Kidney Foundation's (Kidney Disease Outcomes Quality Initiative) 2007 and 2012 updated guidelines advocate blood pressure readings <130/80 mmHg in diabetics with CKD, or even lower in patients with high-level albuminuria (ACR >500 mg/g).<sup>114</sup> The Canadian Society of Nephrology continues to advocate for the lower target of <130/80 mmHg for all diabetics, regardless of CKD or albuminuria.<sup>115</sup> It is probably sufficient to say that low risk diabetics with normoalbuminuria could be treated to a target of <140/90 mmHg, while those at high risk or significant albuminuria should have a lower target of <130/80 mmHg.

### Anti-lipid agents

In the Casale Monferrato study of 1,253 type 2 diabetics, apolipoprotein B and high-density lipoprotein cholesterol levels were independent risk factors for progression to overt nephropathy during 7 years follow-up.<sup>19</sup> In a large multinational case-control study of 2,535 type 2 diabetics with good control of low-density lipoprotein cholesterol, triglycerides and high-density lipoprotein cholesterol were associated with a higher risk of DN.<sup>116</sup> Data from the Joslin Diabetes Center from 439 type 1 diabetics also indicated that elevated cholesterol levels (>220 mg/dL) was associated with progression of DN.<sup>117</sup> Experimentally, statins have been shown to reduce NF- $\kappa$ B activation by p38 MAPK in tubular cells, AGE-mediated ROS activation, and tubular apoptosis and suppress RAS activation and aldosterone production.<sup>118–120</sup>

Despite the epidemiological and experimental data, there is limited data from intervention studies with regards to renal outcomes. In a study of type 2 diabetics, simvastatin reduced albuminuria and improved expression of slit diaphragm proteins compared with cholestyramine despite similar lipid reductions.<sup>121</sup> In an open-label randomized study in 104 type 2 diabetics, rosuvastatin reduced albuminuria and oxidative stress independent of lipid levels.<sup>122</sup> The Heart Protection Study noted that simvastatin treatment was associated with a lesser decline in GFR compared to placebo after an average of 4.6 years, a difference which was bigger in diabetics compared to nondiabetics.<sup>123</sup> The CARDS study of 2,838 type 2 diabetics randomized patients to atorvastatin or placebo, with a median follow-up of 3.9 years. Atorvastatin treatment

improved the annual decline in eGFR, particularly in those with albuminuria.<sup>124</sup> Currently, statins are already recommended for diabetics with DN over the age of 40 years, irrespective of their baseline lipid levels. This is primarily for cardiovascular benefit rather than renal disease per se, as albuminuria has been demonstrated to be an independent risk factor for cardiovascular events and mortality.<sup>125</sup>

### Uric acid

Epidemiological studies demonstrate a strong link between uric acid and DN. The Joslin Diabetes Center study of 355 type 1 diabetics found that higher baseline uric acid levels was associated with early GFR loss over 4–6 years.<sup>126</sup> Data from the Coronary Artery Calcification study, which included 324 type 1 diabetics with normoalbuminuria at baseline who were followed for 6 years, showed that for every 1 mg/dL increase in uric acid levels there was an 80% increased risk of developing micro- or macroalbuminuria.<sup>127</sup> In the Steno Diabetes Center study of 263 type 1 diabetics, baseline serum uric acid at the onset of diabetes predicted development of macroalbuminuria 18 years later.<sup>128</sup>

Does lowering uric acid prevent progression of DN? A post hoc analysis of RENAAL noted that uric acid lowering by losartan may have accounted for 20% of the benefit afforded by the intervention.<sup>129</sup> In diabetic mice, allopurinol attenuated albuminuria and tubulointerstitial injury, suggesting that uric acid is not just a potential marker but a therapeutic target.<sup>130,131</sup> Allopurinol improves endothelial dysfunction and reduces urinary TGF- $\beta$  in DN.<sup>132–134</sup> The PERL study is currently enrolling type 1 diabetics into a randomized trial of allopurinol versus placebo.<sup>135</sup>

### Vitamin D

A low vitamin D level is common in patients with CKD. Vitamin D deficiency is linked to RAS activation and podocyte injury.<sup>136,137</sup> Vitamin D may also play a role in preventing epithelial-to-mesenchymal transformation of tubular epithelial cells.<sup>138</sup> Experimentally, active vitamin D also attenuated oxidative stress by restoring Nrf2 levels, important for cellular protection against oxidative injury. This was associated with reduced NF- $\kappa$ B activation and lower albuminuria.<sup>139</sup>

Observational data from the PRONEDI trial of type 2 diabetics with stage 2–3 CKD showed that vitamin D levels <15 ng/mL was independently a risk factor for the composite outcome of >50% increase in serum creatinine, ESRD, or death.<sup>140</sup> In the VITAL study, type 2 diabetics randomized to paricalcitol (a synthetic D<sub>2</sub> agonist) for 24 weeks achieved significantly lower albuminuria than

placebo treatment.<sup>141</sup> The upcoming VALIDATE-D study will evaluate the effect of calcitriol supplementation in patients on lisinopril to determine if there is a synergistic effect on RAS activity to lower proteinuria.<sup>142</sup> Future randomized trials will hopefully determine the usefulness of targeting the vitamin D receptor in preserving GFR in DN.

## Lifestyle, diet, and alternative medicine

Although moderate-intensity aerobic physical activity is recommended for all diabetics to improve glycemic control and cardiovascular risk, the DCCT study of type 1 diabetics found no evidence that physical activity prevents DN.<sup>143</sup> Exercise may temporarily increase albumin excretion and should be avoided prior to urine collection for albumin excretion. On the other hand, the Look AHEAD study of type 2 diabetics suggested that intensive lifestyle intervention targeting weight loss may reduce progression of CKD, despite no benefit on cardiovascular outcomes.<sup>144</sup>

A low protein diet is advocated by the American Diabetes Association.<sup>21</sup> A recent meta-analysis of 13 randomized controlled trials with 779 type 1 and type 2 diabetics found that a low protein diet was associated with significant improvement in GFR. However, adequate compliance was necessary for this effect on GFR. Interestingly, proteinuria was not different between low protein and regular protein patients but HbA<sub>1c</sub> decreased slightly with low protein intake

(−0.26%; 95% confidence interval −0.35 to −0.18). Low protein intake was defined as 0.6–0.8 g/kg/day and regular protein intake as 1.0–1.6 g/kg/day.<sup>145</sup>

Substituting soy protein for animal protein may also be beneficial in diabetics with proteinuria but studies have not been consistent.<sup>146</sup> A number of alternative medicine supplements have also been studied (Table 3). Lastly, sodium restriction to 50–70 mmol daily may enhance the action of RAS inhibitors and result in a greater reduction in albuminuria in type 2 diabetics.<sup>147,148</sup> However, this degree of sodium restriction is quite difficult for most and some advocate achieving an intake of <100 mmol/day as adequate restriction.

## Multifactorial risk factor reduction

The benefits of intensive multifactorial intervention in type 2 diabetics were shown in the Steno-2 trial of 160 patients with microalbuminuria. Intensive therapy included: reduced dietary fat, light/moderate exercise, smoking cessation, tight glycemic control (<6.5%), tight blood pressure control (<130/80), ACE inhibitors, and anti-lipid medications (cholesterol <4.5 mmol/L). After a mean follow-up of 7.8 years, patients receiving multifactorial intervention had significantly lower risk of overt nephropathy (hazard ratio 0.39; 95% confidence interval 0.17–0.87) than those receiving regular management.<sup>149</sup>

**Table 3** Diet and alternative medicine

Product	Diabetes type (patients, n)	Study design	Potential mechanisms	Key findings	Reference
Silymarin (milk thistle, silybum)	Type 2 (60)	RCT	Antioxidant, anti-inflammatory Antiapoptotic	Silymarin (140 mg three times daily) for 3 months reduced albuminuria, urine TNF- $\alpha$ , urine, and serum malondialdehyde (oxidative stress marker) compared to baseline.	Fallahzadeh et al <sup>218</sup>
Zinc	Type 2 (54)	Non-RCT	Antioxidant, improved glycemic control	Zinc supplement (50 mg elemental zinc) for 12 weeks improved glycemic control, lipids, and albuminuria compared to baseline. Effects on albuminuria were not shown to be independent of other metabolic effects.	Khan et al <sup>219</sup>
	Type 2 (50)	RCT crossover		Zinc supplement (30 mg elemental zinc) for 12 weeks reduced HbA <sub>1c</sub> and albuminuria compared to baseline. A 4-week washout was carried out before crossover.	Parham et al <sup>220</sup>
Curcumin (turmeric)	Type 2 (40)	RCT	Antioxidant	Turmeric capsules 500 mg three times daily for 2 months reduced albuminuria, TGF- $\beta$ , and IL-18 levels compared to baseline.	Khajehdehi et al <sup>221</sup>
Green tea	Recruiting	RCT	Antioxidant	This trial is currently recruiting: Clinical Trials NCT01923597. Diabetic patients randomized to green tea extract, epigallocatechin, or placebo for 3 months. The primary outcome is a change in albuminuria.	None
Fish oil	Type 1 (36)	RCT	Anti-inflammatory Immunomodulatory	1-year fish oil supplementation 4.6 g/day did not affect albuminuria or kidney function.	Rossing et al <sup>222</sup>

**Abbreviation:** HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>; RCT, randomized controlled trial.

## Transplantation

Simultaneous pancreas/kidney transplantation is an effective treatment for type 1 diabetics with ESRD, with most achieving insulin independence and preventing recurrence of DN in the allograft.<sup>150,151</sup> In patients with CKD after 10 years of pancreas transplantation alone, patients with sustained normoglycemia showed reductions in albuminuria and reversal of DN lesions on serial biopsy, including regression of glomerular basement membrane thickening and mesangial matrix deposition.<sup>152</sup> Some of these benefits may be offset by interstitial fibrosis and arteriolar hyalinosis due to calcineurin inhibitor (eg, cyclosporine) use. However, the same authors note that tubulointerstitial remodeling at 10 years had ameliorated some of the interstitial collagen deposition noted at 5 years, although vascular changes were not affected.<sup>153</sup>

## Novel agents

The diabetic milieu is a complex environment where a number of interventions may be utilized to target various pathological processes. As no single therapy completely ameliorates DN, novel strategies are needed to complement existing interventions. Some of these novel agents are described below and summarized in Table 4.

### Renin inhibitors

Renin catalyses the rate-limiting step in the production of angiotensin II. In diabetic rats, aliskiren reduced albu-

minuria and glomerulosclerosis, and was more effective than perindopril in reducing interstitial fibrosis.<sup>154</sup> In type 2 diabetics after a 4-week washout of previous medications, aliskiren reduced blood pressure and albuminuria, with the effects on albuminuria persisting after withdrawal of medication.<sup>155</sup> In the AVOID trial of 599 type 2 diabetics, the combination of aliskiren 300 mg and losartan 100 mg for 6 months reduced the urine ACR independent of blood pressure.<sup>156</sup> However, the much larger ALTITUDE trial, which randomized 8,561 high-risk type 2 diabetics to aliskiren 300 mg or placebo as adjunctive to RAS inhibition, found no significant difference in renal outcomes. It is noted that the trial was terminated prematurely due to excess hyperkalemia and hypotension in the aliskiren group.<sup>157</sup> Due to the lack of good randomized controlled trial evidence supporting the use of aliskiren in combination with ACE inhibitors or ARBs, and the increased adverse effects, the combination is not recommended. From the US Food and Drug Administration perspective, the combination should be contraindicated in patients with diabetes. However, it could be considered as an alternative RAS blocker for blood pressure lowering and proteinuria reduction. More research is needed to demonstrate that aliskiren is as good as ACE inhibitors or ARBs.

### Endothelin inhibitors

In diabetic rats, an ET<sub>A</sub> receptor blockade with atrasentan or avosentan reduced albuminuria and renal fibrosis.<sup>158,159</sup>

**Table 4** Summary of novel agents

Category	Mechanism of action	Drug(s)	Human data
Direct renin inhibitors	Blocks conversion of angiotensinogen to angiotensin I.	Aliskiren	RCT
Endothelin inhibitors	Predominantly blocks ET <sub>A</sub> receptors on vascular endothelium.	Atrasentan Avosentan	RCT RCT
Vasopeptidase inhibitors	Blocks ACE and neutral endopeptidase. Palosuran blocks urotensin II receptor.	Palosuran Omapatrilat Ilepatril	RCT None None
PKC inhibitors	Blocks PKC-β intracellular signaling.	Ruboxistaurin	RCT, pooled
Aldose reductase	Reduces sorbitol formation by the polyol pathway.	Epalrestat Ponalrestat Tolrestat	Non-RCT Non-RCT None
Phosphodiesterase inhibitors	Increases cellular cAMP with broad effects. Cilostazol blocks PDE3, pentoxifylline is nonspecific and also blocks the adenosine receptor.	Cilostazol Pentoxifylline	RCT RCT, MetaAx
AGE inhibitors	Blocks AGE formation, enhances breakdown, or breaks crosslinks.	Aminoguanidine Pyridoxamine Alegebrium	RCT RCT None
Antioxidative stress	Activation of nuclear transcription factor Nrf2.	Bardoxolone	RCT
Glycosaminoglycans	Reduces heparan sulfate degradation in GBM, anti-inflammatory actions.	Sulodexide	RCT
Antifibrosis	Reduces TGF-β signaling and TNF-α levels but exact mechanism unclear.	Pirfenidone	RCT

**Abbreviations:** AGE, advanced glycation end-products; cAMP, cyclic adenosine monophosphate; RCT, randomized controlled trial; MetaAx, meta-analysis; GBM, glomerular basement membrane.

The ASCEND trial of 1,392 type 2 diabetics with overt nephropathy examined the effect of avosentan on time to doubling of serum creatinine, ESRD, or death. Avosentan halved proteinuria but increased fluid retention, edema, and congestive heart failure, resulting in the trial being stopped early.<sup>160</sup> Since ASCEND, two other randomized controlled trials have noted reduction in albuminuria at the cost of edema and congestive heart failure.<sup>160,161</sup> The latter trial involving 1,392 type 2 diabetics was also stopped prematurely after a median follow-up of 4 months. In a randomized trial of 211 type 2 diabetics, atrasentan added to RAS inhibition for 12 weeks reduced albuminuria in association with lowering blood pressure.<sup>162</sup> Fluid overload was reported as manageable, albeit more patients discontinued treatment on the higher dose of atrasentan. The SONAR trial (NCT01858532) with atrasentan is currently in progress to evaluate renal outcomes in type 2 diabetics.

### Urotensin and vasopeptidase inhibitors

Vasopeptidase inhibitors can block ACE and neutral endopeptidase. Palosuran is a competitive antagonist of the urotensin II receptor. In diabetic patients with macroalbuminuria, a 2-week course of palosuran in addition to RAS inhibitors reduced albuminuria by 24%.<sup>163</sup> The PROLONG trial is a prospective, randomized controlled crossover trial in hypertensive type 2 diabetics looking at the effects of palosuran on albuminuria and blood pressure.<sup>164</sup> This study found no significant reduction in albuminuria or blood pressure after 4 weeks of treatment. Other vasopeptidase inhibitors such as omapatrilat and ilepatril (AVE7688) have been shown to attenuate albuminuria in diabetic rats but human data are lacking.<sup>165,166</sup>

### PKC inhibitors

Ruboxistaurin is a selective inhibitor of PKC- $\beta$ . Animal studies with ruboxistaurin showed beneficial effects on reducing mesangial expansion, hyperfiltration, albuminuria, macrophage accumulation, and tubulointerstitial injury.<sup>167,168</sup> Small randomized controlled studies have demonstrated that ruboxistaurin reduced urinary TGF- $\beta$  excretion by >50%,<sup>169</sup> reduced albuminuria, and preserved eGFR at 1 year in type 2 diabetics.<sup>170</sup> However, when pooled data from three large studies of ruboxistaurin from diabetic retinopathy trials were analyzed (n=1,157), ruboxistaurin was no different from placebo after 3 years in reducing the rates of doubling of serum creatinine or stage 4–5 CKD.<sup>171</sup>

### Aldose reductase inhibitors

These inhibitors suppress sorbitol accumulation in tissues. Epalrestat reduced mesangial expansion and

preserved renal function in diabetic rats.<sup>172</sup> Another inhibitor – tolrestat – prevented glomerular hypertrophy and hyperfiltration, mesangial cell hypocontractility, and albuminuria in diabetic rats.<sup>173</sup> A small study of 35 type 2 diabetics showed that epalrestat treatment for 5 years prevented progression of microalbuminuria.<sup>174</sup> A post hoc analysis of the Aldose Reductase Inhibitor–Diabetes Complications Trial concluded that progression of retinopathy/albuminuria was significantly inhibited by epalrestat.<sup>175</sup> This was a re-analysis of the original 3-year, open-label trial using a subset of patients for which data were available. On the other hand, another inhibitor – ponalrestat – did not affect urinary albumin excretion or glomerular filtration in type 1 diabetics.<sup>176</sup>

### Phosphodiesterase inhibitors

Cilostazol inhibits phosphodiesterase III and reduces thrombospondin-1 and TGF- $\beta$  expression, attenuating hyperfiltration, albuminuria, and extracellular matrix deposition in diabetic rats.<sup>177,178</sup> In humans, one study using cilostazol for 3 months in type 2 diabetics demonstrated a reduction in urinary ACR and renal production of thromboxane B2.<sup>179</sup> A small Chinese study randomized 40 type 2 diabetics to cilostazol or placebo for 6 months. Cilostazol reduced albuminuria, serum ICAM-1, and MCP-1 levels but did not affect kidney function.<sup>180</sup>

Pentoxifylline is a methylxanthine-derived phosphodiesterase inhibitor that antagonizes the adenosine receptor and lowers blood viscosity. It also has anti-inflammatory and immunomodulatory properties, and lowers serum and urine TNF- $\alpha$  in diabetic patients with DN. In a Cochrane meta-analysis of 17 randomized trials involving 991 participants, pentoxifylline was better than placebo in reducing albuminuria and preserving serum creatinine but was equivalent to captopril. However, the studies were small and of poor methodology, with no data on ESRD or mortality.<sup>181</sup> Since the meta-analysis, other trials have examined the addition of pentoxifylline to RAS blockers and have consistently found a benefit in reducing proteinuria. Roozbeh et al enrolled 74 patients with type 2 diabetes with overt proteinuria, randomized to pentoxifylline 400 mg daily plus captopril or captopril alone. The reduction in proteinuria from baseline was greater in the pentoxifylline-treated group, associated with a modest reduction in blood pressure.<sup>182</sup> Oliaei et al enrolled 50 type 2 diabetics with proteinuria >500 mg/day despite RAS inhibition, to pentoxifylline 400 mg three times a day versus placebo. The pentoxifylline group had greater reductions in proteinuria but no difference in creatinine clearance.<sup>183</sup> Ghorbani et al enrolled 100 type 2 diabetics

with proteinuria randomized to pentoxifylline 400 mg/day or placebo for 6 months. Both groups received losartan and enalapril in combination. After 6 months, pentoxifylline treatment was associated with lower proteinuria and higher creatinine clearance.<sup>184</sup> The results of the PREDIAN study are still expected.<sup>185</sup>

### AGE inhibitors

AGE inhibitors reduce AGE formation, enhance degradation, or break AGE crosslinks. The prototype AGE inhibitor is aminoguanidine (pimagedine), which acts by scavenging intermediates such as 3-deoxyglucosone and methylglyoxal.<sup>186</sup> Experimentally, aminoguanidine attenuates albuminuria, mesangial expansion, and collagen deposition in diabetic rats.<sup>187</sup> However, the placebo-controlled ACTION trial in 690 type 1 diabetics with overt nephropathy showed no difference in the time taken to double serum creatinine despite a reduction in proteinuria with pimagedine treatment for 2–4 years.<sup>188</sup>

Pyridoxamine inhibits AGE formation and scavenges ROS and toxic carbonyls. When data from two 24-week studies were combined, pyridoxamine reduced the change from baseline creatinine in type 1 and type 2 diabetics without affecting albuminuria.<sup>189</sup> However, in a randomized controlled trial of 317 type 2 diabetics, pyridoxamine treatment for 52 weeks did not significantly affect serum creatinine.<sup>190</sup> GLY-230 is another inhibitor of protein glycation that was studied in 21 diabetic men in a randomized trial for 14 days. GLY-230 reduced glycated albumin and albuminuria compared to baseline but not placebo.<sup>191</sup> AGE crosslink breakers, such as alegebrium, and inhibitors of the AGE receptor have shown benefit in DN models but have not been studied in humans.

### Agents targeting oxidative stress

Cellular and mitochondrial ROS formation is an important contributor to the pathophysiology of DN. Targeted inhibitors of ROS generation are emerging but most have not progressed to clinical trials. Oxidative stress and inflammation in DN may also lead to a reduction in Nrf2, a nuclear transcription factor which plays a key role in antioxidant and cytoprotective mechanisms.<sup>192</sup> Bardoxolone is a potent activator of Nrf2. In the BEAM trial of 227 type 2 diabetics with eGFR 20–45 mL/minute/m<sup>2</sup> randomized to bardoxolone (25, 75, or 150 mg daily) or placebo, bardoxolone was associated with an improvement in eGFR at 24 weeks, which was sustained to 52 weeks of treatment.<sup>193</sup> In the much larger BEACON trial of 2,185 type 2 diabetics with stage 4 CKD, patients

were randomized to bardoxolone 20 mg daily or placebo. The trial was stopped after a median follow-up of 9 months due to a higher rate of cardiovascular events and increased albuminuria, with no reduction in ESRD or cardiovascular death.<sup>194</sup> Subsequently, one animal study in diabetic rats found unfavorable side effects of bardoxolone analogs, further questioning the safety profile in DN.<sup>195</sup>

### Glycosaminoglycans

Sulodexide is a mixture of 80% heparan sulfate and 20% dermatan sulfate. Sulodexide may reduce the enhanced heparan sulfate degradation in the glomerular basement membrane that occurs in DN. It has anti-inflammatory properties and inhibits the hyperglycemia-induced production of ROS, MCP-1, and IL-6 in endothelial cells.<sup>196</sup> It may improve endothelial dysfunction, vascular permeability, and renal hemodynamics. It may also attenuate TGF- $\beta$  gene expression, extracellular matrix expansion, and inhibit HPSE-1, which plays a role in tubular epithelial-to-mesenchymal transition.<sup>197</sup>

The DiNAS trial enrolled 223 type 1 and type 2 diabetics with serum creatinine  $\leq 1.7$  mg/dL in a randomized trial of sulodexide (50, 100, 200 mg/day) versus placebo for 4 months, with a further 4 months follow-up postintervention. There was a dose-dependent effect, with 200 mg/day the most effective in reducing albuminuria. RAS inhibition was not universal in this trial although post hoc analysis indicated the effect of sulodexide was additive to ACE inhibition.<sup>198</sup> The Sun-MICRO trial enrolled 1,056 type 2 diabetics with microalbuminuria in a randomized trial of sulodexide 200 mg/day versus placebo for 12 months. There was no difference between the groups in normalizing albumin excretion or reducing albuminuria by at least 50%.<sup>199</sup> The Sun-MACRO trial enrolled 1,248 type 2 diabetics with renal impairment and overt nephropathy in a randomized trial of sulodexide 200 mg/day versus placebo. The study was terminated mid-enrollment, with data on 1,029 patient-years analyzed. This showed no significant difference in doubling of serum creatinine, ESRD, or creatinine  $> 6$  mg/dL.<sup>200</sup> In both the Sun-MICRO and Sun-MACRO trials, patients were on maximum doses of RAS inhibitors. The latter studies have dampened the enthusiasm for sulodexide in DN.

### Antifibrotic agents

Pirfenidone inhibits TGF- $\beta$  production and TNF- $\alpha$  production in models of DN and non-DN kidney disease. The exact mechanism of action is unclear. In db/db mice with type 2 diabetes, pirfenidone reduced mesangial matrix expansion

but did not affect albuminuria.<sup>201</sup> In a small randomized trial of 77 type 1 and 2 diabetics with established DN, pirfenidone at 1,200 mg/day for 1 year improved eGFR from baseline compared to placebo (mean intergroup difference 5.5 mL/minute/1.73 m<sup>2</sup>). Pirfenidone at the higher dose of 2,400 mg/day did not demonstrate a similar benefit and the dropout rate was high. Pirfenidone did not lower albuminuria. Larger studies are needed to validate the findings.

## Gene and cell-based therapy

Gene therapy involves introducing a gene into cells to increase the production of a protein of interest. A carrier or vector such as modified adenovirus is employed to deliver the gene to the nucleus where the protein coded by the gene is produced by the cellular machinery. Gene therapy targeting TGF- $\beta$ /SMAD signaling has shown promise in reducing kidney injury in diabetic models. Ka et al studied Smad7 gene therapy in the db/db mouse model of type 2 diabetes. Treatment inhibited TGF- $\beta$ /SMAD and NF- $\kappa$ B activation, resulting in a reduction in proteinuria, macrophage infiltration, inflammation, podocyte injury, and renal fibrosis.<sup>202</sup> A similar finding was noted by Zhang et al by using gene therapy to enhance decorin expression in the streptozotocin model of type 1 diabetes. The beneficial effects were attributed

to downregulation of TGF- $\beta$ /SMAD signaling as decorin is a natural inhibitor of TGF- $\beta$ 1.<sup>203</sup> HGF gene therapy has been shown in db/db mice to enhance renal expression of SDF-1, associated with increased numbers of bone marrow-derived monocyte/macrophages with a higher proportion of M2 markers (anti-inflammatory phenotype). This was associated with a reduction in proinflammatory cytokines, reduced histological injury, and preservation of podocytes.<sup>204</sup> Kosugi et al examined soluble Flt-1 gene therapy in db/db mice. sFlt-1 is an endogenous inhibitor of VEGF and treated animals showed reduced VEGF expression in association with elevated sFlt-1 levels in the kidney. Although sFlt-1 gene therapy reduced podocyte injury and albuminuria, tubulointerstitial injury was enhanced, leading the authors to conclude that this approach would not be beneficial in DN.<sup>205</sup> Thus, there are some potential risks with gene therapy, which may be related to the inserted gene itself or the viral vector utilized but this discussion is beyond the scope of this review.

Progenitor (stem) cells are multipotent cells capable of self-renewal and differentiation into specialized cells, and are broadly categorized into embryonic stem cells and adult stem cells. Adult stem cells can be derived from bone marrow, adipose tissue, or peripheral blood. Stem cells can also be harvested from umbilical cord blood at birth.

**Table 5** Stem cell therapy in experimental diabetic nephropathy

Source	Model	Main outcomes	Reference
Human B-MSC	T1DM, mice NOD/SCID	↓ glucose, ↑ insulin and $\beta$ -cells, ↓ mesangial thickening, ↓ macrophage infiltration	Lee et al <sup>223</sup>
Mouse B-MSC	T1DM, mice C57BL/6	↓ glucose, ↑ mouse insulin and $\beta$ -cells, ↓ albuminuria, ↓ glomerular fibrosis and mesangial expansion	Ezquer et al <sup>224</sup>
Mouse B-MSC	T1DM, mice C57BL/6	No effect on glucose, insulin, or $\beta$ -cells, ↓ albuminuria, ↓ glomerular fibrosis and mesangial expansion, ↓ podocyte loss	Ezquer et al <sup>225</sup>
Rat B-MSC	T1DM, rats SD	↓ glucose, ↓ albuminuria, ↓ renal mass index	Zhou et al <sup>226</sup>
Human UC-MSC	T1DM, rats SD	No effect on glucose, ↓ proteinuria, ↓ fibronectin and $\alpha$ -smooth muscle actin, ↑ E-cadherin	Park et al <sup>227</sup>
Human UC-MSC	T1DM, rats SD	No effect on glucose, ↓ proteinuria, ↓ mesangial expansion, ↓ $\alpha$ -smooth muscle actin, TGF- $\beta$ 1, and collagen, ↑ E-cadherin	Park et al <sup>228</sup>
Rat A-MSC	T1DM, rats SD	↓ glucose, ↑ insulin, ↓ lipids, ↓ creatinine, ↓ mesangial expansion, ↓ oxidative stress, ↓ proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6), ↓ MAPK signaling (p38, ERK, JNK)	Fang et al <sup>229</sup>
Rat B-MSC	T1DM, rats SD	No effect on glucose, ↓ albuminuria, ↓ BMP-7, ↓ podocyte injury and loss, ↑ creatinine clearance, ↓ renal mass index	Wang et al <sup>230</sup>
Rat B-MSC	T1DM, rats Wistar	↓ glucose and albuminuria, ↓ glomerulosclerosis, ↓ MCP-1 and macrophages, ↑ HGF, ↓ proinflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ )	Lv et al <sup>231</sup>
Rat B-MSC	T1DM, rats SD	↓ glucose, ↑ insulin and $\beta$ -cells, ↓ albuminuria, ↑ synaptopodin, ↓ TGF- $\beta$ 1, ↑ IL-10	Zhang et al <sup>232</sup>
Human A-MSC	T1DM, rats SD	No effect on glucose or $\beta$ cells, ↓ proteinuria, ↑ creatinine clearance, ↓ cholesterol, ↓ glomerular hypertrophy, ↓ podocyte injury/loss, ↓ interstitial fibrosis	Zhang et al <sup>233</sup>
Rat B-MSC	T1DM, rats Albino	↓ urea and creatinine, ↓ albuminuria, ↓ Bax expression, ↓ TGF- $\beta$ and TNF- $\alpha$ , ↑ VEGF	Abdel Aziz et al <sup>234</sup>

**Note:** a down arrow indicates a reduction or decrease; an up arrow indicates an increase.

**Abbreviations:** A-MSC, adipose-derived mesenchymal stromal (stem) cells; B-MSC, bone marrow-derived mesenchymal stromal (stem) cells; SD, Sprague Dawley; T1DM, streptozotocin-induced model of type 1 diabetes; UC-MSC, umbilical cord blood-derived mesenchymal stromal (stem) cells.

The potential benefits of stem cell treatment in DN include: 1) replacing or regenerating damaged cells, 2) modulating inflammation, 3) reducing oxidative stress, and 4) improving glycemia. There have been a number of experimental studies of stem cell treatment in DN (Table 5). Most studies have demonstrated a blood glucose lowering effect by improved pancreatic  $\beta$ -cell function and insulin levels, whilst some others have not. This may relate to the nature of the cells utilized or the method of delivery. Some of these studies suggest that a paracrine effect is more important as a renoprotective mechanism, rather than regeneration or replacement of injured cells. This is based on observations of low level engraftment of mesenchymal stem cells in the kidney and the production of beneficial growth factors, antifibrotic factors, and factors which protect from oxidative stress.<sup>206,207</sup>

The main issues facing cell-based therapy include: 1) consistency of manufactured cells (phenotypic change occur with repeat passages), 2) cell delivery method (optimize tissue targeting and minimizing passive entrapment), and 3) engrafting and cell survival. Notwithstanding the limitations mentioned, both gene therapy and stem cell therapy are promising areas of research but there are currently no successful human studies to date. Further studies are also needed to confirm that mesenchymal stem cells ameliorate DN independent of its metabolic benefits.

## Conclusion

DN and ESRD remains a significant problem despite best efforts to limit the impact of the disease on such end-organ damage. In such a complex milieu of diabetes where no single treatment can halt DN progression, a multifactorial approach remains the most sensible. This should include optimal glycemic control and single RAS inhibition for hypertension or albuminuria. Based on the evidence, ACE inhibitors are preferred for type 1 diabetics. Second-line antihypertensives include non-dihydropyridine CCBs and diuretics. Lipid management with a statin is prudent for cardiovascular disease even though a direct impact on renal disease has not been conclusively shown other than as part of the multifactorial risk intervention similar to the Steno-2 study (which includes aspirin). No alternative medicines or supplements have been shown to slow GFR decline although effects on albuminuria are reported by some small studies. None can be routinely recommended currently and further studies on vitamin D are awaited. Further data on uric acid management with allopurinol are also awaited. Mild salt and protein restriction may also benefit some patients but strict monitoring and compliance can be problematic.

Understanding the pathophysiology of DN has improved over the years, particularly the molecular biology aspect. Inflammation has emerged as an important theme, while treatment targets and options continue to evolve as knowledge improves. The inflammatory amplification loop mediated by macrophages may be a good candidate for inhibition to reduce DN progression. Leukocyte or monocyte/macrophage culling may not necessarily be the best long-term strategy but manipulation of the macrophage phenotype and the interaction with T-cells should be further investigated. Blocking specific cell signaling pathways involved with inflammation may be useful but can be troubled by off-target effects, which will need to be fully explored before clinical trials can proceed.

A number of potential treatment strategies have shown benefit in improving surrogate markers like albuminuria but the translation to preserving GFR and preventing ESRD has not always followed. Such is the case with dual or triple blockade of the RAS system in DN seen in recent large clinical trials. It is acknowledged that albuminuria as a surrogate marker of disease progression is flawed. Furthermore, experimental interventions which reduce histological injury and inflammation do not always reduce the level of established proteinuria. Novel biomarkers may assist in this area when more data becomes available. Despite these challenges, new strategies to complement existing treatments will nonetheless continue to be looked for.

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## Disclosure

The author reports no conflicts of interest in this work. This manuscript has not been submitted or published anywhere else.

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