Twenty years after ACEIs and ARBs: emerging treatment strategies for diabetic nephropathy

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DIABETIC NEPHROPATHY (DN) is the leading cause of end-stage kidney disease in developed countries (123). Current clinical management is directed at strict control of blood glucose and blood pressure (BP) as well as inhibition of the renin angiotensin system (RAS) using angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). While several smaller human studies had suggested beneficial effects of ACEIs in DN, the beneficial actions of these agents were convincingly demonstrated in a large, randomized, controlled trial in 1993 (83). In this study, captopril attenuated the decline in renal function in Type 1 diabetic patients with heavy proteinuria more effectively than BP control alone (83). Since this landmark study, data from several large human studies have consistently demonstrated renoprotective benefits of ACEIs and ARBs in diabetic patients with kidney disease (13, 84, 107). As a result, ACEIs and ARBs are now a mainstay of management for DN. Despite their clinical effectiveness, diabetic kidney disease continues to progress ultimately causing end-stage kidney disease (ESKD) in ~20% of patients (92, 97). Thus, new treatment strategies are needed. In this review, we examine major cell-signaling pathways implicated in the pathogenesis of DN beginning with G protein-coupled receptors (GPCRs) and proceeding in rough chronological order to more recently identified signaling pathways that might be exploited for the treatment of diabetic kidney disease. Where applicable, we will discuss recently completed or ongoing clinical trials; however, the primary focus will be data arising from in vitro and in vivo experiments that may inform future clinical studies.

Targeting GPCR Signaling Pathways in DN

GPCRs are the target of ~30–40% of all modern medicinal drugs (140). In this section, we discuss promising strategies to maximize RAS blockade, as well as other GPCR systems that might be targeted for the treatment of diabetic kidney disease. Maximizing RAS blockade. While targeting the RAS was a major advance in the treatment of DN (13, 83, 84, 107), initial attempts to maximize RAS blockade produced disappointing results (42, 170). For example, combined ACEI and ARB therapy was associated with an increased risk of the composite primary end-point of death from cardiovascular causes in the ONTARGET clinical trial (170). In a secondary analysis of renal outcomes, dialysis or doubling of the serum creatinine was similar in the monotherapy ACEI or ARB group but increased with combination treatment (94). Other investigators have targeted additional components of the RAS. For example, the direct renin inhibitor aliskiren showed promise as an adjunct to ACEI and ARBs in the treatment of DN (108, 112). Unfortunately, when the effect of aliskiren in combination with the RAS blockade was tested in a large cohort of patients with Type 2 diabetes and chronic kidney disease (CKD), investigators observed no difference in the composite end-point of death from cardiovascular or renal events (106). In this trial, aliskiren reduced albuminuria but increased the rate of decline in renal function and was associated with an increased risk of hypotension and hyperkalemia (106). An alternative approach to enhance RAS blockade is inhibition of the mineralocorticoid receptor (MR), a nuclear receptor that is not a member of the
GPCR family. In vitro, aldosterone promotes apoptosis in podocytes (81) and induces expression of the inflammatory chemokine CCL2 (C-C motif ligand 2, formerly termed monocyte chemoattractant protein 1) in an NF-H9260B-dependent manner in both mesangial and proximal tubule cells (54). Furthermore, administration of MR antagonists to rodents with either type 1 or type 2 diabetes decreases albuminuria and inhibits fibrosis (51, 54). In small clinical trials, short-term treatment with spironolactone decreased 24-h urinary albumin excretion in Type 1 and Type 2 diabetic patients with persistent albuminuria despite RAS blockade (68, 127, 128). Similarly, combined treatment with an ACEI and the selective aldosterone inhibitor eplerenone reduced albuminuria to a greater extent than the ACEI alone without causing a significant increase in hyperkalemia (37). Additional MR antagonists have been developed (Table 1) and phase 2 clinical trials are currently evaluating these agents in patients with Type 2 diabetes and albuminuria (Table 2). Ultimately, MR blockade may prove to be an effective treatment strategy for DN; however, larger trials with longer followup are required to assess its effect on progression to ESRD and/or mortality.

A second promising approach to enhance RAS blockade is activating the vitamin D receptor (VDR), another nuclear receptor system. In addition to its well-known role in calcium homeostasis, the VDR ligand, 1,25-dihydroxyvitamin D3 has also been shown to negatively regulate renin gene expression in vitro (169) and in vivo (86). VDR knockout mice treated with the β-cell toxin streptozotocin (STZ) develop more severe kidney damage than wild-type mice (174), suggesting a protective role for VDR in diabetic nephropathy. Furthermore, treatment of diabetic mice with either paricalcitol or doxercalciferol abrogates renal damage by preserving glomerular basement membrane width, as well as blunting inflammation and fibrosis (30, 173, 175). In both cases, a synergistic effect with ARBs was observed. The mechanisms mediating the beneficial effects of VDR activation in diabetes are not known with certainty; however, it may be due to anti-inflammatory effects (29, 121) and/or by inhibiting the compensatory increase in renin expression that occurs in the setting of ARB therapy (175). VDR activation has also shown promise in clinical trials. In the VITAL (selective VITamin D receptor activator for Albuminuria Lowering) study, paricalcitol (2 μg per day)

Table 1. Selected approved and investigational drug products

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Approved Drug(s)*</th>
<th>Investigational Drug(s)*</th>
<th>Mechanism(s) of action</th>
<th>Major adverse side-effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine GPCRs</td>
<td>Dipyridamole</td>
<td>Adenosine re-uptake inhibitor</td>
<td>Bleeding, Myocardial ischemia at high doses</td>
<td></td>
</tr>
<tr>
<td>AMPK</td>
<td>Metformin</td>
<td>ATL146e, CGS212680 Adiponectin, AICAR**</td>
<td>Lactic acidosis</td>
<td></td>
</tr>
<tr>
<td>Calcineurin</td>
<td>Cyclosporine, Tacrolimus</td>
<td>VIVIT†</td>
<td>Inhibitors</td>
<td>Immunosuppression Nephrotoxicity</td>
</tr>
<tr>
<td>Chemokine GPCRs</td>
<td>CCX140-B, INCB3344, RS504393, RO5234444 BMS-813160 PF-04634817</td>
<td>CR2 antagonists</td>
<td>GI symptoms, Infection</td>
<td></td>
</tr>
<tr>
<td>Endothelin GPCRs</td>
<td>Atrasentan, Avosentan</td>
<td>Endothelin antagonists</td>
<td>Fluid retention, Heart failure</td>
<td></td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>AG400</td>
<td>JAK inhibitors</td>
<td>Anemia, Thrombocytopenia</td>
<td>Hyperkalemia</td>
</tr>
<tr>
<td>MR</td>
<td>Finerenone (BAY94-8862)</td>
<td>MR antagonists</td>
<td>Immunosuppression, Proteinuria</td>
<td></td>
</tr>
<tr>
<td>mTOR</td>
<td>Sirolimus, Everolimus</td>
<td>Inhibitors</td>
<td>Immunosuppression, Proteinuria</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Vitamin E, Probucol, Resveratrol</td>
<td>α-lipoic acid</td>
<td>Antioxidants</td>
<td></td>
</tr>
<tr>
<td>Nrf2</td>
<td>Bardoxolone, RTA405</td>
<td>Nrf2 activators</td>
<td>Heart failure, Proteinuria</td>
<td></td>
</tr>
<tr>
<td>NOX</td>
<td>Apocynin, GKT137831</td>
<td>NOX inhibitor</td>
<td>NOX1/4 inhibitor</td>
<td>GI symptoms, Photosensitivity rash</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Pirfenidone, Anti-TGF-β antibodies LY2382770</td>
<td>Inhibits the TGF-β promoter activity</td>
<td>GI symptoms, Photosensitivity rash</td>
<td></td>
</tr>
<tr>
<td>VDR</td>
<td>VTB2518</td>
<td>TGF-β antisense VDR agonists</td>
<td>Hypercalcemia</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Bevacizumab, Ramucirumab</td>
<td>VEGF antibody</td>
<td>Proteinuria, Hypertension</td>
<td>Thrombotic microangiopathy</td>
</tr>
<tr>
<td>Wnt/β-catenin</td>
<td>Sorafenib Sunitinib</td>
<td>VEGFR2 antibody</td>
<td>Edema, Ascites</td>
<td></td>
</tr>
</tbody>
</table>

*Available drug products are dietary supplements or drugs approved for an indication other than DN. Investigational drug products are drugs used in animal studies or in early phase clinical trials. **5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside, †Valine-isoleucine-valine-isoleucine-threonine.
decreased urine albumin excretion by 18% compared with placebo in patients with Type 2 diabetes (26). As a result of these promising results, VDR agonists are currently being tested in additional clinical trials (Table 2).

**Endothelin receptors.** The endothelin receptors, ET$_A$R (ET$_A$ receptor) and ET$_B$R, are G protein-coupled receptors that mediate the biological effects of endothelin peptides. The ET$_A$R and ET$_B$R are expressed throughout the kidney (12), and endothelin peptides are key regulators of blood pressure and sodium homeostasis (72). Both the ET$_A$R and ET$_B$R cause vasoconstriction; but the ET$_B$R is also a vasodilator due to ET$_B$R-induced generation of the vasodilator prostaglandins and nitric oxide (12). In the kidney, endothelins exert a net natriuresis and decreased urine albumin excretion. The biological effects of endothelins and their receptors in the kidney are complex (72). In addition to effects on vascular tone and natriuresis, endothelin receptors promote hemodynamic and natriuretic actions of endothelins and their peptide catabolism.

**Selected ongoing and recently completed clinical trials in diabetic nephropathy**

<table>
<thead>
<tr>
<th>Trial/phase</th>
<th>Drug</th>
<th>Mechanism(s) of action</th>
<th>Patient characteristics</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01712061 (Phase 2)</td>
<td>PF-04634817</td>
<td>CCR2/5 antagonist</td>
<td>Type 2 diabetes, CKD stages 2–4, urine ACR* &gt;300 mg/gram</td>
<td>Change in urine ACR* from baseline</td>
</tr>
<tr>
<td>NCT01752985 (Phase 2)</td>
<td>BMS-813160</td>
<td>CCR2/5 antagonist</td>
<td>Type 2 diabetes, urine ACR* 200–3500 mg/gram</td>
<td>Change in urine ACR* from baseline</td>
</tr>
<tr>
<td>NCT01447147 (Phase 2)</td>
<td>CCX140-B</td>
<td>CCR2 antagonist</td>
<td>Type 2 diabetes, urine ACR* 100–3000 mg/g, eGFR† &gt;25 ml/min/1.73m$^2$</td>
<td>Safety and tolerability</td>
</tr>
<tr>
<td>NCT01440257 (Phase 2)</td>
<td>CCX140-B</td>
<td>CCR2 antagonist</td>
<td>Type 2 diabetes, urine ACR* &gt;200–3000 mg/g, eGFR† &gt;25 ml/min/1.73m$^2$</td>
<td>Change in 24 h urine ACR*</td>
</tr>
<tr>
<td>NCT02410499 (Phase 2)</td>
<td>MLN1202</td>
<td>CCR2 monoclonal antibody</td>
<td>Type 2 diabetes, albuminuria, eGFR† 25–59 ml/min/1.73m$^2$</td>
<td>Change in urine ACR*</td>
</tr>
<tr>
<td>NCT01858532 (Phase 3)</td>
<td>Atrasentan</td>
<td>Selective ETA antagonist</td>
<td>Type 2 diabetes, urine ACR* ≥300 and &lt;5000 mg/g, eGFR† 25–75 ml/min/1.73m$^2$ and BNP ≤200 ng/l</td>
<td>Doubling of serum creatinine or progression to ESRD</td>
</tr>
<tr>
<td>NCT01683409 (Phase 2)</td>
<td>Baricitinib</td>
<td>JAK1/2 inhibitor</td>
<td>Type 2 diabetes, albuminuria, eGFR† 25–70 ml/min/1.73m$^2$</td>
<td>Change in urine ACR* after 24 wk</td>
</tr>
<tr>
<td>NCT02345057 (Phase 2)</td>
<td>CS-3150</td>
<td>MR antagonist</td>
<td>Type 2 diabetes, urine ACR* ≥45 and &lt;300 mg/g, eGFR† &gt;30 ml/min/1.73m$^2$</td>
<td>Change in urine ACR*</td>
</tr>
<tr>
<td>NCT01968668 (Phase 1/2)</td>
<td>Finerenone (BAY94-8862)</td>
<td>MR antagonist</td>
<td>Type 2 diabetes, urine ACR* ≥300 ml/min/1.73m$^2$</td>
<td>Change in urine ACR* at 90 days</td>
</tr>
<tr>
<td>NCT01874431 (Phase 2)</td>
<td>Baricitinib</td>
<td>JAK1/2 inhibitor</td>
<td>Type 2 diabetes, albuminuria, eGFR† 25–70 ml/min/1.73m$^2$</td>
<td>Change in urine ACR* at 90 days</td>
</tr>
<tr>
<td>NCT01889277 (Phase 1/2)</td>
<td>MT-3995</td>
<td>MR antagonist</td>
<td>Type 2 diabetes, albuminuria, eGFR† ≥50–300 ml/min/1.73m$^2$</td>
<td>Safety and tolerability</td>
</tr>
<tr>
<td>NCT02517320 (Phase 2)</td>
<td>Probucol</td>
<td>Antioxidant</td>
<td>Type 2 diabetes, urine ACR* ≥300 mg/g, eGFR† 15–90 ml/min/1.73m$^2$</td>
<td>Change in urine ACR from baseline after 16 wk</td>
</tr>
<tr>
<td>NCT02316821 (Phase 2)</td>
<td>RTA 402</td>
<td>Nrf2 activator</td>
<td>Type 2 diabetes and chronic kidney disease</td>
<td>Change in GFR as measured by inulin clearance</td>
</tr>
<tr>
<td>NCT02010242 (Phase 2)</td>
<td>GKT137831</td>
<td>NOX1/4 inhibitor</td>
<td>Type 2 diabetes, urine ACR* ≥300–3500 mg/g, eGFR† ≥30 ml/min/1.73m$^2$</td>
<td>Change in urine ACR* at 8.10 and 12 wk</td>
</tr>
<tr>
<td>NCT01113801 (Phase 2)</td>
<td>LY2382770</td>
<td>TGF-β antibody</td>
<td>Type 1 or type 2 diabetes, urine ACR ≥800 mg/g, eGFR† 20–60 ml/min/1.73m$^2$,</td>
<td>Change in serum creatinine from baseline after 12 mo</td>
</tr>
<tr>
<td>NCT00552409 (Phase 2/3)</td>
<td>Cholecalciferol</td>
<td>Precursor of the VDR agonist calcitriol</td>
<td>Type 2 diabetes, urine ACR* ≥30–1000 mg/g, eGFR† ≥60 ml/min/1.73m$^2$</td>
<td>Change in 24 h urine ACR*</td>
</tr>
<tr>
<td>NCT01393808, (Phase 2)</td>
<td>Paricalcitol</td>
<td>VDR agonist</td>
<td>Type 2 diabetes, low or high salt diet, urine albumin ≥300 mg/24 h, creatinine &lt;2 mg/dL</td>
<td>Change in urine ACR* after 4 mo</td>
</tr>
<tr>
<td>NCT02410005 (Phase 2/3)</td>
<td>Calcitriol</td>
<td>VDR agonist ARB§</td>
<td>Type 2 diabetes, urine ACR* &gt;300 mg/g, eGFR† 30–90 ml/min/1.73m$^2$</td>
<td>Change in 24 h albuminuria</td>
</tr>
</tbody>
</table>

*Albumin creatinine ratio (ACR), †Estimated glomerular filtration rate (eGFR), §Angiotensin receptor blocker. Additional information on these studies is available at https://clinicaltrials.gov/.
rosis and tubulointerstitial fibrosis (59). Infusion of ET-1 into rats produces a dose-dependent increase in glomerular permeability to albumin and increases glomerular expression of the inflammatory chemokine CCL2. These effects are blocked by a selective ET_{AR} antagonist (120). Furthermore, deletion of both ET_{AR} and ET_{BR} from podocytes protects against glomerulosclerosis (82), while diabetic ET_{B} receptor-deficient rats exhibit higher levels of albuminuria and enhanced glomerular injury compared with wild-type controls (115). Taken together, these data suggest that ET_{AR} receptor activation promotes podocyte damage and albuminuria.

This differential role of the ET_{AR} and ET_{BR} in the kidney is reflected in the effects of receptor antagonists. For example, treatment with the nonspecific ET_{AR}/ET_{BR} antagonist bosentan did not improve renal pathology or albumin excretion in hypertensive diabetic rats (69). In contrast, selective ET_{AR} blockade with avosentan, alone or in combination with lisinopril, improved albuminuria and glomerulosclerosis in uninephrectomized, diabetic rats (43). ET_{AR} antagonists have also shown promise in clinical trials. Avosentan significantly reduced albuminuria in patients with either Type I or Type 2 diabetes and macroalbuminuria on standard RAS blockade (162). Similarly, treatment with the highly selective ET_{AR} antagonist atrasentan significantly reduced the urinary albumin to creatinine ratio in patients with diabetic nephropathy already receiving stable doses of RAS inhibitors (28, 71). The main impediment to using ET_{AR} antagonists for the treatment of DN is fluid retention, which results in edema and may aggravate heart failure (28, 71, 93, 162). While promising, it remains to be seen whether these off target effects can be overcome by more selective ET_{AR} antagonists.

**Chemokine receptors.** Another promising target for the treatment of diabetes includes the family of chemokine receptors, which are predominantly expressed on T cells and monocytes (163). These GPCRs play a critical role in chronic inflammatory diseases (163). Accumulating evidence suggests that chronic inflammation also plays an important role in metabolic diseases linked to obesity, including insulin resistance and diabetes mellitus (163). Adipose tissue produces both CCL2 and CCL5 (46, 143, 165), which bind to the chemokine receptors CCR2 and CCR5, respectively (163). Both ligands are upregulated in obese rodents (46, 143, 165). In obese mice, enhanced chemokine levels are associated with an increased number of inflammatory cells in adipose tissue (161, 165), which ostensibly causes insulin resistance by indirect mechanisms. In addition, the CCL2-CCR axis may have a direct role in insulin resistance because treatment with CCL2 reduces insulin-stimulated glucose uptake in cultured adipocytes (125). In support of an important role for CCR2 signaling in diabetes, Affaw et al. (4) found that a selective A2A agonist reduced albuminuria, mesangial expansion, and glomerular basement membrane width in diabetic rats. Moreover, knockout of the A2A receptor aggravated diabetic kidney disease in this same model (4). Similarly, A2A receptor activation reduced proteinuria and promoted an anti-inflammatory phenotype characterized by reduced proinflammatory tumor necrosis factor-α (TNF-α) levels and enhanced anti-inflammatory IL-10 levels, as well as decreased macrophage infiltration and glomerular injury (113). Taken together, these data suggest that the A2A receptor may be a novel future therapeutic target for the treatment of DN.

### Regulation of Inflammation and Fibrosis by Transforming growth Factor-β

The secreted protein-transforming growth factor-β (TGF-β) is a master regulator of inflammation and fibrosis. Activation of TGF-β in the kidney initiates a profibrotic program characterized by extracellular matrix production, cellular hypertrophy, basement membrane thickening, and apoptosis. These changes eventually lead to glomerulosclerosis and tubulointerstitial fibrosis (89). In CKD, the link between TGF-β and fibrosis has been well established, and a full discussion of the topic is beyond the scope of this review. Therefore, we will focus on the therapeutic potential of TGF-β inhibition. To assess the role of TGF-β in early diabetes, Sharma et al. (131, 133) treated mice with STZ and then measured the expression of TGF-β. Within 3 days of STZ administration, the expression of TGF-β increased in the kidney cortex. Treating the mice with an anti-TGF-β antibody blocked the increase and also inhibited glomerular hypertrophy and upregulation of collagen α1, suggesting that TGF-β induction by hyperglycemia in vivo is an early event and highlighting the potential of a neutralizing anti-TGF-β antibody. In a long-term experiment, this same anti-TGF-β antibody was administered to db/db mice (a genetic model of Type 2 diabetes) starting at the time when the mice become hyperglycemic. Again, anti-TGF-β antibody blocked the increase in collagen-α1 and fibronectin in the kidney cortex (179). However, it had no effect on albuminuria (179). Given that DN is often well established before renoprotective therapies are initiated, strategies to ameliorate established DN are required. In this regard, treatment with an anti-TGF-β antibody reduced glomerular basement membrane thickness and mesangial expansion in db/db mice with established diabetic kidney disease (17). In another study, diabetic rats were treated with either a murine or human anti-TGF-β antibody in combination with lisinopril (8). In the absence of additional treatment, both murine and human antibodies de-
increased blood pressure, albuminuria, and fibrosis. The human anti-TGF-β antibody was also able to abrogate glomerulosclerosis and had a synergistic effect with lisinopril (8). As a result, a phase 2 clinical trial is studying an anti-TGF-β antibody in patients with DN (Table 2) (1). The TGF-β pathway has also been targeted with pirfenidone, a synthetic compound that inhibits TGF-β promoter activity in vitro (117). Treatment of mouse mesangial cells with pirfenidone inhibited both TGF-β signaling and reactive oxygen species (ROS) production in a dose-dependent manner. Additionally, pirfenidone decreased mesangial expansion and the expression of collagen IV and fibronectin in db/db mice (117). A small randomized controlled trial to determine the effect of pirfenidone in diabetic nephropathy patients already on RAS blockade demonstrated a significant improvement in estimated glomerular filtration rate compared with placebo, but there was a high dropout rate in the pirfenidone group, which complicated interpretation of the study results (130).

Despite these promising findings, the use of TGF-β inhibitors for the treatment of fibrotic disease processes is tempered by the dual biological roles of TGF-β as both a profibrotic and anti-inflammatory mediator (167). For example, the use of a TGF-β-neutralizing antibody in patients with systemic sclerosis was complicated by serious adverse effects, including several deaths, with no evidence of a beneficial treatment effect (31). As a result, investigators have explored other approaches. One promising strategy is amplifying bone morphogenetic protein (BMP) 7 signaling (167). BMP7 activates members of the TGF-β receptor superfamily and inhibits both inflammation and fibrosis (167). BMP7 is highly expressed in the kidney and overexpression of BMP7 in podocytes and proximal renal tubular cells (RTCs) reduces albuminuria, prevents podocyte loss, and decreases tubulointerstitial fibrosis (154). Similarly, pharmacological doses of BMP7 reduce albuminuria and glomerulosclerosis in diabetic rats (153). Unfortunately, the bioavailability of BMP7 in the kidney is quite low (167). A potential strategy to circumvent this problem is targeting the predominant BMP7 receptor in proximal RTCs, activin-like kinase 3 or Alk3 (141). Consistent with an important role for Alk3 in regulating fibrosis, knockout of Alk3 in proximal RTCs enhanced TGF-β signaling and increased kidney fibrosis (141). Other treatment strategies include targeting endogenous inhibitors of TGF-β signaling, such as gremlin and uterine sensitization gene-1 (167). Thus, while much work remains to be accomplished, significant advancement has been made in exploiting the TGF-β superfamily to treat the fibrotic disease process, including diabetic kidney disease.

Reducing Oxidative Stress in Diabetes

A large body of evidence links oxidative stress to the development of diabetic kidney disease (14, 139). The increase in reactive oxygen species is the result of both increased production, as well as reduced and/or inadequate antioxidant function (139). Sources of ROS include enzymatic activity, including xanthine oxidase, cytochrome P-450 enzymes, cyclooxygenases, and uncoupled nitric oxide synthase (NOS), as well as NADPH oxides and mitochondrial superoxide production (139, 166). A large component of the increase in ROS generation in diabetes appears to be caused by hyperglycemia (139). Although not universally accepted (34), a prevailing theory suggests that hyperglycemia causes enhanced mitochondrial ROS generation (15). In this model, metabolism of glucose generates the electron donors NADH and FADH2, which donate their electrons to the mitochondrial electron transport chain leading to the production of ATP and water (15). Under hyperglycemic conditions, metabolism of glucose leads to excessive electron transport, eventually overloading the pathway and resulting in donation of electrons to molecular oxygen, generating superoxide (15). Another major source of ROS is NADPH oxidase (NOX) protein complexes (14, 139). Diabetes promotes increased expression of NOX proteins, resulting in enhanced superoxide generation. In diabetic animals, this pathway has been shown to be a major source of ROS (7, 15, 48). In support of an important role for NOX proteins in the pathogenesis of DN, NOX inhibitors reduce renal injury in animal models of diabetic kidney disease (142).

Counterbalancing excessive ROS generation is the antioxidant system, which includes superoxide dismutase, catalase, and the glutathione system (glucose-6 phosphate dehydrogenase, glutathione reductase, and glutathione peroxidase) (139). Numerous abnormalities of the antioxidant system have been described in animal models, including increased, decreased, and unchanged antioxidant levels (139). Overall, however, there does appear to be an inadequate antioxidant response, either as a result of altered levels and/or abnormal function (139). One consequence of enhanced ROS generation is excessive conversion of NAD to NADH (see above), resulting in depletion of cellular NAD. This cofactor is required to convert gyceraldehyde 3-phosphate to pyruvate during glycolysis, resulting in blockade of the glycolytic pathway and accumulation of pyruvate precursor compounds (15, 139). The metabolic intermediates can then be metabolized to advanced glycation end products (AGE), sorbitol, glucose-6-phosphate, and diacyl glycerol, an activator of PKC (15, 139). These compounds have numerous adverse effects, including enhancing expression of TGF-β, vascular endothelial growth factor (VEGF) and NADPH oxidases, activating NF-κB and decreasing expression of endothelial ROS (eNOS) (15). Exacerbating the decrease in eNOS expression is depletion of cofactors required for eNOS enzymatic activity as a result of oxidation by ROS (101). This leads to uncoupled eNOS activity, which results in the generation of superoxide instead of nitric oxide (NO) and, in turn, enhanced ROS generation (101).

The consequence of enhanced ROS generation is to oxidize adjacent molecules, including protein, lipids, nucleic acids, and carbohydrates (139). As a result, multiple cellular processes are disrupted, including organelle function, gene regulation, and cellular signaling, which ultimately may affect cell survival (139). A variety of genetic and pharmacological strategies have been used to “boost” the antioxidant system in diabetes. These strategies have shown promise in animal models of diabetic kidney disease. For example, treatment with the antioxidants vitamin E or probucol reduced ROS generation in STZ-treated diabetic rats (74). Another antioxidant α-lipoic acid inhibits podocyte loss, decreases albuminuria, and attenuates glomerulopathy in animal models of diabetes (137, 164). Alternatively, overexpression of an ubiquitously expressed superoxide dismutase transgene ameliorated kidney injury in diabetic mice (22, 32). Other groups have tried to target specific sources of ROS generation. This strategy has been used to reduce uncoupled eNOS activity in diabetes by supplementing cofactors
oxidized in the diabetic environment (19). These supplements reduced albuminuria and glomerular basement membrane thickness in a mouse model of Type 2 diabetes (19). Unfortunately, the beneficial effects of antioxidant supplementation in animal models have not translated to beneficial effects in diabetic humans. For example, diabetic patients were treated with 400 IU vitamin E per day in the MICRO-HOPE clinical trial (microalbuminuria cardiovascular renal outcomes arm of HOPE) (90). In this study, vitamin E had no effect on either the development or progression of diabetic kidney disease (90). In contrast, treatment with the lipid-lowering and antioxidant probucol demonstrated a trend toward improved renal function, reduced proteinuria and less ESKD in diabetic patients over a three-year time period (36). This trial was, however, a small, open-label study, and it is difficult to discern whether the effects were directly related to probucol’s antioxidant properties. Results of additional clinical trials (Table 2) may provide more definitive information on the efficacy of probucol in DN.

Another promising strategy to enhance the antioxidant system is targeting the transcription factor Nrf2 (nuclear factor (erythroid-derived 2)-like 2, also known as NFE2L2 or Nrf2), a key regulator of antioxidant protein expression (44). Nrf2 interacts with Keap 1 (Kelch-like-ECH-associated protein 1), which promotes ubiquitination and proteosomal degradation of Nrf2 (44). In diabetic rats, proteosomal inhibition upregulates expression of both Nrf2 and antioxidant genes (91). Thus, Nrf2 and the negative regulator Keap1 are attractive targets for modulating antioxidant expression in diabetes. In support of an important role for Nrf2 in DN, Nrf2 deletion exacerbates diabetic kidney disease in mice (66). Enthusiasm for this pathway in treating human disease stimulated a number of clinical trials examining Nrf2 agonists and Nrf2 inducers in diverse disease processes (reviewed in Ref. 44). In this regard, bardoxolone (Table 1) had shown great promise in an early-phase 2 clinical trial in patients with moderate to severe diabetic kidney disease (110, 111). Unfortunately, the phase 3 study was terminated early due to a higher rate of cardiovascular events (27). In the phase 3 study, treatment with bardoxolone improved glomerular filtration rate but increased albuminuria (27). Similarly, bardoxolone-treated monkeys also demonstrated enhanced proteinuria, likely as a result of decreased megalin-mediated protein uptake by the proximal tubule (119). It has been suggested that compounds such as bardoxolone may have a narrow therapeutic window and that higher dosages of the drug may stimulate expression of proinflammatory genes (53). Therefore, it is possible that titrating the dosage to maintain renal function, as opposed to improving renal function, may have a more favorable side effect profile (53). There is, however, a general concern that activation of the Nrf2 pathway may cause cancer cells to acquire a growth advantage (157). Indeed, Keap1 expression is downregulated in some cancers (157, 171). Thus, targeting the Nrf2/Keap1 system holds great promise, but further studies in animal models are needed to optimize this therapeutic approach.

Lastly, while there is strong evidence that ROS contribute to diabetic kidney disease, it is important to understand that ROS also play a key role in normal physiology. The system is designed to maintain ROS generation at some optimal level. For example, ROS play a role in cellular proliferation, and overexpression of antioxidant proteins inhibits cell growth and increases apoptosis (139). Another caveat is that blood glucose levels are often markedly and chronically elevated in the animal models used to study diabetic kidney disease compared with diabetic patients, who are usually receiving treatment for hyperglycemia. If ROS generation is largely driven by the severity of hyperglycemia, then the beneficial effects of antioxidant therapy in animal models may overestimate the beneficial effects of antioxidants in humans with diabetes, at least over the short term. Although we acknowledge that the time-averaged effect over decades of the disease could be similar, if may be difficult to design clinical trials to study the effects of antioxidant therapies in diabetic humans if detectable effects of the treatment may take decades to discern.

**Dysregulated VEGF Expression in Diabetes**

VEGF was discovered in 1983 and was initially named vasopermeability factor due to its ability to increase permeability of tumor-associated blood vessels (96). VEGF is now recognized as the critical angiogenic factor regulating endothelial cell migration, proliferation, differentiation, and cell survival by inhibiting apoptosis (96, 104). The VEGF family is composed of secreted, dimeric glycoproteins, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (21, 96, 129). The VEGF-A (hereafter referred to as VEGF) is recognized as the prototypical family member and is the key regulator of physiological and pathological angiogenesis. Within the kidney, VEGF is expressed predominantly by podocytes but can also be detected in distal tubules, collecting ducts, and, to a lesser extent, the proximal tubules (21, 96, 129). Alternative splicing of VEGF results in multiple isoforms (96), which act by binding to receptor tyrosine kinases (RTKs) on target cells. The VEGF receptor 2 (VEGFR2, also termed Flk1 or KDR for kinase-insert domain receptor) is the RTK responsible for the known biological actions of VEGFR2 on endothelial cells (96, 104). A second receptor (VEGFR1 or Flt1) is also found on endothelium and seems to play a role in regulating endothelial cell VEGFR2 activity (104). VEGFR1 is, however, functional on other cell types. For example, VEGFR1 promotes hematopoietic stem cell recruitment and monocyte/macrophage migration (96, 104). In the kidney, VEGFR2 expression has been reported on glomerular endothelial cells, peritubular endothelial cells, medullary interstitial cells, mesangial cells, and glomerular podocytes (10, 21, 96, 147). Expression of VEGFR2 on podocytes is, however, controversial with several groups unable to detect VEGFR2 mRNA or protein in cultured podocytes (18, 41) or in vivo (136), although all of these studies were able to detect podocyte expression of VEGFR1 (18, 41, 136). Indeed, the biological effects of VEGF on podocytes may be mediated by activating VEGFR1, including antiapoptotic actions (41) and stimulating synthesis of glomerular basement membrane components (18). Given the proximity of podocytes to glomerular endothelial cells and mesangial cells, both autocrine and paracrine signaling has been proposed to contribute to VEGF actions within the glomerulus (96). Although recent studies have questioned the presence of autocrine VEGF signaling under normal physiological conditions (136), the presence of both paracrine and autocrine signaling is supported by both cell culture experiments (18, 41) and in vivo studies (147) and might be mediated by activating VEGFR1.
In diabetes, renal VEGF expression is markedly upregulated with prominent VEGF expression in glomerular podocytes (21, 96, 129). This enhanced expression is, in part, related to activation of mammalian target of rapamycin (mTOR) target genes, as well as blockade of glycolytic pathways [see oxidative stress above and (15)]. Within the diabetic kidney, the effects of VEGF are complex. In endothelial cells, VEGF induces eNOS expression and NO release, which under normal conditions, promotes vasorelaxation, inhibits VEGF-induced capillary growth, blocks endothelial cell activation by inflammatory cytokines, such as TNF and inhibits release of prothrombotic von Willebrand factor from Weibel-Palade bodies (101). In the diabetic milieu, however, VEGF stimulates “uncoupled” NO generation and, in turn, NO production is decreased, and generation of ROS is enhanced (101). In this model, enhanced VEGF generation in diabetes might have adverse effects on endothelial cells by stimulating apoptosis-promoting ROS generation, endothelial cell activation, and intravascular thrombosis. In podocytes, the proapoptotic effect of ROS may be mitigated by prosurvival, autocrine VEGF signaling. In this scenario, inhibition of paracrine VEGF signaling in endothelial cells would be predicted to have beneficial effects in diabetic kidney disease. In contrast, inhibition of autocrine VEGF signaling in podocytes would be predicted to enhance podocyte loss. Thus, global inhibition of VEGF in diabetes might have both adverse and beneficial effects on the disease process.

The role of VEGF inhibition in diabetic kidney disease has been studied using rodent models of diabetes. In this regard, VEGF inhibition attenuates albuminuria and some histological features of diabetic kidney disease in Type 1 and Type 2 diabetes (25, 39, 76). For example, a VEGF antibody ameliorates diabetic kidney disease in rodent models of Type 2 diabetes (25, 39). Moreover, treatment with a soluble splice variant of VEGFR1 (sFlt1) reduced albuminuria, mesangial expansion, and glomerular basement membrane (GBM), thickening in diabetic mice (76). In support of a pathogenic role for VEGF in diabetic nephropathy, overexpression of VEGF in podocytes causes proteinuria, GBM thickening, and mesangial expansion (146, 147). Enthusiasm for these promising findings is, however, tempered by the observation that the absence of podocyte VEGF enhances glomerular injury in diabetic mice (138), suggesting some basal level of VEGF expression is required for glomerular maintenance.

The effects of VEGF are also regulated by additional angiogenic mediators. Angiopoietin-1 (Angpt1) is produced by specialized pericytes, such as podocytes and mesangial cells (65). It binds to the Tie-2 receptor on endothelial cells and maintains endothelial cell quiescence and constrains angiogenesis and fibrosis following vessel injury (65). A second Angpt family member, Angpt2, is produced by endothelial cells and is the natural antagonist of Angpt1 (24). In diabetes, expression of Angpt1 and Angpt2 is altered, resulting in an elevation in the Angpt2/Angpt1 ratio (65). In support of an important role for the Angpts in DN, deletion of glomerular Angpt1 enhances albuminuria and the severity of glomerular injury in diabetic kidney disease. Moreover, enhancing glomerular expression of Angpt1 ameliorates glomerular damage in diabetic mice (33, 80). Thus, angiogenic signaling is a complex process with multiple, mutually antagonistic mediators.

While the Tie2 receptor system is also a potential target for developing therapeutic agents, a large number of VEGF inhibitors have been developed and are used extensively for the treatment of solid tumors, including renal cell carcinoma, hepatocellular carcinoma, gastrointestinal stromal tumors, and soft tissue sarcomas (40). These drugs include blocking antibodies and small-molecule inhibitors (Table 1) (40). While the clinical use of these agents has been associated with a low risk of adverse events (40), 1–2% of patients develop proteinuria (178), and a small percentage develop thrombotic microangiopathy (38). A pathogenic role for VEGF in the latter is suggested by the observation that animals lacking glomerular VEGF develop a thrombotic glomerular disease (38). Given adverse effects of current VEGF inhibitors, as well as complexity of VEGF signaling within the glomerulus, a more complete understanding of VEGF biology in diabetes will be essential for designing effective treatment strategies that target angiogenic signaling in DN.

**Activation of Janus Kinases and Signal Transducers and Activators of Transcription in DN**

JAKs are a group of tyrosine kinases that mediate cellular response to cytokines and chemokines through interactions with STAT transcription regulators (47). The JAK/STAT pathway is involved in several developmental processes, including hematopoiesis, immune system development, and cell growth (47). In canonical JAK/STAT signaling, the pathway is activated by extracellular ligand binding to its receptor. This receptor-ligand interaction results in JAK phosphorylation. Phosphorylated JAKs then phosphorylate and activate cytoplasmic STAT proteins. Activated STATs translocate to the nucleus, where they bind to target genes (57). One group of STAT target genes, the suppressors of cytokine signaling (SOCS) family of proteins, inhibit JAK/STAT phosphorylation, thus creating a negative feedback loop (57). Altered JAK/STAT signaling has been implicated in several human diseases. For example, activating mutations in JAK2 were identified in myeloproliferative disorders (6, 75). In DN, a role for the JAK/STAT pathway in disease pathogenesis was described over a decade ago in a series of studies by Marrero et al. (95). These findings have more recently been supported by microarray analysis of RNA isolated from human kidney biopsies, which demonstrated significant upregulation of multiple JAK/STAT gene family members in both the glomerular and tubulointerstitial compartments (9). In this study, there was a significant inverse correlation between expression of JAK/STAT genes and glomerular filtration rate (9). The mechanisms promoting JAK/STAT activation in DN have not been fully elucidated; however, work by several groups found that ANG II and high glucose are upstream activators of JAK2, STATs 1, 3, and 5, as well as SOCS 1 and 3 in vitro (2, 95, 156). In vivo, ANG II infusion increases phosphorylation of JAK2 and STAT1 (58). Furthermore, treating diabetic rats with candesartan or the JAK2 inhibitor, AG490, decreases albuminuria (5). Taken together, these data implicate JAK/STAT family members in the pathogenesis of DN and suggest that inhibition of JAK/STAT might be beneficial. Currently, there are two JAK inhibitors on the market, tofacitinib, which preferentially inhibits Jakbs 1 and 3 over JAK2, and ruxolitinib, which blocks JAK2 (Table 1) (122, 148). A major
hurdle to using JAK inhibition in patients with DN is the risk of myelosuppression (148). JAK2, in particular, mediates signaling by several important hematopoietic cytokines, including erythropoietin and GM-CS (105). If these agents are proven effective for the treatment of human diabetic kidney disease (Table 2), there is likely to be a significant risk-benefit ratio to consider and, in turn, this class of medications may only be considered for the patients with the greatest risk for disease progression.

Enhanced Calcineurin/Nuclear Factor of Activated T-Cell Signaling in DN

CN is a calcium-activated phosphatase that plays a key role in diverse disease processes. An important CN substrate is the family of NFAT transcription factors (60). NFAT family members were originally discovered in cells of the lymphoid lineage, but abundant evidence indicates that NFAT isoforms are expressed in nonimmune cells with some family members expressed ubiquitously (61). In quiescent cells, NFAT isoforms are phosphorylated and located in the cytoplasm (61). CN dephosphorylates NFAT, which permits translocation to the nucleus and stimulation of gene transcription.

A growing literature suggests that CN is activated in kidneys of diabetic rodents (50, 150). This increase in CN activation may contribute to the apoptosis of cell types that play an important role in the pathogenesis of DN, including renal tubular cells (20) and podocytes (85, 150). CN directly promotes apoptosis through dephosphorylation of the proapoptotic protein BAD (155) and Drp1 (134). CN may also indirectly cause apoptosis by stimulating gene transcription. In this regard, a cell-permeable peptide inhibitor termed VIVIT that specifically blocks CN-dependent NFAT activation attenuates apoptosis of cultured podocytes (85, 150). Indeed, expression of a constitutively active NFAT isoform specifically in podocytes promotes proteinuria, glomerulosclerosis, and a decrease in podocyte numbers (159). Important gene targets of CN include TRPC6 (transient receptor potential cation channel C6), COX2 (cyclooxygenase 2), and RCAN1 (regulator of CN 1) (77, 152). These CN gene targets are upregulated in animal models of diabetic kidney disease and in patients with diabetes (73, 109). In support of a pathogenic role for CN in DN, inhibition CN ameliorates animal models of diabetic kidney disease (50, 116, 172). For example, CN is required for glomerular hypertrophy and extracellular matrix accumulation in diabetic rats (50). In diabetic rodents, albuminuria, extracellular matrix accumulation, and interstitial injury are attenuated by either pharmacologic CN inhibition (116) or the CN inhibitor VIVIT (172). The beneficial effects of VIVIT suggest that NFAT-mediated gene transcription is, at least in part, responsible for the beneficial effects of CN inhibition in diabetic kidney disease.

Currently available pharmacological CN inhibitors include cyclosporine A and tacrolimus/FK506 (Table 1) (45). Both of these drugs are immunosuppressive agents (45). As with JAK-STAT and mTOR inhibitors, the risk-benefit ratio will have to be carefully considered if CN inhibition is undertaken for treatment of DN. Moreover, both cyclosporine A and FK506 have adverse effects, such as nephrotoxicity and promoting the development of posttransplant diabetes. The development, however, of pharmacological agents that selectively inhibit CN-NFAT signaling (3) without affecting dephosphorylation of other CN substrates may provide a therapeutic strategy for inhibiting the CN-NFAT signaling pathway, while avoiding the adverse side effects of pharmacologic CN inhibitors (168). In this regard, the cell-permeable peptide NFAT inhibitor VIVIT prevented rejection of pancreatic islets in vivo without the adverse effects on glucose metabolism (102). Further research is needed but, if these initial findings are confirmed, then the results might provide the impetus for the development of highly specific pharmacological agents that block CN-dependent NFAT signaling without some of the adverse side effects of current pharmacological CN inhibitors. Indeed, a recent study identified dipyridamole as a small-molecule inhibitor of CN-dependent NFAT activation that does not affect CN phosphatase activity (99).

Regulation of Cell Metabolism by mTOR/AMPK

The serine/threonine kinase mTOR regulates cell growth and metabolism. mTOR forms two distinct signaling complexes called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (78). mTORC1 integrates input from both intracellular and extracellular signals to regulate several processes, including lipid metabolism, protein synthesis, and autophagy (35). Upstream regulators of mTORC1 include oxygen, growth factors, and amino acids (35, 78). Emerging evidence supports the role of mTORC1 in renal development and kidney disease. Embryonic deletion of the mTORC1 component Raptor in mouse podocytes causes robust albuminuria and glomerulosclerosis (49). This phenotype is present, although less severe when Raptor is deleted in adult mice (49). Moreover, genetic activation of mTORC1 by deletion of an upstream inhibitor in podocytes recapitulates histologic features of DN, including mesangial expansion and glomerulosclerosis (62), and genetic inhibition of mTORC1 by deletion of one copy of Raptor confers resistance to albuminuria and mesangial expansion in a mouse model of Type 2 diabetes (62). In support of an important role for enhanced mTORC1 activity in diabetic kidney disease, phosphorylation of a downstream target of mTORC1 (p70S6 kinase) is enhanced in glomeruli from diabetic mice and in biopsy specimens from patients with DN (49, 98). Taken together, these findings support an important role for mTORC1 in diabetic kidney disease and suggest that a balance of mTORC1 signaling is required for maintenance of glomerular integrity. Targeting this pathway using pharmacological inhibitors may, therefore, have a narrow therapeutic window. Preclinical studies in rodents, however, suggest that this strategy is feasible because mTORC1 inhibition ameliorates mesangial expansion and interstitial fibrosis in STZ-treated Sprague-Dawley rats (88). There are currently two mTOR inhibitors in clinical use, sirolimus (also known as rapamycin) and everolimus (Table 1). Unfortunately, these drugs are immunosuppressive agents (149), which may limit their use in all but high-risk patients. In addition, both drugs have been associated with development of proteinuria (100), consistent with the narrow therapeutic window required to obtain a beneficial therapeutic effect. While the mechanisms of this proteinuric effect are not known with certainty, mTORC1 target genes include VEGF isoforms (49). As discussed above, VEGF plays a key role in maintenance of glomerular filtration barrier integrity. Alternatively, mTORC2 is required for acti-
viation of prosurvival Akt signaling (16, 118). While mTORC2 is classically thought to be insensitive to current mTOR inhibitors, recent studies suggest that both mTOR complexes can be inhibited by these agents (78, 124). Thus, one possible mechanism to reduce toxicity may be the development of more selective mTOR inhibitors, which preferentially target mTORC1 (149). mTORC2, however, also activates Rac 1 (64), and aberrant activation of Rho GTPases family members cause proteinuria (11, 151, 177). Thus, inhibition of mTORC2 signaling may also contribute to the beneficial effects of mTOR blockade in diabetic kidney disease. Additional studies will, therefore, be necessary to optimize this therapeutic approach.

Another therapeutic approach is to target upstream regulators of mTORC1 activity, such as AMPK. In this regard, AMPK is a potent negative regulator of mTORC1 (56, 132), and strategies to augment AMPK activity could theoretically have therapeutic benefits in DN, perhaps without immunosuppressive effects. In this regard, the adipocyte-secreted hormone adiponectin is a potent activator of AMPK activity (132). Adiponectin is a circulating plasma protein, and adiponectin serum levels negatively correlate with Type 2 diabetes, coronary artery disease, and obesity (132). In support of a role for adiponectin in DN, Sharma et al. (132) found that plasma adiponectin levels were inversely correlated with albuminuria in obese patients. Moreover, knockout of adiponectin caused albuminuria and foot process effacement in mice, which was improved with adiponectin treatment in association with AMPK activation. These beneficial effects were not the result of improved glucose control because this knockout model has normal glucose tolerance and insulin sensitivity. These data suggest that AMPK activation may be a useful therapeutic strategy for ameliorating the development of diabetes and DN. In support of this notion, treatment with the AMPK activator AICAR (5-aminoimidazole-4-carboxamide-1-D-ribonucleoside) (Table 1) improved albuminuria and kidney histology in two mouse models of diabetic kidney disease without altering blood glucose levels (34). Similarly, treatment with another AMPK activator metformin (Table 1) (56) reduced albuminuria and mesangial expansion in a rat model of Type 2 diabetes (70). Unfortunately, this study did not control for the beneficial effects of reduced hyperglycemia observed in the metformin-treated group. While these data are promising, additional basic research is needed. Moreover, given that metformin is already used clinically, examining this agent in humans with diabetic kidney disease may also be of benefit. Unfortunately, current guidelines limit the use of metformin in patients with CKD (63, 87), but carefully monitored clinical trials that control for effects of hyperglycemia in patients with DN and relatively well-preserved kidney function might be feasible.

Induction of Wnt/β-Catenin Signaling in DN

Wnt/β-catenin signaling was initially identified as a proto-oncogene in a mouse model of breast cancer (103). Since then, activated Wnt signaling has been identified in a variety of cancers and diverse biological processes, including embryonic patterning, bone biology, and stem cell maintenance (103). Recent studies have implicated Wnt/β-catenin signaling in the pathogenesis of DN. In this regard, components of the Wnt/β-catenin signaling cascade are upregulated in proteinuric renal diseases, including animal models of diabetic kidney disease and humans with DN (23). The mechanisms of Wnt/β-catenin gene induction are not known with certainty, but CN is activated in diabetic kidneys (50, 150), and expression of a constitutively active NFAT isoform in podocytes in vivo upregulates Wnt genes (159). Moreover, Wnt/β-catenin upregulates multiple genes of the RAS, suggesting a potentially important role for this pathway as a major mediator of kidney damage in DN (175). Small molecule inhibitors of Wnt/β-catenin signaling have been developed (Table 1), and treatment with these inhibitors has beneficial effects in animal models of glomerular disease (175). Thus, while the studies are still in their early stages, the Wnt/β-catenin signaling pathway looks promising as a potential target for the treatment of DN.

Summary and Conclusions

We have attempted to summarize a large body of literature on emerging treatment strategies for DN. For this review, we focused predominantly on signaling pathways that have been implicated relatively recently and selected pathways in which therapeutic agents and/or small molecule inhibitors were readily available (Table 1). Unfortunately, many of these inhibitors have significant risks associated with their use, including currently available inhibitors of JAK/STAT, mTOR, CN, and VEGF signaling. As a result, using inhibitors of these pathways to treat diabetic patients will likely require identifying patients at high risk for disease progression. In contrast, other treatment strategies look more promising as a general approach to patients with DN. Combined therapies using aldosterone antagonists, endothelin inhibitors, and chemokine receptor blockers with RAS blockade are an obvious approach that is currently being studied in clinical trials (Table 2). Another approach is the use of AMPK activators. Metformin activates AMPK and could be studied in clinical trials, but it may be difficult to control for effects of treatment on hyperglycemia and insulin sensitivity. Moreover, the use of metformin is restricted to patients with relatively normal renal function (63, 87), although a number of investigators have suggested that the guidelines for using this agent in patients with CKD could be liberalized (63, 87). Additional AMPK activators are available that have beneficial effects in animal models of diabetic kidney disease without affecting blood glucose levels (34), but these agents would need to be approved for use in humans if clinical studies are considered. Vitamin D is another agent that has demonstrated beneficial effects in animal models of diabetic kidney disease (30, 158, 160, 173) and has been shown to lower albuminuria in macroalbuminuric patients with Type 2 diabetes treated with RAS blockade (26). Vitamin D supplementation might, therefore, be a useful adjunction to current therapeutic approaches in DN that could also be rigorously tested in clinical trials (Table 2). Lastly, there may be strategies to reduce toxicities of other pharmacologic agents with a significant risk of adverse effects. For example, the development of mTOR inhibitors, which are more selective for mTORC1 or CN inhibitors that specifically block CN-NFAT signaling without affecting other signaling cascades (see Regulation of Cell Metabolism by mTOR/AMPK and Enhanced CN/NFAT Signaling in DN). Modification of the dosing regimens used for bardoxolone-like drugs may also be of benefit (see Reducing Oxidative Stress in Diabetes). In summary, promising new therapeutic approaches for the treatment of
diabetic kidney disease are rapidly expanding. While more research is needed, a major challenge to the development of new therapies will be deciding which promising ideas to pursue and then translating these ideas into innovative health care strategies for treating patients with diabetic kidney disease.

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REFERENCES
35. Gaston RS.
38. Gerhardt 49.
43. Gerhardt 49.
44. J Am Soc Nephrol


81. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angio-


84. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. I, 2,5-Dihydroxyvi-


93. Mulero MC, Auberea A, Orzaez A, Messegue J, Serrano-Cande-

94. Murakami N, Riella LV, Funakoshi T. Risk of metabolic complica-


98. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF recep-


103. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Al-

104. Peiris H, Raghuwahari R, Jessup CF, Zanin MP, Mohanasundaram D, Mackenzie KD, Chataway T, Clarke JN, Brealay J, Coates PT, Pritchard MA, Keating DJ. Increased expression of the glucose-


