CLINICAL INVESTIGATION

Treatment of albuminuria due to diabetic nephropathy: recent trial results

Diabetic nephropathy is overwhelmingly the most common cause of chronic kidney disease, and is generally characterized by albuminuria in those patients destined to progress to end stage renal disease. To date, there are few interventions other than glucose and blood pressure control that have reliably been shown to modify albuminuria and alter the course of diabetic nephropathy. This review highlights recently completed and ongoing diabetic nephropathy clinical trials that have employed albuminuria as a primary end point. There is a trend toward development of drugs that target non-traditional mechanisms, especially anti-inflammatory, antioxidant and antifibrotic agents, which raises optimism that abrogation of multiple pathophysiologic pathways will yield additive benefits and improve diabetic nephropathy outcomes.

Keywords: anti-oxidant • chronic kidney disease • clinical trials • diabetes • fibrosis • inflammation

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Albuminuria as a marker of diabetic nephropathy

Diabetes is a major public health problem, with an estimated prevalence of 25.8 M in the USA [150]. Diabetic nephropathy (DN) is one of the most serious complications, and the etiology for over 40% of incident end stage renal disease (ESRD) in the USA. Because DN is a chronic disease that develops over decades and approximately one-third of diabetic patients develop DN, much effort is being devoted to the discovery of biomarkers to accurately predict which patients will develop DN before glomerular filtration rate (GFR) has begun to decline. Hyperfiltration is the earliest measurable biomarker in clinical use for DN progression in types 1 and 2 diabetes. However, in type 2 diabetes, which comprises the vast majority of DN, the mean duration between disease onset and diagnosis commonly exceeds the window for hyperfiltration detection [1].

Albuminuria is a practical and widely employed alternative biomarker for DN [2], as well as mortality [3]. Multiple studies have shown that the degree of albuminuria correlates with the likelihood of DN progression. However, neither the US FDA [4], National Kidney Foundation/Kidney Disease Outcomes Quality Initiative [5], nor Kidney Disease Improving Global Outcomes [6] guidelines recommend albuminuria as a sole, reliable biomarker for DN progression, due mainly to lack of evidence that modulation of albuminuria independently affects GFR decline and progression to ESRD, or that albuminuria reduction results in sustained, beneficial histologic alterations after drug withdrawal. In addition, microalbuminuria (in the 30–300 mg/g creatinine range) commonly and spontaneously reverts to normoalbuminuria [7], and typical DN histopathology can occur in the absence of albuminuria [8,9]. Multiple groups are therefore in pursuit of a more reliable biomarker for the identification of early, progressive DN [10]. Nevertheless, the urine albumin:creatinine ratio is still recommended for routine monitoring of DN progression and Kidney Disease Improving Global Outcomes guidelines suggest treatment approaches based upon a

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risk assessment that takes into account the combination of albuminuria and estimated GFR (eGFR) [6]. A recent working group acknowledged the limitations of albuminuria as a biomarker, but concluded that it might be satisfactory for evaluating primary prevention or complete remission of DN, as determined by progression from normoalbuminuria (<30 mg/g) to macroalbuminuria (>300 mg/g), or regression from macroalbuminuria to normoalbuminuria, respectively, provided that there is persistence of efficacy after intervention withdrawal [4]. Furthermore, for the purposes of drug development, even if albuminuria is not the most desirable primary end point, it is difficult to imagine approval of a drug for DN that does not have albuminuria-reducing properties.

Although there have been some recent disappointments with DN therapies that were close to being marketed, there are several recently completed and ongoing trials that are promising. In particular, there has been recent attention devoted to disparate mechanisms of DN, which raises the possibility of additive or synergistic effects, if combined with existing therapies (Figure 1). The discussion of these trials has been organized according to eight mechanistic classes: vasoactive interventions, new drugs for glycemic control, anti-inflammatory agents, antioxidants, antifibrotic agents, lipid-lowering drugs, vitamin D analogs and mesenchymal stem cells (Figure 1 and Table 1).

Vasoactive interventions

Renin–angiotensin–aldosterone system blockers

Inhibition of the renin–angiotensin–aldosterone system (RAAS) has emerged as one the most effective strategies for reducing albuminuria and slowing the progression of DN. Several large randomized clinical trials have shown that ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) preserve kidney function in patients with DN, reduce the risk of progressing from microalbuminuria to macroalbuminuria (overt DN), and may even impact on the primary prevention of DN, by decreasing the likelihood that a diabetic subject with hypertension will develop microalbuminuria [81]. It has been over 20 years since the first landmark trial involving RAAS inhibitors in DN demonstrated that captopril slowed the decline of renal function in subjects with type 1 diabetes and baseline albuminuria [11]. Randomized controlled trials examining the impact of ARBs in type 2 diabetic subjects with proteinuria also demonstrated that losartan (RENAAL) and irbesartan (IDNT) reduced albuminuria and adverse renal events [16–17]. In addition to slowing progression of renal disease in patients with established DN, multiple studies, including HOPE and IRMA-2, have shown that ACEi and ARBs can also reduce the risk for developing overt DN [12,18]. Randomized controlled studies have also demonstrated that in hypertensive type 2 diabetic patients, RAAS inhibitors can reduce the risk for developing microalbuminuria (trandolapril in BENEDICT), and in diabetic subjects with at least one cardiovascular disease (CVD) risk factor, they can delay the development of microalbuminuria (olmesartan in ROADMAP) [13,19]. Despite the benefit on albuminuria in ROADMAP, olmesartan was associated with an increased risk for fatal cardiovascular events [19]. While most data suggest clear benefit when an ACEi or ARB is used in diabetic patients with hypertension or increased levels of albuminuria, the benefit of RAAS inhibition in non-hypertensive patients with normalbuminuria is not proven [14,15]. Taken together, these results reflect why many physicians view ACEi and ARBs (with the exception of olmesartan) as the standard of care in the treatment of hypertension in the context of diabetes.

Dual therapy with ACEi plus ARB

Initial studies examining the benefit of dual RAAS blockade with ACEi plus ARB in patients with DN were encouraging. For example, a regimen of candesartan and lisinopril decreased albuminuria in patients with DN more than either agent alone [28]. However, subsequent, larger, randomized controlled trials failed to demonstrate that dual RAAS blockade was superior to a single RAAS blocking agent for reducing mortality, renal or cardiovascular outcomes. In fact, some of these studies were stopped prematurely, due to safety concerns about hyperkalemia and acute kidney injury. The ONTARGET study enrolled more than 25,000 subjects, many of whom had diabetes (n = 9612), to investigate the benefit and safety of dual RAAS blockade compared with ACEi or ARB monotherapy [30]. This study found that dual blockade did not reduce risk for a combined outcome, but did increase risk for renal dysfunction and hyperkalemia. The recently published VA NEPHRON D trial enrolled 1448 subjects with type 2 diabetes and macroalbuminuria. The risk for hyperkalemia and acute kidney injury, without improved renal outcomes, was increased in the group randomized to receive lisinopril plus losartan compared with the control group that received placebo with losartan [31]. A similar, smaller (n = 577), randomized controlled trial involving proteinuric diabetic patients from Hong Kong and Japan, reached the same conclusion [34]. Most enrolled subjects were already being treated with an ACEi (73.1%). Patients who were then randomized to receive olmesartan demonstrated improved albuminuria and GFR outcomes, but failed to achieve secondary CVD outcomes, including increased mor-

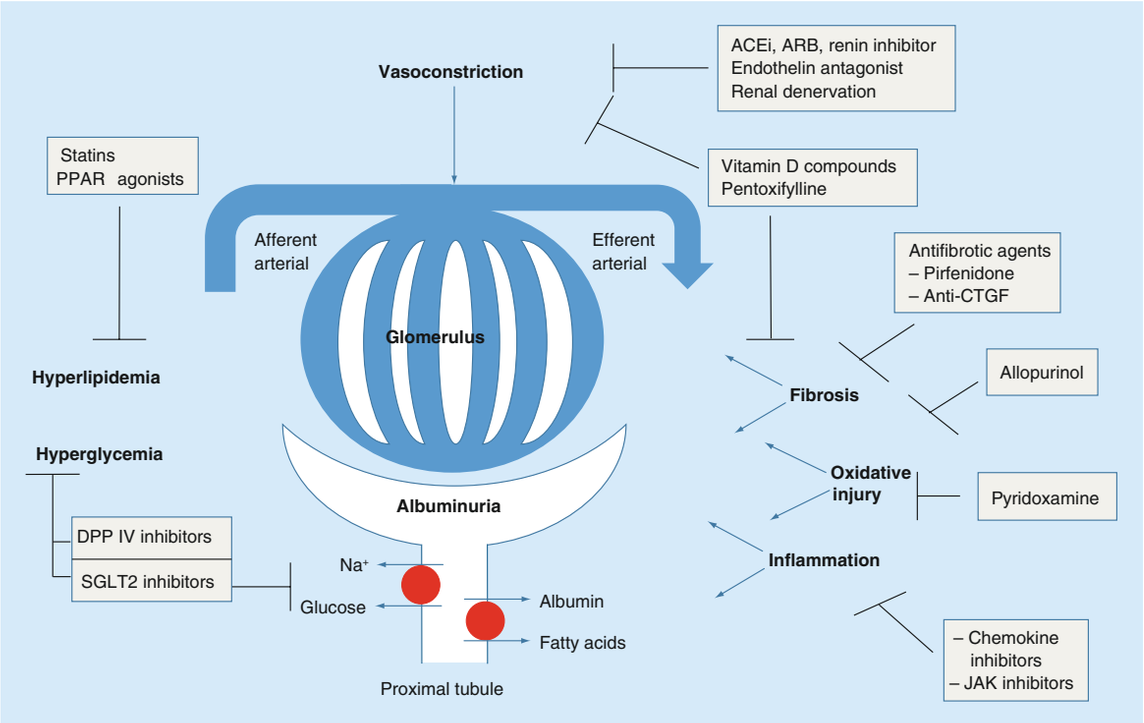

Figure 1. Multiple mechanisms of nephron injury in diabetic nephropathy with albuminuria.
ACEi: ACE inhibitors; ARB: Angiotensin receptor blockers; DPP IV: Dipeptidyl peptidase-4.

tality from cardiovascular causes [34]. These data are consistent with a recent meta-analysis of 33 randomized controlled trials focusing on dual RAAS blockade that included over 68,000 patients. Although most of the included studies enrolled patients without chronic kidney disease (CKD) or albuminuria, and albuminuria was not a primary outcome in the meta-analysis, the authors nevertheless found an increased incidence of hyperkalemia and renal failure without a significant reduction in all-cause and cardiovascular mortality [32]. The ongoing VALID trial [35], which is scheduled to be completed by December 2015, is investigating the potential benefit of dual RAAS blockade with the ACEi benazopril and the ARB valsartan compared with treatment with either agent alone.

While most large randomized controlled trials have concluded that dual RAAS blockade with ACEi, ARBs and renin inhibitors is not beneficial, and may be harmful, certain clinical scenarios, such as persistent nephrotic range proteinuria, despite maximum doses of a single RAAS inhibitor, were not specifically addressed by these trials. It is conceivable that combined treatment with ACEi and ARBs may be reasonable in such circumstances, though further studies will be necessary. At present, many international hypertension organizations, including the Eighth Joint National Committee [82], the American Society of Hypertension/International Society of Hypertension [83], the European Society of Hypertension and the European Society of Cardiology [84], the Canadian Hypertension Education Program (www.hypertension.ca), and the British Hypertension Society (National Institute for Health and Clinical Excellence guidelines [85]) have recommended against prescribing an ACEi plus ARB for the treatment of hypertension.

Renin inhibitors

Interest has also emerged for using inhibitors of the renin-angiotensin-aldosterone system besides ACEi and ARBs to treat DN. These agents include direct renin inhibitors such as aliskiren. Several small studies have demonstrated that renin inhibitors reduced albuminuria in patients with DN due to type 2 diabetes [20,21]. However, since ACEi and ARBs are well established as first line therapy for DN, larger trials investigating the benefit of renin inhibitors have mainly involved randomizing these agents as additive therapy to DN patients already receiving an ACEi or ARB. For example, the AVOID trial, which randomized 599 subjects with type 2 diabetes already taking losartan, to aliskiren or placebo, found that dual RAAS blockade reduced proteinuria and was not associated with an increased risk of adverse events [29]. However, similar to the ACEi plus ARB experience, the ALTITUDE study, which randomized 8561 diabetic subjects,

Table 1. Summary of clinical trials for treatment of albuminuria in diabetic nephropathy.

Agent	Completed studies showing benefit	Completed studies showing no benefit	Ongoing clinical trials
Vasoactive agents			
RAAS inhibitors	The Collaborative Study Group [11] HOPE [12], BENEDICT [13]	Mauer *et al.* [14], Bilous *et al.* [15],	–
ACEi	–	–	–
ARB	RENAAL [16], IDNT [17], IRMA-2 [18], ROADMAP [19]	–	–
Renin inhibitor	Persson *et al.* [20], Fogari *et al.* [21]	–	–
Mineralocorticoid receptor antagonists antagonist	Bertocchio *et al.* [22]	–	NCT01874431 [23], NCT01968668 [24], NCT01756716 [25], NCT01756703 [26], NCT01889277 [27]
Dual RAAS inhibitor	CALM [28], AVOID [29]	ONTARGET [30], VA-NEPHRON D [31], Makani *et al.* [32], ALTITUDE [33], Imai *et al.* [34]	NCT00494715 [35]
Endothelin antagonists	Kohan *et al.* [36]	ASCEND [37]	NCT01356849 [38]
Renal denervation	–	–	NCT01588795 [39]
Anti-fibrosis agents			
Anti-CTGF	–	–	NCT00754143 [40], NCT00913393 [41]
Pirfenidone	Sharma *et al.* [42]	–	
Anti-inflammatory			
Chemokine inhibitors	–	–	NCT01440257 [43], NCT01712061 [44]
	–	–	NCT01547897 [45], NCT01109212 [46]
JAK inhibitor	–	–	NCT01683409 [47]
Bardoxolone	Pergola *et al.* [48]	De Zeeuw [49]	
Antioxidants			
Pyridoxamine	Williams *et al.* [50]	Lewis *et al.* [51]	
Oral diabetic medications			
Dipeptidylpeptidase (DPP-IV) inhibitors	Hattori [52], Groop *et al.* [53]	–	NCT01792518 [54], NCT01968044 [55]
SGLT2 inhibitors	–	–	NCT01032629 [56], NCT01131676 [57]
Pioglitazone	Sarafidis *et al.* [58], Morikawa *et al.* [59]	–	–
Lipid-lowering medications			
Fenofibrates	FIELD [60]	–	–
Statins	Fried *et al.* [61], Sandhu [62]	ALLHAT [63]	–

ACEi: ACE inhibitors; ARB: Angiotensin receptor blockers; RAAS: Renin–angiotensin–aldosterone system.

Table 1. Summary of clinical trials for treatment of albuminuria in diabetic nephropathy (cont.).

Agent	Completed studies showing benefit	Completed studies showing no benefit	Ongoing clinical trials
Simvastatin + Ezetimibe	SHARP [64]	–	
Multiple Mechanisms			
Allopurinol	Momeni *et al.* [65], Goicoechea	–	NCT01575379 [66]
	Goicoechea *et al.* [67]	–	
Pentoxifylline	McCormick *et al.* [68]	–	PREDIAN [69]
CTP-499	–	–	NCT01487109 [70]
PF-00489791	–	–	NCT01200394 [71]
Roboxistaurin	–	Tuttle *et al.* [72,73]	–
Vitamin D compounds	Kim *et al.* [74], Molina *et al.* [75]	–	NCT01169259 [76]
Cholecalciferol	–	–	–
Paricalcitol	de Zeeuw *et al.* [77], Fishbane *et al.* [78], Alborzi *et al.* [79], Agarwal *et al.* [12]	–	–
Mesenchymal stem cells	–	–	NCT01843387 [80]

ACEi: ACE inhibitors; ARB: Angiotensin receptor blockers; RAAS: Renin–angiotensin–aldosterone system.

already being treated with ACEi or ARB, to aliskiren or placebo, failed to demonstrate significant benefit for dual RAAS blockade [33]. Importantly, there was also a higher risk for hyperkalemia in the group that received dual RAAS blockade [33].

Mineralocorticoid receptor antagonists

The role of mineralocorticoid receptor antagonists, such as spironolactone or eplerenone, for the treatment of DN has recently been reviewed [22]. A meta-analysis by Navaneethan *et al.* also demonstrated that addition of aldosterone antagonists to regimens in proteinuric patients (many with DN) already taking ACEi or ARB significantly reduced proteinuria [86]. However, with the exception of aldosterone antagonists added to ACEi or ARBs in patients with systolic heart failure, dual therapy with a mineralocorticoid receptor antagonist plus another RAAS inhibitor has frequently resulted in excess complications (mainly hyperkalemia) in studies enrolling subjects with or without diabetes, which may outweigh benefits [31–32,87–88].

The toxicity associated with dual RAAS therapies may be ameliorated with a new generation of non-steroid mineralocorticoid receptor antagonists, such as BR-4628 [89] and SM-368229 [90]. *In vivo* rat studies demonstrated that SM-368229 potently inhibits albuminuria, with minimal effect on serum potassium concentration. Studies are ongoing to characterize the dissociation between the albuminuria- and potassium-sparing effects in humans. Current clinical trials with other mineralocorticoid receptor antagonists in patients with DN include two studies with dihydronaphthyridine, also known as BAY94-8862 [23,24], as well as three, combined Phase I and II trials enrolling subjects to evaluate safety, tolerability and pharmacology of MT-3995 [25,26,27]. All five of these trials are examining change in albuminuria as one of the end points.

Endothelin receptor antagonists

Endothelin has long been implicated as a possible factor in the pathogenesis of DN, particularly through the ET_A receptor, which mediates vascoconstriction and Na reabsorption in the distal nephron, as well as stimulation of pro-fibrotic TFG-β secretion. Avosentan was the first endothelin receptor antagonist to be studied as a therapy for DN. In ASCEND, which was a placebo-controlled trial involving nearly 1400 patients, avosentan had a salutary effect on albuminuria, but the trial was discontinued prematurely due to increased mortality and congestive heart failure, presumably due to nonselective inhibition of ET_B, which regulates natriuresis [37]. In a trial of patients with types 2 diabetes and albuminuria, already on RAAS-blocking therapy, the additive effect of more selective ET_A inhibition with atrasentan resulted in a modest decrease in albuminuria over 8 weeks [36]. A larger, randomized, placebo-controlled trial (RADAR [38], targeted n = 149), tested the efficacy and safety of two different atrasentan

doses in patients with DN. The primary outcome was change in albuminuria at 12 weeks. The study has been completed, though the results have not been published.

Renal denervation

Based upon pilot studies that demonstrated large decrements in blood pressure with renal denervation [91,92], and the recognition that albuminuria is at least partly mediated by hemodynamic alterations, there is some recent interest in renal denervation as a therapy for diabetic albuminuria. The Renal Denervation in Diabetes (DERENEDIAB) study is a proof-of-concept, multicenter, prospective, randomized, controlled study of the effectiveness of renal denervation in addition to standardized medical treatment, compared with medical treatment alone, in subjects with DN and resistant proteinuria [39]. Bilateral renal denervation will be performed through delivery of catheter-based radiofrequency energy to the luminal surface of the renal artery. The primary outcome is change in urine protein:creatinine ratio at 1 year.

Oral hypoglycemic agents

Multiple studies have demonstrated that glycemic control with insulin and/or oral hypoglycemic agents slows the progression of microvascular disease, including albuminuria and other measures of DN, in type 1 and 2 diabetes [93–97]. However, an important caveat is that randomization to aggressive glycemic control has consistently been shown to either have no effect [94,98–99], or in the case of ACCORD [100], increased overall and cardiovascular mortality in type 2 diabetes, suggesting that diabetes-associated microvascular and macrovascular complications may be regulated by different mechanisms. The optimum hemoglobin A1c target in type 2 diabetes has therefore been debated.

Dipeptidylpeptidase-IV (DPP-IV) inhibitors prevent the degradation of GLP-1, which results in enhanced insulin and reduced glucagon secretion, with a net effect of lower plasma glucose concentration. Potentially beneficial effects of DPP-IV inhibitors on albuminuria and DN progression are likely to be mediated largely by sustained lowering of serum glucose. However, other purported mechanisms include enhanced GLP-1 effects of renal target cells, and blunted cleavage of other DDP-IV substrates, such as meprin-β and HMGB1 [101]. In a 6-month trial involving 36 patients with type 2 diabetes, sitagliptin reduced albuminuria by 20% [52]. A study of four pooled trials, involving 217 patient with type 2 diabetes, all of whom were already receiving a RAAS inhibitor, demonstrated that addition of linagliptin caused a 28% reduction in albuminuria over a 6-month time period [53]. MARLINA is a current Phase III trial that is examining the effect of linagliptin on albuminuria at 24 weeks in diabetic subjects with baseline macroalbuminuria [54]. Gemigliptin is being tested in a Phase IV trial that is enrolling patients with moderate to severe DN, and analyzing change in albuminuria over a 40-week period as a secondary outcome [55].

SGLT2 inhibitors, which reversibly inhibit glucose uptake by the proximal tubule SGLT2 Na–glucose transporter, represent another new mechanism of glucose control. In addition to the effect on maintaining euglycemia, SGLT2 inhibitors blunt Na reabsorption in the initial portion of the proximal tubule, which has been implicated in the pathogenesis of hyperfiltration, tubular hypertrophy, and DN progression [102]. A recent report in the Akita mouse model of type 1 diabetes, demonstrated that the SGLT2 inhibitor empagliflozin decreased albuminuria, blood pressure and inflammatory biomarkers [103]. Several groups presented similar results, with a variety of SGLT2 inhibitors and diabetic animal models, in abstract form at the 2013 American Society of Nephrology meeting, suggesting that a number of peer-reviewed, pre-clinical studies could be published in the near future. A trial involving 534 patients with type 2 diabetes, with inadequate glycemic control on metformin, and randomized to receive dapagliflozin or placebo, achieved better glucose control with combined therapy [104]. Large clinical trials with dapagliflozin (no renal outcomes) [105], canagliflozin (CANVAS trial [56], urine albumin:creatinine ratio end points over 9 years) and empagliflozin (onset of incident albuminuria over 8 years [57]) are ongoing to characterize the long term safety, as well as renal and cardiovascular efficacy of SGLT2 inhibitors. Two small trials examining the pharmacokinetics, pharmacodynamics and safety with TA-7284 [106] and canagliflozin [107] in patients with moderate renal impairment have recently been completed.

Anti-inflammatory agents

Chemokine inhibitors

With the relatively recent recognition that inflammation pathways may play a role in the pathogenesis of DN there are several reports implicating chemokine (C-C motif) ligand-2 (CCL2) (also known as MCP-1) and its receptor CCR2 [108–115], with the rationale that CCL2–CCR2 binding in cells of the monocyte/macrophage lineage activates pro-inflammatory pathways. In addition, CCL2 secretion is stimulated by glucose. A decade-old clinical trial in subjects with type 2 diabetes suggested that lisinopril effects on albuminuria may be mediated by CCL2 [116]. Some data that urinary CCL2 correlates with albuminuria, and several studies in diabetic mouse models have demonstrated a beneficial effect of the CCR2 inhibitor, CCX140-B, on albumin-

uria. As a result, human trials are ongoing, including a Phase II trial in diabetic patients with macroalbuminuria, which will be examining the effect of CCX140-B on albuminuria as the primary outcome [43]. A Phase II, randomized, double-blind, placebo-controlled trial is ongoing to evaluate the efficacy and safety of a chemokine CCR2/5 receptor antagonist (PF-04634817) in adults with type 2 diabetes and macroalbuminuria [44]. The primary end point is reduction in albuminuria after 12 weeks. NOX-E36 is a Spiegelmer, which is a 40-nucleotide, non-natural, L-RNA oligomer, that is delivered subcutaneously to inhibit CCL2 [117]. A Phase II clinical trial, with a planned enrollment of 75 patients with DN, will soon be initiated to evaluate the efficacy of NOX-E46 on albuminuria reduction [45]. Bindarit is a compound that acts primarily to inhibit CCL2 synthesis, though it also exhibits selective inhibition against MCP-3/CCL7 and MCP-2/CCL8. The drug was tested in a Phase II trial involving 100 patients with DN, as defined by persistent micro- or macro-albuminuria, who concomitantly received irbesartan therapy [46]. The primary outcome measure was change in albuminuria at 12 weeks; the results have not yet been published.

JAK inhibition

The JAK–STAT signaling pathway mediates many of the downstream intracellular effects of cytokines. Baricitinib (formerly INCB28050, LY3009104) is an oral JAK1 and JAK2 inhibitor that is in Phase III development as a treatment for rheumatoid arthritis. It is also being tested in a Phase II trial as a potential treatment for DN. The trial is designed to recruit 250 patients, with a primary end point of albuminuria at 24 weeks [47].

Bardoxolone methyl is a synthetic triterpenoid that activates the transcription factor Nrf2, which in turn regulates anti-inflammatory processes, by inhibition of NFκB and decreasing oxidative stress. The initial trial enrolled 227 patients with type 2 diabetes, baseline eGFR between 20 and 45, and an equal proportion of subjects with normo-, micro-, and macro-albuminuria [48]. Subjects were randomized to three different bardoxolone doses or placebo. The primary outcome, eGFR at 6 and 12 months, was improved with all three drug doses compared with placebo. However, albuminuria was slightly increased in all groups over the course of the study, and positively correlated with eGFR. Because of the encouraging eGFR results a larger, placebo-controlled study (2185 subjects with baseline eGFR = 15–30) was recently conducted, which examined a composite end point of ESRD or cardiovascular mortality [49]. Unfortunately the study was halted after nine months due to increased incidence of cardiovascular events in the bardoxolone group, with no difference in the composite outcomes between groups. At baseline, the median urine albumin:creatinine ratio was 320 mg/g, and similar to the prior study, bardoxolone caused significant increases in albuminuria. The effects of RTA 405, a synthetic triterpenoid analog of bardoxolone methyl, has been tested in the obese Zucker rat model of diabetes [118]. In 3 month old rats randomized to RTA 405 and/or ramipril, RTA 405-treated mice developed multiple adverse outcomes, including hypertension, worsening of glomerulosclerosis, tubular atrophy and proteinuria, as well as biochemical and histologic evidence for hepatotoxicity. In addition, the salutary effects of ramipril were negated by addition of RTA 405.

Anti-oxidants

Pyridoxamine

Results from extensive preclinical studies have revealed that advanced glycation end products, reactive oxygen and carbonyl species, such as methylglyoxal, which are generated in diabetes modify extracellular matrix proteins and abrogate binding with resident glomerular cells. Elegant *in vitro* studies have shown that the vitamin B6 derivative, pyridoxamine, acts as a scavenger and reverses the extracellular matrix modifications [69,119]. Two groups have demonstrated in animal models of DN that pyridoxamine reversed glomerular pathology and preserved renal function, as determined by improved albuminuria [120,121]. A small Phase II clinical trial showed a significant interaction between pyridoxamine and eGFR preservation, though albuminuria was unaffected [50]. A subsequent, double-blind, randomized, placebo-controlled trial, involving 317 patients with advanced type 2 DN (mean serum creatinine = 2.2 mg/dl, range 1.3–3.5, mean urine protein:creatinine ratio = 3 g/g) with 1 year follow-up showed no benefit of pyridoxamine, with respect to change in eGFR or proteinuria [51]. However, a subgroup analysis indicated a trend toward therapeutic benefit among patients with lower serum creatinine values, suggesting that pyridoxamine treatment may be effective in diabetic patients with relatively preserved GFR.

Allopurinol

Serum uric acid concentration has been proposed as an independent risk for albuminuria and GFR decline in type 1 diabetes, and cardiovascular mortality in type 2 diabetes, primarily due to inflammatory and oxidant properties, which cause endothelial dysfunction [122]. Two recent trials in cohorts with type 1 diabetes have demonstrated an association between baseline serum uric acid concentration and subsequent development of albuminuria [123,124]. Elevated baseline serum uric acid

has also been linked to early GFR decline over a mean 5 year period in a study involving 355 patients with type 1 diabetes, though interestingly, there was no relationship between uric acid and albuminuria progression [125]. An association between uric acid and rate of eGFR decline, or progression to ESRD, has been less reliably shown in studies with larger, nondiabetic populations [126–128], suggesting that uric acid may be a biomarker rather than a cause of CKD.

An obvious, pragmatic appeal of urate as a therapeutic target is that allopurinol received FDA approval for the treatment of gout nearly 50 years ago. It is therefore available in generic forms, which are inexpensive, and could be tested relatively quickly for off-label indications, such as DN. In studies from Kosugi *et al.* in db/db mice with type 2 diabetes, treatment with allopurinol resulted in a 65% decrease in albuminuria over 8 weeks, which was accompanied by decreased inflammation and fibrosis [129]. One randomized, double blind, placebo control trial, which enrolled 40 patients with type 2 diabetes, demonstrated that allopurinol treatment for 4 months resulted in a significant decrease in 24 h urine protein [65]. In a prospective, randomized trial that enrolled 113 patients with CKD (mean eGFR: 40), approximately a third of whom had diabetes, the allopurinol-treated group experienced a significantly slower rate of eGFR decline over 24 months [67]. A *post hoc* analysis of a relatively large (n = 1348) trial of patients with type 2 diabetes revealed that after adjusting for confounders, 20% of the renoprotective effect of losartan was attributed to its uric acid-lowering properties [130]. A clinical trial in patients with type 1 diabetes has been initiated at the Joslin Clinic [66]. The primary outcome is change in GFR over a 2 year period, and secondary outcomes include median urinary albumin excretion rate during the last 4 months of the treatment, time to serum creatinine doubling or ESRD, time to fatal or nonfatal cardiovascular events, and GFR trajectory.

Antifibrotic agents

A cardinal feature of DN is accumulation of extracellular matrix in glomeruli and the tubulointerstitium, which manifests as mesangial matrix expansion, Kimmelstiel–Wilson nodules and/or interstitial fibrosis. As a result, there is recent interest in the role of antifibrotic agents, some of which have been initially introduced for scarring diseases of the lung or liver. With the recognition that the biochemical pathophysiology may be similar with DN, some of these agents are being considered as therapies for DN. In a small study of 52 patients with DN primarily from type 2 diabetes, randomized to placebo or two different doses of the oral antifibrotic agent pirfenidone, the treatment groups showed benefit for the primary outcome, change in eGFR [42], but the mean change in albuminuria did not differ across groups (p = 0.19). To our knowledge, there are no further trials with this drug in DN.

Similar to other methyl xanthine derivatives, pentoxifylline acts by phosphodiesterase inhibition. Like many of the drugs being tested for DN (Table 1), pentoxifylline has many purported salutary properties, including vasodilation, as well as anti-inflammatory, anti-oxidative and antifibrotic effects. In a meta-analysis of 476 patients with DN, pentoxifylline caused a significant decrease in albuminuria in patients with baseline macroalbuminuria, but the decrease in albuminuria was not significant in those patients with pre-existing microalbuminuria [68]. In the PREDIAN study [131], which was initiated in 2011, diabetic patients with baseline eGFR = 30–60 and mean urine albumin:creatinine ratio = 1.3 g/g were randomized to pentoxifylline 1200 mg/day versus placebo. The primary outcome is eGFR change, though albuminuria will be evaluated as well. Largely because of the encouraging results with pentoxifylline and albuminuria, a Phase II randomized, placebo-controlled trial has been undertaken to determine whether CTP-499, a pentoxifylline metabolite, exerts antialbuminuric effects in 170 patients with type 2 diabetes and nephropathy [70]. An ongoing Phase II trial with another phosphodiesterase inhibitor, PF-00489791, is examining change in albuminuria at 12 weeks as the primary end point [71].

FG-3019 is a humanized monoclonal antibody, which is immunoreactive to CTGF. A Phase I clinical trial, examining safety and efficacy, as determined by albuminuria, was completed [40] and a Phase II trial in DN with albuminuria as the end point, in patients already on an ACE inhibitor or ARB, was terminated [41]. Results from neither of these trials were published. There are a number of investigators testing neutralizing anti-TGFβ antibodies as therapy for non-renal diseases, and we speculate that there will be future studies in DN.

PKC-β is a signaling protein thought to play a role in the development of diabetic microvascular complications, such as DN. Although multiple preclinical trials have shown benefits of the PKC-β inhibitor ruboxistaurin (RBX) in animal models of DN, only one randomized trial in humans has been published. This study enrolled 123 subjects on stable doses of ACEi or ARB for 6 months, who were then randomized to RBX or placebo for 1 year [72]. The RBX group experienced greater declines in albuminuria, and lesser declines in eGFR, but the differences between groups were not statistically significant. In a subsequent combined analysis of three Phase III randomized placebo controlled trials of RBX for diabetic retinopathy as a primary outcome, RBX had no significant effect on secondary renal outcomes (incidence of ESRD, doubling

of serum creatinine, death) compared with placebo [73]. However, albuminuria data were not included in these three trials.

Lipid-lowering drugs

In proteinuric kidney diseases, such as DN, injured glomeruli permit filtration of albumin, which is complexed with fatty acids. Vulnerable, downstream tubular epithelial cells then become exposed to luminal fatty acids, which leads to tubular lipotoxicity, apoptosis and DN progression [132,133]. This concept is supported by *in vitro* and animal model experiments demonstrating that lipidated, but not delipidated, albumin is nephrotoxic [133–135], and suggest that a major pathophysiologic role of filtered albumin is to enhance delivery of lipotoxic fatty acids to proximal tubules.

In humans with DN, a typical serum lipid profile is elevated fatty acids and triglycerides, increased total and decreased HDL cholesterol [136], and serum triglycerides and saturated fatty acids are associated with CKD progression in cohorts enriched for diabetes [137–139]. The recent FinnDiane study demonstrated that poor glycemic control and serum fatty acids were the best predictors of early, accelerated DN progression [139].

Treatment of humans and animal models of DN with PPAR-α nuclear receptor agonists, which stimulate fatty acid oxidation through multiple pathways [140], improved albuminuria and glomerular histology [151,141], and halted GFR decline [60]. Although not prescribed specifically for lipid lowering, thiazolidinediones are ligands for a related nuclear receptor, PPAR-γ. A meta-analysis of randomized, controlled trials incorporating 2860 patients concluded that pioglitazone treatment significantly reduced albuminuria [58]. A subsequent trial of 63 patients on RAAS therapy, randomized to pioglitazone or metformin, demonstrated significantly greater reduction of albuminuria at 52 weeks in the pioglitazone group [59].

In CVD intervention trials, statins have salutary effects on renal function [61–63,137]. In the largest trial that will probably ever be attempted to address the role of serum lipids in kidney disease (SHARP), 9270 diabetic and non-diabetic subjects with CVDs were randomized to simvistatin plus etizimibe versus placebo. There was a trend ($p = 0.09$) toward benefit on CVD progression, a secondary outcome in the study [64].

Vitamin D

In addition to the established role in bone, vitamin D has myriad effects on other organs, including purported benefits to the kidney [142]. A significant amount of experimental and observational data suggest that vitamin D reduces proteinuria and plays a role in regulating the RAAS, vascular health, immune function and insulin sensitivity [142]. Interactions and cross talk between vitamin D molecules, the RAAS, FGF 23/klotho system, phosphorus, and parathyroid hormone may also contribute to proteinuric renal disease pathogenesis by modulating fibrosis, inflammation and proteinuria [143]. Several small clinical studies in diabetic and non-diabetic patients with CVD have been undertaken that demonstrate that various formulations of vitamin D may decrease albuminuria. For example, two studies found that cholecalciferol (nutritional vitamin D) supplementation reduced albuminuria in patients with DN from type 2 diabetes, and already on RAAS inhibitors [74,75]. Active vitamin D analogs, in particular paricalcitol, have also been shown in multiple, small, randomized, controlled studies to lower albuminuria in diabetic [77] and non-diabetic [78–79,144] patients concomitantly taking RAAS inhibitors. The VITAL is a randomized clinical trial of vitamin D (cholecalciferol) and ω3 fatty acid supplements in the primary prevention of cancer and CVD, with a targeted enrollment of 20,000. In an ancillary trial to VITAL [76], participants with a history of diabetes will be examined for the prevention of DN, with albuminuria at 3 years as the primary end point.

Bone marrow-derived mesenchymal stem cells

The potential for treating DN with mesenchymal stem cells is an intriguing concept, with some preclinical studies suggesting merit [145]. Because innate tubular repair appears to result from proliferation of local progenitor cells [146,147], the mechanism driving stem cell therapies may be unrelated to direct differentiation into kidney cells, but rather, as a source of growth factors and other secreted molecules. There is a single, combined Phase I and II trial, that has been initiated to investigate safety and efficacy of mesenchymal stem cells in DN [80]. The trial is designed to enroll 30 patients, with secondary outcomes to include changes in albuminuria, eGFR and glycemic control.

Conclusion & future perspective

In preclinical and early clinical trials of DN, albuminuria is frequently employed as a surrogate marker of disease, despite a lack of recognition by the FDA or advisory guideline panels that albuminuria represents a sole criterion for DN drug approval. Nevertheless, albuminuria may be important for DN pathogenesis, and it is therefore included as a parameter in most DN clinical trials. After many years of disappointing clinical trial results [31,37,49,51,148–149], with the conclusion that there are few therapeutic options for DN other than ACEi, ARBs, glucose and blood pressure control, new therapies may be on the horizon. In our opinion, the most exciting ongoing trials are focused on new mechanisms

of disease, such as inflammation and fibrosis, with the hope that pharmaceutical agents affecting these pathways may have an additive benefit to existing therapies. However, we do not discount the possibility that new vasoactive and oral glycemic agents, such as endothelin receptor antagonists and SGLT2 inhibitors, which work by distinct mechanisms, and are in late-phase clinical trials, may also be additive or synergistic with currently available treatments.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Diabetic nephropathy (DN) is the most common cause of end stage renal disease.
- Albuminuria is a commonly employed biomarker for DN, but it is unacceptable as a sole criterion for assessing drug efficacy or approval.
- The only established therapies for DN are blood pressure (most data with ACE inhibitors, angiotensin receptor blockers) and glycemic control.

Preclinical evidence for new DN mechanisms that have led to new ongoing trials

- The untoward effects of dual RAAS blockade may be ameliorated by a new class of non-steroid mineralocorticoid receptor inhibitors that are currently in clinical trials.
- Multiple groups are investigating SGLT2 inhibitor efficacy in reducing albuminuria in animal models of DN.
- Extensive evidence has emerged to support a role for chemokine-induced inflammation in the pathophysiology of DN, and early clinical trials with chemokine inhibitors have been initiated.
- Substantial data are consistent with uric acid-induced inflammation and oxidation, which has led to one large clinical trial to investigate efficacy of allopurinol in DN.

Accumulated & pending clinical evidence for DN therapies

- While each type of RAAS blocker appears to have efficacy in the treatment of DN, recent large randomized controlled trials suggest that in most situations, when RAAS blockers are used in combination, the benefits are outweighed by potential complications, most commonly hyperkalemia.
- A small clinical trial demonstrated efficacy of the endothelin receptor antagonist, atrasentan, which is now in late-phase US FDA trials.
- Renal denervation has resulted in impressive antihypertensive effects, and clinical trials for DN are ongoing.
- Several small trials have shown reduction in albuminuria with DPP-IV inhibitors in patients with DN.
- Impressive *in vitro* data with pyridoxamine have translated to mixed results in clinical trials.
- Modest effects of pentoxifylline on diabetic albuminuria have led to further clinical trials.
- While there may be compelling reasons for treating hyperlipidemia in diabetic patients with cardiovascular disease risk, a salutary effect with existing lipid lowering drugs on DN *per se* has not been convincingly demonstrated.
- Small trials suggest efficacy of vitamin D analogs for diabetic albuminuria and a large clinical trial has been started.

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