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# THE NEXT GENERATION OF THERAPEUTICS FOR CHRONIC KIDNEY DISEASE

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## Abstract

Chronic kidney disease (CKD) represents a leading cause of death in the United States. There is no cure for the disease, with current treatment strategies relying on blood pressure control through blockade of the renin angiotensin system and glycemic control. Such approaches only delay end stage kidney disease development, and can be associated with significant side effects. Recent identification of several novel mechanisms contributing to CKD development - including vascular changes, loss of podocytes and renal epithelial cells, matrix deposition, inflammation and metabolic dysregulation – has revealed new potential therapeutic approaches for CKD. This review will assess emerging strategies and agents for CKD treatment, highlighting associated challenges in their clinical development.

## Introduction

Approximately 20 million people in the United States are currently affected by CKD, with half a million of these presenting with the most severe form; end stage renal disease (ESRD). The only treatment for ESRD is dialysis or transplantation. However, the mortality of patients on dialysis can be as high as 20% per year[1,2] and transplantation is limited by organ shortage.

The most common cause of chronic and ESRD in the United States is diabetes, which accounts for roughly 50% of all cases, followed by hypertension (25%), with other causes including glomerulonephritides and polycystic kidney disease[1,3]. Cardiovascular disease (CVD) remains the leading cause of mortality in patients with CKD[4]. Despite tremendous progress in reducing CVD death in the general population, this has not translated into CKD patients. The reduction in CVD in the general population correlates strongly with serum cholesterol, smoking status and blood pressure, but the mortality of CKD of subjects is much higher than non-CKD subjects when compared by traditional CVD risk calculations[5,6]. So called “non-traditional risk factors” may result from the build-up of different toxins and metabolites which likely contributes to this increased mortality of patients with CKD.

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The most commonly used definition for CKD is purely based on estimation of the glomerular filtration rate (eGFR)[7], with a 40% decline to a GFR of less than 60ml/min/1.73m2 for more than 3 months being used to diagnose CKD. Different stages of CKD have been proposed, based on GFR criteria. These include stage G1 when GFR>90 cc/min, stage G2 when GFR is 90-60cc/min, stage G3 when GFR is 60-30 cc/min, stage G4 when GFR is 30–15 cc/min, while stage G5 is when GFR is below 15 cc/min. The stages were developed mostly for research purposes and at present we are not aware of any clear distinction between these stages, rather GFR decline represents a continuously increased risk for death. A broader definition of CKD is also in use, which takes structural, functional, pathological, laboratory or imaging abnormalities into consideration. Leakage of albumin or protein in the urine is one such functional abnormality that has gained more prominence in the new classification guidelines[8,9]. Most clinical studies use albuminuria and proteinuria interchangeably as albumin and its degradation products represent the overwhelming majority of urinary proteins. The presence of albuminuria correlates strongly with the development of ESRD, increased CVD and mortality, while reduction of albuminuria is usually associated with protection from functional decline[10].

Sclerosis of the glomerulus and interstitial fibrosis are the common histopathological and structural features of CKD[11]. Glomerular changes are usually specific for disease etiology and as indicated above, are therefore used for diagnosis and disease classification[12]. Tubulointerstitial fibrosis strongly correlates with kidney function and represents a common complex architectural change in the kidney[11,13], which includes matrix and collagen production by epithelial cells and activated myofibroblasts[11]. Mouse genetic studies have highlighted the key role of epithelial cells in the development of fibrosis[1415,16]. Indeed, genetic overexpression of Notch, Wnt, KIM and HIF in tubule epithelial cells was sufficient to induce epithelial damage, dedifferentiation and the full spectrum of fibrosis[1415,16], while genetic deletion of these pathways protected from fibrosis development. Fibrosis is a reactive process that develops mostly in response to epithelial injury and is almost always accompanied by inflammation, manifested by increased cytokine expression and accumulation of macrophages and inflammatory cells[17]. Vascular injury and loss of capillaries exacerbates epithelial injury at later stages by limiting the nutrient availability to epithelial cells. Our understanding of fibrosis has significantly improved over the past several years, revealing new potential therapeutic targets.

At present there is no cure for most forms of chronic kidney disease. The list of conditions associated with reversible renal failure is short, comprising of decreased renal perfusion, nephrotoxic drugs and urinary obstruction. Although steroids and other immunosuppressive measurements can halt or reverse some diseases of the kidney, such as IgA neuropathy[18], lupus nephritis, membranous nephropathy, focal segmental glomerulosclerosis, vasculitis and MCD, they have not shown benefit in kidney disease in patients with diabetes and hypertension[19].

Hemodynamic changes play a critical role in CKD development and strategies to control high blood pressure have had a significant beneficial impact on disease[20]. An increase in systemic blood pressure can increase glomerular filtration, leading to hyperfiltration, an increase in glomerular size and glomerular cells must endure the increased stretch[21]. In

addition, as the vessels that supply the kidney with nutrients originate from the efferent portion of the glomerulus, these changes reduce the post-glomerular blood flow leading to ischemic changes in the kidney[22, 23]. Inhibiting the renin angiotensin system, which is involved in the regulation of blood pressure and fluid balance[19], using either angiotensin convertase enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB), reduces glomerular hyperfiltration and albuminuria, and slows the decline in kidney function[19]. These agents have therefore become the mainstay of CKD treatment. However, this approach only slows the decline in kidney function and does not cure CKD. In addition these agents can be associated with significant side effects, such as an increase in serum potassium levels, which can limit their therapeutic use[24].

There are several additional drugs in development that target hemodynamic changes in CKD. Smoking cessation also slows the rate of progression[25]. Many practitioners recommend protein restriction, statin therapy and the correction of metabolic acidosis, but at present the benefit of these measures have not been unequivocally demonstrated in large randomized trials[26]. As our understanding of CKD pathogenic mechanisms has improved, new therapeutic approaches, including targeting inflammation, fibrosis or podocytes, are emerging. In this Review, we will assess current and emerging strategies for the treatment of the major types of CKD - diabetic and hypertensive CKD -and address key challenges and considerations in the development of novel therapies and future clinical trial design.

## Current treatments for CKD

Angiotensin converting enzyme inhibition (ACEi) and angiotensin receptor blockade (ARB) have been the mainstay of therapy for chronic kidney disease for over twenty years (Figure 1). The initial rationale for testing ACEi therapy was based on preclinical studies showing a reduction in intraglomerular hemodynamic pressure accompanied by proteinuria reduction in hyper-filtering rat kidney disease models, including diabetic nephropathy and partial renal ablation[2327]. Clinical testing of the ACEi, captopril, in patients with type I diabetes and nephropathy revealed that captopril improved renal outcomes beyond that achieved with alternative methods of blood pressure reduction[28], establishing captopril and ACEi as the standard of care for patients with diabetic nephropathy (DN). Subsequent studies extended the observed benefit to patients with other causes of kidney disease including those with late stage CKD[29,30].

Similar beneficial effects of Angiotensin type 1 receptor blockade (ARB) were subsequently observed in patients with type 2 diabetes and nephropathy[31–33], establishing the renin angiotensin pathway as a key target for the treatment of CKD. Notably, the therapeutic benefit of ACE/ARB therapy is closely associated with an early reduction in estimated GFR[34], coupled with reduction in proteinuria (or albuminuria) (Figure 1)[35,36], consistent with the capacity of these agents to reduce intra-glomerular filtration pressure as noted above[27].

However, although ACEi/ARB therapy slows the progression of renal disease, it does not halt disease progression. In addition, such therapy has been associated with side effects - including cough for ACEi and serious hyperkalemia, that often limits their use[37,38].

Although the introduction of ACEi and ARB therapy has decreased the incidence of renal failure in the US[1], the prevalence of CKD has increased, with more than 100,000 patients developing renal failure each year[1,39]. Furthermore, the dramatic decrease in adverse cardiovascular outcomes, stroke and amputations, seen over the past twenty years in diabetes patients has not been accompanied by a similar reduction in diabetic ESRD[39]. At present there are no therapies that reverse the loss of renal function in CKD, in stark contrast to more acute inflammatory glomerulonephritis, where immunosuppression can potentially even cure disease.

Nevertheless ACEi or ARB therapy is now considered the standard of care for chronic proteinuric kidney disease, therefore any novel therapy must prove added benefit on the background of ACEi or ARB therapy.

## New approaches to modulate blood pressure, hemodynamics

### Combination of ACEi and ARB

Given the evident role of the renin angiotensin system (RAS) in the progression of renal disease, it was hypothesized that continued disease progression could be prevented by more complete RAS blockade[38,40]. To investigate this, several trials investigating the combination of both an ACEi and an ARB have been performed (discussed below).

The ONTARGET trial compared the benefit of the ACEi Ramipril, the ARB telmisartan or their combination in 25,920 patients with vascular disease and/or high-risk diabetes investigators[41]. Notably, most of the subjects enrolled in ONTARGET did not have microalbuminuria or macroalbuminuria at baseline, so renal benefit in proteinuric patients afforded by combination ACEi/ARB therapy could not be established[41]. During the study, 784 patients permanently discontinued randomized therapy because of hypotensive symptoms, with the majority of these being on combination therapy. The number of subjects achieving primary renal outcome of dialysis, doubling of serum creatinine, or death was significantly increased in the combination therapy group compared to those treated with monotherapy. Many of the dialysis events were due to acute renal failure, which was particularly increased in normotensive patients. These disappointing, but not entirely unpredictable results, underscore the safety risks associated with ACEi/ARB therapy.

Similarly, the VA-Nephron D trial studied whether the combination of an ACEi and ARB (Lisinopril and losartan, respectively) further improved renal outcomes in subjects with type 2 diabetes mellitus, but only enrolled patients with significant residual proteinuria[37]. Upon enrollment, subjects were all placed on Losartan. During the initial up-titration of losartan, there was a statistically significant decline in the median urinary albumin-to-creatinine ratio. Subjects were then randomized to additionally receive either Lisinopril or placebo. In the year following randomization, a further decline in albuminuria was observed, which was significantly greater in the combination-therapy group compared to patients receiving monotherapy. While there was a trend towards reduced renal failure events using the ACEi/ARB combination, this was not statistically significant. Moreover, the combination was associated with doubling of the risk for hyperkalemia and acute kidney injury compared to monotherapy[37]. Based on these results, combination therapy of ACEi/ARB cannot be

widely recommended for use in clinical practice, although fastidious management of serum potassium might allow this combination to provide some benefit for select patients with residual proteinuria[37].

### Renin Inhibition

An alternative approach to RAS blockade is to inhibit the first step in the renin-angiotensin-aldosterone cascade, renin mediated cleavage of angiotensinogen to angiotensin-1 (Figure 1). The effect of the renin inhibitor, Aliskerin, on renal outcomes was tested in patients with diabetic nephropathy (ALTITUDE trial)[42]. Aliskerin added to either ACEi or ARB therapy resulted in greater albuminuria reduction than placebo, but did not decrease renal events or improve the rate of eGFR loss and was associated with increased hyperkalemia and stroke. The ALTITUDE trial was prematurely terminated due to increased adverse events and lack of benefit on renal function decline. Taken together these findings raise significant concerns regarding the safety of complete RAS inhibition as a strategy for treating progressive renal disease.

### Mineralocorticoid receptor (MR) antagonists

The mineralocorticoid, aldosterone, is an important mediator of the effect of angiotensin, as angiotensin causes aldosterone to be released from the adrenal gland (Figure 1). Aldosterone increases tubule secretion of potassium and reabsorption of sodium and chloride, thereby expanding intravascular volume and increasing blood pressure[43]. Aldosterone also plays a role in directly regulating the expression of several profibrotic molecules; for example plasminogen activator inhibitor-1[44,4546]. Despite the preceding findings for the combination of ACEi plus ARB, and the known risks of hyperkalemia following mineralocorticoid receptor blockade[47], trials of mineralocorticoid receptor inhibitors for the treatment of diabetic nephropathy are underway. An open label study investigating the aldosterone receptor antagonist spironolactone plus ARB, versus ACEi plus ARB, suggested superior ACR reduction in subjects with microalbuminuria and diabetic nephropathy taking spironolactone[48], although the interpretation was confounded by lower blood pressures in this group[49]. Additional studies of MR antagonists are ongoing and include efficacy studies of Finerenone (Bayer)[50], CS-3150 (Diachii Sankyo) and MT-3995 (Mitsubishi Tanabe), to determine the effects of these agents on ACR in subjects with diabetic nephropathy[51]. Whether appropriate dosing regimens can be identified which will provide efficacy yet circumvent the risk of hyperkalemia remains to be seen. It seems however that approaches that target pathways orthogonal to the RAAS should have the advantage of non-overlapping safety profile concerns.

## Targeting the vasculature

Diabetic kidney disease is grouped under the microvascular complications of diabetes. Endothelial cell dysfunction, including proliferation and abnormal angiogenesis, can be observed in both experimental diabetes and human disease[52]. Similar endothelial pathology has been reported in the retina, which likely underlies the strong association between renal disease and retinopathy in diabetes[53,54]. Early phases of experimental diabetes are characterized by an increase in glomerular size, which may be a consequence of cellular

endothelial hypertrophy and hyperplasia, as well as podocyte hypertrophy (Figure 2)[55,56]. Podocytes are a rich source of VEGF, angiopoetins and SDF, generating a bidirectional signaling pathway between endothelial and glomerular epithelial cells across the filtration barrier (Figure 2)[57]. This podocyte endothelial cross-talk plays a pivotal role in mediating filtration barrier perm-selectivity and albuminuria development[58]. The interaction between podocytes and endothelial cells has been the focus of robust preclinical experimentation, but most targets have not yet advanced into clinical development.

## Restoring the endothelial glycocalyx

The glycocalyx is a glycoprotein-polysaccharide rich coat that covers mammalian cells (Figure 2). Glomerular endothelial glycocalyx protects from proteinuria by limiting the entry of plasma proteins into the filtration barrier[59,60]. Perturbation and loss of the endothelial glycocalyx has been proposed to be an early event in diabetes[61,62]. Sulodexide is a highly purified mixture of glycosaminoglycans composed of 80% fast moving heparin and 20% dermatan sulphate, which exhibits anti-thrombotic and profibrinolytic properties and may inhibit endothelial glycocalyx breakdown[6364]. However, while sulodexide reduced urine albumin excretion in patients with type 2 diabetic nephropathy, it failed to show benefit in a multicenter placebo-controlled double-blinded phase 3 study[65]. Although other approaches to restore the endothelial glycocalyx, such as the use of chemical heparanase inhibition, are being investigated, they have not yet been put into clinical development[66].

## Endothelin Receptor Antagonists

Preclinical studies of animal models with kidney disease have suggested that selective blockade of the endothelin A (ETA) receptor is associated with renal protection, when used in addition to existing therapies, such as RAS interventions (Figure 2)[67]. Several potential mechanisms for ETA receptor blockade providing renal protection have been proposed. ETA receptor blockade has vascular effects, which cause glomerular vasodilation[68], and the ETA receptor antagonist atrasentan has been shown to reduce albuminuria (Table I)[69]. Second, endothelin has been associated with renal inflammation, which is reduced by ETA receptor blockade, perhaps by mitigating the inflammatory effects of albuminuria[70–72]. Finally, endothelin has been implicated in the deposition of collagen and fibrosis and, thus, ETA receptor antagonists may directly reduce fibrosis in the kidney[72–74].

However, a renal outcomes study using the ETA antagonist Avosentan was prematurely terminated because of a three-fold increase in fluid retention and heart failure compared with placebo[75], and therefore it was no determined whether Avosentan slowed the progression of eGFR decline. It has been hypothesized that fluid retention is driven by the endothelin B receptor blocking capacity of the drugs, although there are also reports of sodium retention induced by ETA receptor blockade[69,76]. Based on this hypothesis, a hard renal outcome trial (SONAR) using the more selective ETA receptor antagonist Atrasentan, has been initiated. In this trial 4100 patients will be treated for 48 months with a primary outcome of creatinine doubling, ESRD or death. Because of the previous association of these drugs with congestive heart failure (CHF), the trial will specifically exclude patients with a previous history of CHF, pedal edema or a current BNP level >200pg/ml. The anticipated completion is in 2017[77].

## Anti-inflammatory Therapy

An association between inflammation and diabetic renal failure has long been recognized (Figure 3)[17,78]. Bohle et. al. described the association of increased tubulointerstitial inflammatory cell infiltrate in diabetic nephropathy human kidney biopsies, with higher serum creatinine levels and longer duration of disease[79]. More recent mRNA profiling of renal biopsies from patients with diabetic nephropathy confirmed that inflammatory pathways are significantly increased in glomeruli and tubules[17,78] and have provided evidence for stimulation of macrophage and dendritic cell maturation pathways and cytotoxic T lymphocyte mediated apoptosis, in glomeruli as well as in the tubulo-interstitial compartments. In later stages of kidney disease, leukocyte migration and complement system signatures were identified in glomeruli[17]. However, even patients with early stages of disease had evidence of activation of inflammatory pathways including the JAK/STAT and NFKB pathways[78,80].

### Tumor Necrosis Factor inhibition

The findings above are congruent with earlier studies suggesting hyperglycemia associated advanced glycosylation end-products (AGEs) activate TNFα production by macrophages in diabetes (Figure 3)[81]. Increased macrophage TNF and IL1 production were shown to be induced by glomerular basement membrane (GBM) from streptozotocin treated rats, compared to control GBM[82,83]. These findings are of particular note, since it has recently been demonstrated that circulating levels of soluble TNFR1 and TNFR2 (sTNFR1, sTNFR2) predict a higher rate of progression to ESRD over the following decade in patients with either type 1 or type 2 diabetes and nephropathy[84,85]. Membrane associated TNFR1 and TNFR2 play important roles in activating chemokine and cytokine release[86]. Interestingly, baseline circulating TNFα levels were only moderately increased in subjects who later developed ESRD, consistent with the possibility that membrane-associated TNF could serve as the activating ligand[87,88]. It is notable that both forms of the circulating sTNFRs are increased and predictive of progression[89–91]. TNFR2 appears to be selectively expressed in endothelium and leukocytes, whereas TNFR1 is widely expressed[89,91]. In addition TNFR1 and TNFR2 can be associated with different functions. A Renal protective role for TNFR1 is postulated, since deletion of this receptor is associated with higher systolic pressure and urinary albumin excretion, as well as an altered GFR[9293]. TNFR2 has been shown to exert positive inotropic effects in cardiac myocytes via enhanced calcium handling[94]. Moreover, TNFR2 may play a role in increased susceptibility to albuminuria[89]

Whether TNF or its receptors play pathogenic roles in CKD progression or diabetic nephropathy remains uncertain. TNF neutralizing antibodies or TNF receptor knockouts have reduced severity in preclinical rodent models of kidney disease[92,95]. Nevertheless, despite anti-TNF agents having been utilized clinically for more than two decades, there have been few studies examining their clinical activity in kidney disease. Any studies have been relatively small and have mainly focused on lupus nephritis and FSGS, leaving their potential activity in other forms of CKD progression unaddressed[89,92].

## MCP1/CCR2 inhibition

Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine (C-C motif) ligand 2 (CCL2), is a 99 amino acid residue secreted protein that interacts with the CCR2 receptor on T-cells and macrophages, recruiting these cells to sites of tissue injury (Figure 3)[96,97]. Renal expression of MCP1 is increased in patients with proteinuric diabetic nephropathy, being predominantly expressed in the tubulo-interstitium rather than the glomerulus[98,99100]. Urinary excretion of MCP1 is also increased in patients with diabetic nephropathy and higher urine MCP1 appears to be predictive of worse renal outcomes[101]. Similarly, in animal models, blockade of CCR2, the receptor for MCP1, ameliorated progression of diabetic nephropathy in db/db mice as well as diabetic $Ins2^{C96Y}$ Akita mice[102,103].

Several companies have established clinical programs to test the efficacy of CCR2 inhibitors in human diabetic nephropathy. Chemocentryx and Pfizer have ongoing clinical development programs of oral receptor antagonists for MCP1/CCR2 (CCX140) and CCR2/CCR5 (PF489791), respectively, and are testing them for their ability to reduce proteinuria in patients with diabetic nephropathy (Table I)[104105].

A 52-week phase II clinical trial of CCX140 in 332 patients with diabetic nephropathy[106,107] met its primary endpoint, demonstrating that treatment with CCX140 added to an ACEi or ARB statistically significantly reduced the urinary albumin creatinine ratio (UACR), beyond that achieved with standard of care. The maximum treatment effect, an 18 percent reduction in UACR, was seen in the 5mg dose group at 12 weeks, and sustained reduction in albuminuria induced by CCX140 relative to SOC alone was observed over the full year[106]. Whether the reported reduction in albuminuria will translate into long term improvements in patient outcomes and reduce the rate of renal function deterioration remains to be tested. At the time of writing this review, results from Pfizer's Phase II trial of the CCR2/5 antagonist PF489791 were not publically available (Table I).

## JAK/STAT inhibition

Janus Kinase (JAK) and signal transducer and activators of transcription (STAT) are important intracellular mediators of receptors for erythopoetin, growth hormone, EGF, as well as inflammatory signaling interferons alpha, IL6, IL12, and IL23 (Figure 3)[108,109]. Ligand binding to their receptors leads to multimer or homodimer assembly, activating the auto-phosphorylation activity of JAK and subsequent STAT phosphorylation and its translocation to the nucleus[110], resulting in the transcription of additional proinflammatory target genes including MCP1, as well as GATA3, IL24, LTB, and SOCS1[111,112113114]. Increased expression of these genes comprise a major genetic signature in diabetic nephropathy and lupus nephritis[78,80,115,116]. Evidence of JAK/STAT activation in experimental models of kidney disease, including diabetic mice and rats, has also been observed[17,78,80] and the use of a non-selective JAK inhibitor (AG-490) significantly reduced urinary protein excretion in diabetic rats[117].

Recently, JAK inhibitors including tofacitinib and baricitinib have been tested in the clinic and found to be efficacious in autoimmune inflammatory diseases, including rheumatoid arthritis and ulcerative colitis[113,118116,119,120]. The documented clinical anti-inflammatory

efficacy of JAK inhibitors, together with the gene pathway signature in diabetic nephropathy have prompted a phase 2 exploration to test the clinical efficacy of these JAK inhibitors in kidney disease[96,121]. The effects of 24 week treatment of the JAK1 and JAK2 inhibitor baricitinib (Eli Lilly/Incyte) on albuminuria has been investigated in 129 subjects with proteinuric diabetic nephropathy already taking ACEi or ARB[121]. Baricitinib treatment was associated with a 30–40% dose-dependent reduction in albuminuria. However, the side effect of reduced hemoglobin associated with this class of drugs was observed[121]. It will be necessary to determine whether or not these effects on albuminuria reduction translate into long-term benefit on kidney function and mortality.

## Complement inhibition

Complement activation plays a well-established role in the pathogenesis of diverse renal disease, including membranoproliferative glomerulonephritis (MPGN), post-infectious glomerulonephritis, hemolytic uremic syndrome and IgA nephropathy[107,122]. Eculizumab (Alexion Pharmaceuticals) is a monoclonal antibody to C5, which blocks the cleavage of the C5 complement protein to C5a and C5b, thereby preventing the generation of the pro-inflammatory peptide C5a and the membrane-attack complex C5b-9 (Figure 3)[123]. Eculizumab treatment improved eGFR in patients with atypical hemolytic uremic syndrome and the associated thrombotic microangiopathy[123124]. Although these were small phase two trials, the effects were sufficiently profound to support registration of eculizumab, for which it is now approved.

In addition to immune glomerulonephritides, complement activation has been proposed to contribute to the pathogenesis of diabetic nephropathy, possibly through glucose-associated production of neoepitopes activating the lectin complement pathway[125,126]. This hypothesis has received additional support from molecular profiling studies of human diabetic nephropathy kidney biopsies and plasma, which show complement to be among the most activated pathways[17,127]. C3 is robustly expressed in kidney biopsies from patients with diabetic nephropathy[17]. Although complement inhibition has not been clinically tested in diabetic nephropathy, inhibition of complement in animal models of the disease support potential therapeutic benefit[128]. However, the potential risk of increased infections with this approach must be considered[124].

## Apoptosis signaling kinase 1 (ASK1)

ASK1 is a mitogen activated protein kinase kinase kinase (MAPK3K) stress responsive kinase, that can be activated by multiple stimuli including reactive oxygen species (ROS) and TNFα activation of TNFR1[129,130]. ASK1 signals through a cascade of downstream kinases including p38 and c-Jun N-terminal kinase (JNK), leading to target gene expression including inflammatory cytokines[131132]. A preliminary report suggests that ASK1 inhibition substantially improves glomerulosclerosis in a diabetic animal model[133]. Gilead Sciences has initiated a 300 patient phase 2 program to test the efficacy of the selective ASK1 inhibitor GS-4997 in diabetic nephropathy. The primary outcome is the change in eGFR following 48 weeks of treatment. A secondary outcome will determine the proportion of participants achieving at least a 30% reduction in albuminuria at week 48 from baseline[134,135] .

### Vascular adhesion protein 1 (VAP1) inhibition

Accumulating evidence pointing to renal inflammation in the pathogenesis of progressive kidney disease[136] has generated interest in strategies to impede trans-capillary migration of inflammatory cells into the diseased kidney. Vascular adhesion protein 1 (VAP-1) is an endothelial sialoglycoprotein, whose cell surface expression is induced under inflammatory conditions[137,138]. VAP-1, also known as amine oxidase, copper containing 3 (AOC3), possesses mono-amine oxidase activity and also interacts with leukocyte adhesion molecules including Siglec-9 and 10[139]. Both these functions are important in facilitating leukocyte egress into inflamed tissue[137]. Based on these findings, Astellas has initiated a phase 2 study of ASP8232 - a small molecule inhibitor of AOC3 activity, in 110 patients with diabetic nephropathy[140]. The trial will explore urine ACR reduction following twelve weeks of treatment and is anticipated to read-out in June 2016 (Table I).

## Targeting fibrosis

Tubulointerstitial fibrosis is the common histological manifestation of CKD, almost regardless of disease etiology (Figure 3). The degree of fibrosis is a strong predictor of future GFR decline and often an important reason why biopsies are performed[79]. Tubulointerstitial fibrosis shows similarities to other fibrotic conditions including lung fibrosis and liver cirrhosis[141]. Common mechanisms identified in fibrosis include: epithelial injury, fibroblast activation, matrix deposition and inflammation, which each provide potential therapeutic targets[142].

### TGFB1 inhibition

Cell culture and mouse model data strongly supporttransforming growth factor beta (TGFB1) as the key driver of collagen and activated myofibroblast accumulation, which is characteristic of fibrosis[143]. Inhibiting TGFB signaling therefore represents a promising antifibrotic therapeutic approach (Figure 3). However, clinical trials with CAT-192 (Genzyme), a recombinant human antibody that neutralizes TGFB1 in the treatment of early-stage diffuse cutaneous systemic sclerosis, failed to show benefit in diabetic kidney disease (Figure 3). Similarly, Phase II trials with a TGFB1 neutralizing antibody (Lilly, LY2382770) did not show clinically significant improvement in patients with diabetic kidney disease[144]. Systemic toxicity associated with TGFB inhibition may limit the maximal tolerated dose[145]. Further studies are needed to understand the complex role of TGFB in fibrosis and identify approaches that would avoid systemic inhibition.

One such approach that has recently emerged involves targeting the alpha(v)beta (6) integrin. αvβ6 is an epithelial restricted integrin that binds and activates latent TGFbeta[146]. An anti-αvβ6 neutralizing antibody developed by Biogen (STX100) has shown benefit in multiple fibrosis models[146,147] and is currently undergoing Phase II trials in idiopathic pulmonary fibrosis (IPF) (Figure 3).

### Pirfenidone

Pirfenidone has recently been approved for the treatment of IPF as it has shown statistically significant benefit in reducing pulmonary function decline in Phase III Trials[148]. The

mechanism of action of pirfenidone is not fully understood. In cell culture studies it suppresses collagen expression and has an anti-inflammatory effect[149,150]. It showed modest clinical benefit in a randomized, double-blinded, placebo-controlled Phase II study in 77 subjects with diabetic nephropathy who had elevated albuminuria and reduced eGFR. Among the 52 subjects who completed the study, a significant increase in the mean eGFR in the pirfenidone group compared to the placebo group was reported[151].

### Galectin-3

Galectin-3 is a member of the lectin family, with antimicrobial activity against bacteria and fungi. It has been shown to be involved in an array of biological processes including cell adhesion, growth, differentiation, fibrosis, heart disease, cancer and stroke (Figure 3)[152]. Human studies indicate a correlation between galectin 3 levels and ESRD, pulmonary fibrosis, heart disease and cancer[153]. Galecto Biotech is currently developing galectin-3 inhibitors for the treatment of fibrosis, specifically IPF[154]. It will be interesting to also investigate their effects on kidney disease progression.

### Lysophosphatidic acid receptor (LPAR) antagonists

Fibrosis is associated with both increased lysophosphatidic (LPA) production as well as with increased expression of its receptor (mainly LPAR1) in a number of organs (Figure 3)[155]. LPA is a bioactive mediator which acts via specific G-protein coupled receptors[156]. Genetic (using knock-out mice) and pharmacological approaches demonstrated the contribution of the LPA1R subtype to the development of kidney, lung, vascular and dermal fibrosis[157]. To date, LPA1R antagonists have passed Phase I and Phase II clinical trials for IPF and systemic sclerosis[158], and therefore may be tested in kidney disease in the future.

### Tranilast

Tranilast (Kissei Pharmaceutical Co. Ltd.) has been marketed in Japan for the treatment of allergic diseases since 1982 and more recently for the treatment of keloid/hypertrophic scars (Figure 3)[159,160]. In pilot studies, one of its derivatives, 3- methoxy-4-propargyloxycinnamoyl anthranilate (FT011, Fibrotech Therapeutics) was shown to reduce albuminuria in a rat model of diabetic nephropathy[161]. In cell culture studies, FT011 inhibits both TGFB- and PDGF-induced collagen production[162]. At present we are not aware of an active development program for tranilast/FT011 in DKD.

### Lysyl oxidase inhibition

Lysyl oxidase (LOX) is an extracellular copper-dependent amine oxidase that catalyzes the first step in the formation of crosslinks in collagens and elastin[163,164]. Collagen cross-linking is critical for fibrosis development (Figure 3). Gilead pharmaceuticals have developed a new humanized monoclonal antibody (simtuzumab) that binds and inhibits LOXL2, which is currently in clinical trials for lung and liver fibrosis. Based on its mechanism of action, it may also show benefit in kidney fibrosis[165].

### Targeting microRNAs

Mouse models of IPF have indicated decreased expression of microRNA-29 (miR29) in bleomycin-induced fibrosis[166]. Additional studies have indicated that miR-29 is an important regulator of TGFβ expression[166]. miRagen Therapeutics have developed miR-29 mimics that are now being tested for the treatment of IPF. Notably, miR29 has showed similar expression patterns in mouse models of kidney fibrosis[167], indicating that this could be a potential therapeutic target. Another microRNA - miR-21 - might represent another important target in the kidney[168] (see below).

### Developmental pathways

Unbiased transcriptome analyses studies have highlighted the reactivation of multiple developmental pathways including Wnt/Notch and Hedgehog pathways (which play critical roles in kidney development) in patient samples and animal models of CKD (Figure 3)[1714,169170]. . While transient activation of any of these pathways is likely to be important in regeneration and repair after an acute injury, sustained activation of these pathways have been shown to induce fibrosis development in mouse models[171]. Increased chronic expression of Notch in tubule epithelial cells induced fibrosis development in mouse models, while genetic deletion of Notch from tubule cells or pharmacological inhibition of Notch ameliorated kidney disease development[14]. Notch seems to play an important role in mediating epithelial dedifferentiation, which is a key aspect of fibrosis. The Gli Hedgehog pathway on the other hand seems to play an important role in myofibroblast proliferation and transdifferentiation[170]. Genetic or pharmacological inhibition of Hedgehog signaling in fibroblasts was not only able to halt, but also to reverse fibrosis in a mouse model[14,170,172]. While these pathways may therefore represent new and potentially attractive therapeutic targets, at present, further studies are needed to better differentiate the profibrotic and regenerative activities.

## Metabolic dysregulation in diabetes and CKD

Metabolic dysregulation, particularly poor glucose control, is associated with an increased rate of development of complications, including kidney disease, in patients with type 1 and type2 diabetes[173,174]. Indeed, cells incubated in high glucose or increased fatty acids show significant changes in their metabolic profiles[175]. Increased generation of reactive oxygen species from mitochondrial or plasma membrane oxidase has been proposed to be the unifying theme leading to cell damage (Figure 4)[176].

However, recent studies exploring the effects of glucose normalization on diabetic kidney disease have presented unexpected observations. Findings from the Diabetes Complication Cohort Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) indicate that subjects with initial poor glycemic control continue to develop a higher rate of kidney and cardiovascular disease despite several decades of improved glycemia[173]. Furthermore, we have also learned that in most patients with type2 diabetes and existing kidney disease, targeting a glycated hemoglobin level below 6.0% - almost to the degree of “normalization” of glucose levels - does not translate into reduced development of complications[177,178]. The term “metabolic memory” has been proposed to explain these results, indicating that cells

are reprogrammed by prior episodes of metabolic derangements and continue to respond differently[179]. Epigenetic changes might provide the mechanistic basis for this metabolic memory effect[180]. Epigenetic enzymes require substrates of intermediate metabolism such as methyl and acetyl groups, which is the likely molecular mechanism by which environmental changes and nutrient availability can have a long-term influence on cellular behavior even after the insult is removed[181]. Animal model studies and some human observations indicate epigenetic differences in patients with DKD and CKD[179,182]. Targeting metabolism therefore represents a promising therapeutic strategy as: a) metabolic dysregulation is likely one of the most proximal mechanisms in diabetes and known to play a role in kidney disease development b) acute metabolic derangements likely have a long-term impact on the development of complications by epigenetic reprogramming and c) at later stages, insufficient energy availability is an important mechanism contributing to CKD development[180,183].

## SGLT2 inhibition

The sodium/glucose cotransporter 2 (SGLT2) is the major glucose renal re-uptake mechanism in the apical membrane of the proximal tubule (Figure 4)[184]. SGLT2 inhibitors have entered clinical practice as glucose control agents for diabetics, through their activity to inhibit renal glucose absorption and reduce hyperglycemia[185,186]. Studies in type 1 and type 2 diabetic mice show that SGLT2 inhibition not only reduces hyperglycemia but also has beneficial effects in DKD, including reduced glomerular hyperfiltration, renal hypertrophy and albuminuria, as well as decreased systolic blood pressure[187,188]. These findings were interpreted as being consistent with a critical role for suppression of tubulo-glomerular feedback in hyperfiltration in diabetes. By blocking glucose-coupled Na+ reabsorption, SGLT2 inhibition is thought to increase Na+ delivery to the macula densa, activating constrictor signals to the afferent arteriole, thereby reducing glomerular filtration pressure[189]. This proposed effect of SGLT2 inhibitors to reduce glomerular filtration pressure therefore overlaps with the mechanisms attributed to ACEi/ARB, although SGLT2 inhibition may target the afferent arteriole more selectively.

Like ACEi/ARB, a phase 2 trial of the SGLT2 inhibitor canagliflozin in type 2 diabetics with CKD showed an acute reduction in eGFR, followed by eGFR stabilization[190]. This was accompanied by a significant and dose-dependent reduction in albuminuria. To determine whether the observed decrease in albuminuria translates to improved outcomes in subjects with diabetic nephropathy, Janssen has initiated a phase 3 study of canagliflozin. The CREDENCE trial will enroll 3700 subjects at 531 sites globally[191]. The primary composite endpoint of the study includes end-stage kidney disease, doubling of serum creatinine, and renal or cardiovascular death. Results from this trial are expected in early 2020 (Table I).

## Lipid metabolism

Renal tubular and glomerular epithelial cells are high energy demanding cells with dense mitochondria. Their primary source of energy comes from fatty acid oxidation. Genetic mutations in mitochondrial enzymes have been shown to result in severe tubulointerstitial fibrosis and glomerulosclerosis (Figure 4)[192,193]. As non-transformed cells do not use glucose in high amounts, decreased fatty acid oxidation induces accelerated cell death and

dedifferentiation of the cells[183]. Nuclear receptors, including PPARA, PPARGC1A and LXR pathways have been shown to be key regulators of fatty acid metabolism in tubule cells[194]. Indeed, transgenic expression of PPARA was able to ameliorate CKD development in animal models[195]. Pharmacological activators of PPARA (mostly fibrates) have been in clinical use for treatment of serum lipid abnomalities for many years. Two clinical trials - FIELD and ACCORD - have examined the effectiveness of a PPARA agonist (fibrates) on kidney disease development, both reporting a statistically significant reduction in albuminuria development in diabetic patients (Figure 4)[196,197]. Unfortunately, fibrates raise serum creatinine levels, possibly by interfering with creatinine secretion, therefore determining their effectiveness in GFR decline based on serum creatinine levels cannot be directly studied. The follow-up wash-out study indicates that the GFR decline was statistically slower in the fibrate treated group[198,199]. Upstream targeting of the pathway at the level of AMPK or liver kinase B1 (LKB1), which is an important regulator of AMPK and downstream metabolism, might also offer therapeutic benefits (Figure 4)[200]. An AMP kinase agonist has shown significant benefit in mouse models of diabetic kidney disease and other forms of CKD[201].

### miR-21 antagonism

miR-21 is one of the first discovered and fairly abundant microRNAs, which like many other mRNAs, has pleotropic functions associated with PTEN, TGFβ and tumor suppression[202]. Mouse model studies have reported a protective role for miR21 antagonism in heart fibrosis development (Figure 4)[203204]. Moreover, small molecule antagonism of miR21[205] has shown a marked protective effect in several mouse fibrosis models[168]. MiR-21 in the kidney affects multiple metabolic pathways converging on PPARA and ultimately improving mitochondrial function and metabolism[205]. However, it is interesting to note that genetic deletion of miR-21 does not seem to recapitulate the effect of pharmacological inhibition[206]. Further studies are therefore needed to determine whether miR-21 could potentially be modulated in the treatment of kidney disease.

### Reactive oxygen species: NADPH oxidase inhibition

Increased generation of reactive oxygen species from mitochondrial sources has been proposed as a unifying hypothesis for the development of diabetic complications[207]. Both mitochondrial and plasma membrane NADPH oxidase contribute to ROS increase in diabetes[175]. In addition, inhibition of the plasma membrane NADPH oxidase has shown significant benefit in different rodent models of fibrosis[175]. The kidney specific NADPH isoform, NOX4, has recently received significant attention as specific NOX4 inhibitors appear to show benefit in mouse models of diabetic kidney disease (Figure 4)[208]. NOX4 inhibitors seem to be broadly effective in fibrosis as recent studies have also indicated beneficial effects in models of pulmonary and liver fibrosis[209–211]. In 2012, Genkyotex announced the successful completion of a Phase Ia study with GKT137831, a first in class inhibitor targeting NOX1 and NOX4 enzymes (Table 1)[208]. GKT137831 appears to be safe and well tolerated following oral administration. Phase II studies for diabetic kidney disease with GTK-137831 have been completed but results remain to be reported. Pyridorin (pyridoxamine) (NephroGenex) is a novel compound that is a derivative of vitamin B6, shown to target and reduce pathogenic oxidative chemistries[212]. However, a phase 2 efficacy

study failed to show a robust effect in subjects with diabetic nephropathy[213]. A Phase 3 trial (PIONEER) of pyridorin in patients with earlier stage diabetic nephropathy is ongoing (Table 1).

### Epigenetic targets/HDAC and cytosine methylation

The effect of acute metabolic changes often may not be detected until later stages of disease, as they can result in long-term epigenetic modifications (Figure 4). Several groups have reported differences in cytosine methylation levels between control and disease human kidney samples[214]. Increased methylation of the RASAL1 gene, which encodes an inhibitor of the Ras oncoprotein, has been associated with fibroblast activation and fibrogenesis in the kidney[214]. RASAL1 hypermethylation is mediated by the methyltransferase Dnmt1 and in mouse models of kidney fibrosis, genetic deletion of the Dnmt1 or pharmacological inhibition of the enzyme protected animals from kidney fibrosis development[214]. Studies from our laboratory have also shown that methylation differences target key profibrotic pathways, indicating that they are functionally important[182].

Histone modifications represent another important epigenetic regulator of gene transcription. Inhibitors of histone deacetylases (HDACs) have been reproducibly shown to ameliorate fibrosis development in different models (Figure 4)[215,216]. However, as these drugs broadly interfere with transcription, extended safety studies are needed to understand their side effect profiles.

## Targeting podocytes and albuminuria

The presence of albuminuria in a patient with diabetes and CKD currently fulfills the diagnostic criteria for diabetic CKD (Figure 2). Albuminuria used in conjunction with gender, GFR and serum phosphorus level is an excellent predictor of future glomerular function decline, therefore most Phase 2 studies use albuminuria as an intermediate outcome[7]. Human genetic studies of patients with rare monogenic diseases, Focal Segmental Glomerulosclerosis (FSGS) and Minimal Change Disease (MCD)[193] indicate that podocytes play a critical role in albuminuria and glomerulosclerosis, as almost all monogenic mutations are podocyte specific proteins[193]. Mouse genetic models have further substantiated the role of podocytes in proteinuria[169,217]. Furthermore, podocytes are unable to divide and they have limited (if any) capacity to be replaced[218]. The slit diaphragm which is a junction between podocyte foot processes, the glomerular basement membrane and the fenestrated endothelial cells, represent the key barriers to albumin leakage into the urine (Figure 2)[219]. Over the years, we have learned that the slit diaphragm acts as a signaling platform[220]. Proteinuric disease conditions are almost uniformly associated with foot process simplification of podocytes, caused by reorganization of the actin cytoskeleton[193]. These changes are identified as foot process effacement by histopathological examination. Nephrin clustering and endocytosis is associated with changes in the actin cytoskeleton, cell adhesion and endocytosis[221–223], playing a key role in proteinuria development. Although proteinuria and foot process effacement are reversible conditions, the critical point of no return is reached upon 20% podocyte loss, due to apoptosis, detachment or

dedifferentiation[224,225]. At this point glomerulosclerosis develops leading to a decrease in GFR.

### Abatacept

B7 is a membrane protein found on activated antigen presenting cells that can produce a costimulatory signal to enhance the activity of the T cell[226]. Interestingly, B7 expression is increased in podocytes in a subset of patients with FSGS, a relatively rare glomerular disease (Figure 2)[227]. Abatacept is a clinically approved inhibitor of B7 signaling for the treatment of rheumatoid arthritis, which acts by blocking its interaction with its receptors[228]. Abatacept treatment of 5 subjects with B7-positive FSGS resulted in a significant reduction of proteinuria, but the results were not replicated in other cohort[229]. Mouse model studies indicate that a similar mechanism might be present in models of diabetic kidney disease[230]. Larger clinical studies will be needed to determine the clinical benefit in these disease conditions, which are currently hindered by difficulties of reliable detection of B7 expression in kidney tissue samples[229].

### Actin-cytoskeleton

Recent reports indicate that Bis-T-23, a compound that promotes actin-dependent dynamin oligomerization and thus increases actin polymerization in injured podocytes, improves renal health both in transient and CKD models (Figure 2)[231]. Bis-T-23 has been reported to interact with the GTPase dynamin[222], which has been implicated in the maintenance of cellular architecture in podocytes, through its direct interaction with actin. Dynamin oligomerization appears to play an important role in actin polymerization and global organization of the actin cytoskeleton in the cell[231]. Further studies into the potential of this approach for the treatment of kidney disease are therefore warranted.

### Integrin inhibition

VPI-2690B (Vascular Pharmaceuticals) is a monoclonal antibody which binds to the C-loop domain sequence of integrin αVβ3[232]. This antibody has been shown to reduce albuminuria in diabetic rats and atherosclerosis in diabetic pigs[233,234]. It has been proposed that these effects are mediated by blocking the action of integrin αVβ3 to stimulate insulin-like growth factor-I (IGF-I) signaling in vascular smooth muscle cells and mesangial cells[232–234]. αVβ3 might also play role in podocyte and matrix interaction. VPI-2690B is currently in phase 2 clinical testing for the treatment of diabetic nephropathy[235]. This placebo-controlled trial will enroll 300 diabetic subjects and is projected to complete in August 2017 (Table 1).

## Additional agents in development

There are several other agents, which do not fall into the classes discussed above, that are also being clinically investigated for the treatment of chronic kidney disease.

### Phosphodiesterase inhibitors

Pentoxifylline, a methylxanthine derivative and non-specific phosphodiesterase inhibitor, has long been proposed as a therapy for diabetic nephropathy[236]. The recently completed PREDIAN study tested the effects of 24 months of pentoxyfylline treatment on proteinuria

and eGFR decline in 160 subjects with type 2 diabetes and nephropathy[237]. Subjects randomized to pentoxifylline experienced a marked reduction in albuminuria, but only a relatively small effect on eGFR. Similarly, in a preliminary report, Pfizer carried out a Phase 2 trial of the PDE4/5 inhibitor, PF-00489781, in 256 subjects with diabetic nephropathy[238]. Following 12 weeks of treatment, the urine albumin to creatinine ratio was significantly reduced compared to placebo. In addition, phase 2 testing of phosphodiesterase inhibition with CTP-499, a deuterated analog of 1-(S)-5-hydroxyhexyl-3,7-dimethylxanthine (an active metabolite of pentoxifylline), in patients with type 2 diabetes mellitus and nephropathy has been undertaken by CoNCERT pharma (Table 1). Although this trial has been completed, the full results have not yet been reported. Thus, while phosphodiesterase inhibition appears to have beneficial effects on albuminuria in ACEi/ARB treated patients, it remains to be determined whether these observations will translate into a benefit in renal outcomes and slowing of dialysis progression.

### Allopurinol

Clinical observational studies indicate a strong correlation between serum uric acid levels and the loss of kidney function in diabetic patients[239]. However, whether high uric acid levels actually cause renal function decline remains unclear. Two small clinical trials have provided proof of concept for using allopurinol to lower the risk of kidney function decline. One of the studies included only 51 subjects with high serum urate levels and although the other study had more than 100 participants, the average age in that cohort was unusually high[240–242]. It is therefore not yet clear whether these results can be generalized to all patients with DKD. A large NIH-funded clinical trial - Preventing Early Renal Function Loss (PERL) Consortium - has been formed to test this hypothesis[243]. This study specifically targets patients with type 1 diabetes and relatively preserved kidney function, who are at high risk for progression (Table 1).

### Sodium bicarbonate

One key function of the kidney is to excrete acids that are generated after burning proteins. In the urine, most protons are excreted as ammonia. Ammonia-genesis is decreased in early stages of CKD and many patients with CKD develop metabolic acidosis[26]. As most enzymes operate within a narrow pH range, even small changes could have a significant effect. Metabolic acidosis can activate the complement pathway, leading to low-grade persistent complement activation and inflammation[244]. Small clinical trials aiming to correct acidosis have indicated a benefit of treating patients with sodium bicarbonate[245]. Larger double blinded placebo controlled trials are currently being conducted to determine whether sodium bicarbonate treatment may slow progression of renal disease.

## Challenges in the development of novel therapies for chronic kidney disease

There are a number of challenges facing the development of new treatments for CKD. In particular, several factors must be considered in the design of future clinical trials, including issues regarding patient recruitment and selection, as well as the identification of appropriate endpoints and efficacy biomarkers. It must be noted that testing of novel therapies aimed at

slowing progression of proteinuric CKD must be performed in subjects already receiving the standard of care, including angiotensin converting enzyme inhibition or angiotensin receptor blockers.

## Disease awareness and clinical trial recruitment

The number of randomized controlled trials in nephrology is lower than that for almost any other medical sub-specialty[246]. The specific factors contributing to this gap are complex, but certainly[7,9] include lack of patient disease awareness and self-advocacy[247]. Research has shown that disease awareness in patients with CKD stages 1–3 is as low as 5% even in patients that have seen a physician within the last year[247–250]. Physicians may neglect mentioning to patients the fact that they have kidney disease, perhaps in part because they don't believe they can offer effective therapeutic options.

These issues undoubtedly contribute to major operational difficulties in executing trials designed to slow the progression of kidney disease to dialysis - especially low recruitment rates[251,252]. Overall recruitment rates for diabetic kidney disease trials approximate ~0.20 patients per site per month which is about a quarter of the number of patients enrolled per month for a diabetes trial. While the size of clinical trials may be similar to diabetes mellitus studies, the rate at which patients enroll into diabetic kidney disease trials significantly delays timelines, increases cost, and negatively impacts the willingness of major pharmaceutical companies to invest in these trials. Clinical trial networks and patient registries can significantly facilitate patient identification and recruitment rate in clinical trials for testing therapies. Although clinical trial networks for acute kidney injury have been established, chronic kidney disease trial networks including the two major causes of CKD (diabetic nephropathy and hypertension) are just now being initiated in Canada and Italy but have not included the US, the rest of Europe or Asia[248,253].

## Patient selection

Pre-screening of patients and clear inclusion and exclusion criteria, should balance the desire to eliminate likely biological non-responders that decrease trial efficiency with the need to rapidly accrue patients who are representative of the disease population. These design choices require a sophisticated understanding of the pathophysiology of the disease process. In some circumstances, biomarker-based target engagement can help drive such study enrichment, as exemplified by the significant impact of precision medicine approaches being implemented in the oncology field.

There is a growing appreciation of patient heterogeneity with respect to the rate of loss of renal function. Accordingly, efficient identification and enrollment of patients who are most likely to progress and benefit from the candidate therapeutic intervention remains a significant challenge. Recent results of the large Chronic Renal Insufficiency Cohort (CRIC) have indicated that when using a general patient population, it could take more than a decade to achieve hard end-points used in clinical trials[48]. This has raised the question as to whether trials should be focused on a subset of patients who follow a more rapid kidney function decline, considered "rapid progressors" (Box 1).

One key inclusion criteria used to enrich for CKD patients at high risk for progression has been a high level of proteinuria. Baseline proteinuria is a strong predictor of poor renal outcomes[254–256]. Furthermore the extent of treatment associated proteinuria reduction in Phase 2 studies was highly predictive of a favorable outcome[257258]. These observations have driven trials of new therapeutics to recruit patients with high baseline levels of proteinuria. However, with more widespread use of ACEi/ARB therapy, proteinuria levels are already decreased in many patients, and recruiting subjects with overt proteinuria is increasingly challenging in the implementation of renal trials.

Furthermore, clinical experience working with patients with minimal change disease indicates that proteinuria can be reversible and is not always associated with progressive kidney function decline[259]. There are increasing numbers of DKD patients who progress without developing overt albuminuria – perhaps due to ACEi/ARB induced proteinuria reduction. More than a decade ago, a study showed that close to 25% of subjects do not follow the "classic" paradigm for DKD progression, where micro albuminuria is followed by macroalbuminuria and progressive GFR decline[260].

The development of novel biomarkers that supplement proteinuria in predicting progression of renal disease in patients already taking ACEi/ARB comprises a new challenge for the renal research community. Recent studies indicate that levels of plasma inflammatory markers may have some predictive value[72, 73, 76] (Box 1).

## Clinical Trial Endpoints

Traditional drug registration trial endpoints in kidney disease include doubling of the serum creatinine, initiation of renal replacement therapy (dialysis, transplant) or death. Using the CDK-EPI equation, doubling of serum creatinine level is equivalent to a 57% decline in estimated glomerular filtration rate (eGFR) (Box 2)[261]. Several recent studies have explored the predictive validity of using lesser eGFR decrements (i.e. −30% or −40%) of ESRD events in CKD trials[7262–264]. These analyses support the strong predictive value of these intermediate eGFR decrements as surrogates of the currently accepted endpoints. Because there are greater numbers of these intermediate events, use of these surrogate endpoints could enable smaller and/or shorter trials[265]. However, adopting this design would have to match with the appropriate mechanism of action as many drugs (ACE and ARB) acutely reduce GFR while providing protection from ESRD after a longer-term follow-up[32].

While the data supporting the use of these intermediate surrogates is clear, the acceptability of these endpoints to regulatory agencies and payers has not yet been tested. Ultimately payers shoulder the disproportionate economic burden of CKD patients as they transition onto dialysis, however simply shifting the costs from dialysis (ESRD) to pre-dialysis care is not an attractive proposition. New therapies will not only need to demonstrate reduced ESRD events in the treated population, but that the patient number-needed-to-treat (NNT) to prevent one patient per year from going on to dialysis justifies the cost of that therapy. Perhaps the medical community has grown accustomed to accepting the wrong type of CKD therapeutic –one that slows disease, but does not remit disease.

## Conclusions and future directions

The need for development of novel therapeutics for CKD development has never been greater. While mortality has been dropping from most disease conditions, it has actually increased for patients with chronic kidney disease. No new drug has been registered for the treatment of chronic kidney disease since 2001. However, recent research has identified several potential novel therapeutic targets, including inflammation, fibrosis, cellular metabolism, vascular and podocyte alterations, with some related agents already undergoing Phase II and III clinical trials.

At present, most renal clinical trials continue to enroll subjects with stage 3 CKD and a relatively high level of albuminuria, as these are subjects who will likely reach trial end-point (death, dialysis or doubling of serum creatinine) the fastest. The SONAR study is the only study that uses a more individualized approach and enriches for subjects that are more likely to respond to treatment and less likely to develop side effects[77,266]. The targeted approach is only slowly gaining popularity. For example, drugs that target metabolism would likely need to be introduced early, which is a difficult issue as these trials require large cohorts studied for long durations.[188]. Other strategies, such as targeting inflammation, are likely to be effective even in later stages of the disease[267]. Therapeutics that specifically target fibrosis but not albuminuria are especially challenging as clinically validated biomarkers linked to decreased fibrosis are not available to assess efficacy in short phase II studies

Once an individual drug exhibits clinical benefit by delaying the progression of functional decline, it will be critical to determine whether drug combination strategies could achieve not only prevention but remission. In this regard combining hemodynamic targeting with, metabolic targets, podocyte specific targets and an anti-inflammatory might provide benefits above and beyond single drug treatments.

Opportunities in the field of “genetic medicine” are also emerging in the treatment of kidney disease. Two genetic diseases for which this represents a promising approach are polycystic kidney disease (PKD) and kidney disease associated with a mutation in Apolipoprotein L1 (APOL1) (Box 3). Tailoring of specific therapy combinations towards these genetically defined diseases may help optimize efficacy and reduce the number of patients needed to treat.

Cost remains a key limitation in the development of novel CKD therapies. Foremost among the development costs is the barriers to rapidly identifying and enrolling the appropriate patients into these trials. Patient registries and new trial designs may help address some of the issues discussed above, as well as validation of novel trial end-points. Adaptive clinical trial design - which evaluates patients' reactions to a drug beginning early in a clinical trial and modifies the trial in accord with those findings - might also be beneficial in CKD. Indeed, according to industry estimation this design can reduce the cost of entering a drug to the market to one third.

In summary, care of patients with CKD is emerging as one of most costly and rapidly increasing disease burdens facing society. Although drug development for CKD has lagged

behind other therapeutic areas, recent activity in this area has increased, due in part to recognition of this unmet medical need. Nevertheless systemic engagement of the pharmaceutical industry has been lacking, partly due to lack of success, operational difficulties (e.g. slow patient enrollment) and long duration outcomes trials. Early patient centric discovery approaches and broad academic, industry and patient advocacy partnerships can provide avenues to enhance patient therapeutic response, patient/physician disease awareness, and improve clinical trial efficiencies. Integrated engagement of scientists and stakeholders across the spectrum of medical delivery and payer systems is achievable but critical to stem the tide of kidney disease in the twenty first century.

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### Box 1: Should clinical studies focus on rapid progressors?

Kidney function decline in patients follows a highly heterogeneous pattern. Recent results of the large Chronic Renal Insufficiency Cohort (CRIC) Indicates that the mean GFR decline in patients with stage 3 diabetic CKD is around 1.5-3 cc/year[268]. However, a subset of patients follows a more rapid kidney function decline and can be considered "rapid progressors". Unfortunately, at present there is no consensus definition for "rapid progression". Expert consensus suggested the use of >5 cc/min/1.73m2/year decline[269]. Results from the CRIC cohort indicate that >3cc/min/1.73m2 is already 2 standard deviations above the mean GFR decline, therefore can be used to identify patients with rapid functional decline[268].

Multiple studies have been undertaken to identify clinical, biochemical and genetic markers that can detect rapid progressors. Recent studies indicate that in the stage 3–5 CKD group, the 4 variables: creatinine, proteinuria, phosphate and sex have C-statistics of 0.91 to predict ESRD[270,271]. This means that while it is interesting to identify new biomarkers, it will be challenging to meaningfully add to the identification of people who progress to ESRD especially in disease conditions associated with proteinuria.

Levels of plasma soluble TNFR1 and TNFR2 may potentially be used as predictors of renal failure in patients with either type 1 or type 2 diabetes[84,85,272]. An association between increased circulating TNFR levels and poor renal disease prognosis has also been observed in both IgA nephropathy and idiopathic membranous nephropathy[273,274]. Data suggests that circulating sTNFR could derive from exosomes shed into plasma[86], however the precise tissue and cells responsible for the increased circulating levels remain unclear. Increased urinary excretion and blood levels of kidney injury molecule-1 (KIM-1), also known as TIM1 and hepatitis A virus cellular receptor 1 (HAVCR1), have been associated with acute kidney injury, IgA nephropathy and diabetic nephropathy[275,77,78]. KIM-1 is relatively selectively expressed in liver and renal proximal tubule cells, and appears to play a role in modulating T cell immunity[276277,278]. Interestingly, treatment with the angiotensin receptor antagonist irbesartan reduces u-KIM-1 in patients with diabetic nephropathy[279]. Notably, recent studies have demonstrated that plasma KIM-1 also increases with AKI and importantly, plasma KIM-1 levels are predictive of progression of CKD in patients with type 1 diabetes mellitus[275]. The predictive value of these plasma inflammatory markers supports potential involvement of specific inflammatory pathways in the pathogenesis of diabetic nephropathy.

While identification of rapid progressors is an important endeavor, some critically important questions remain to be addressed. First, and most importantly, what drives rapid progression in these subjects? Are the pathways that drive rapid progression the same as those that drive moderate or slow progression, just with an increased magnitude or activation of different pathways that are responsible for rapid progression? Will trials actually benefit from enrichment strategies or will we be simply selecting for resistant, potentially less compliant subjects, where intervention will make less of an impact.

### Box 2: Trial Efficacy: measured versus estimated GFR

Although almost all-current treatment trials have used serum creatinine based estimated GFR (eGFR) to evaluate the beneficial effect of drugs[32], several studies have shown that estimated GFR does not correlate perfectly with tracer based measured GFR (mGFR)[280]. This correlation is thought to be particularly insufficient at higher GFR ranges, where creatinine based estimations performs very poorly. Several researchers therefore advocate for mGFR in clinical trials as a way to improve precision and thereby reduce cost[281]. While small single center studies reported variable results[282,283], investigation of the Chronic Renal Insufficiency Cohort (CRIC) cohort –a large multi-center trial that resembles most Phase 3 treatment studies[282,284,285] - showed that one-time measures of mGFR do not have stronger cross–sectional associations with concurrent metabolic consequences of decreased renal function (such as hyperphosphatemia or hyperkalemia) than eGFR[284], indicating that in this population mGFR is not a superior surrogate for CKD. Longitudinal measures of mGFR were also not more strongly associated with future clinical sequelae of decreased renal function than repeated measures of eGFR[285]. Considering the cost, the laborious nature of measuring GFR and the invasiveness of such techniques, mGFR should ideally provide superior prediction of clinically relevant CKD outcomes compared with estimated measures of renal function. However, iGFR did not outperform eGFR in this study or others. These results therefore do not appear to support the use of mGFR in order to improve kidney function change estimation, at GFR levels below 60 cc/min/1.73m2. Future studies might be needed to study this at higher GFR levels, however high GFR groups are not typically targeted for intervention trials.

## Box 3: Genetic stratification or precision medicine: are the nephrons ready?

The “genetic revolution” has transformed several areas of medicine, of which oncology has been the most prominent. Large collaborative efforts such as The Cancer Genome Atlas (TCGA), is enabling the systematic analysis of the genetics, genomics, epigenetics and proteomics of all cancer types, collecting hundreds of patient samples and associated clinical information[286].

Implementing such a collaborative team approach could be highly relevant for CKD, which is unlikely to be a homogenous disease and diagnosis is almost exclusively based on clinical description. Although kidney biopsy is not performed on everyone with CKD, the procedure appears to be increasingly safe and tissue samples are reasonably accessible.

Even though the genetic effect size is expected to be small in CKD, this does not mean that they do not highlight critically important and potentially targetable pathways. Two genetic diseases offer an important potential therapeutic opportunity in nephrology. The first is polycystic kidney disease (PKD), which is caused by a relatively common dominant mutation[287]. The mutation leads to epithelial cell proliferation and fluid secretion causing a cystic appearance of the kidney. Arginine vasopressin and its second messenger cAMP act as important promoters of cyst cell proliferation and fluid secretion into cysts[288].Recent clinical studies indicate that treatment with tolvaptan, which is a vasopressin antagonist, shows some clinical benefit in patients with PKD[289]. The second is kidney disease associated with a mutation in Apolipoprotein L1 (APOL1). APOL1 mutation has a high allele frequency in West African population and its diaspora, including African Americans. People with 2 risk alleles have 3–100 times increased risk of developing CKD, indicating a strong effect size, further highlighting its clinical significance[290]. As of yet, studies were unable to demonstrate increased disease risk in patients with only single APOL1 risk allele, indicating a recessive mode of inheritance[268,290]. Although most recessive mutations are loss of function variants, people who have APOL1 null mutations are phenotypically normal and do present with kidney disease[291], suggesting a recessive gain of function variant. While the mechanism of APOL1 induced cytotoxicity remains to be explored, we are presented with a unique therapeutic opportunity, whereby simply reducing APOL1 levels would likely be associated with therapeutic benefit. In this aspect it is important to understand the transcriptional regulation of APOL1. APOL1 appears to be part of the innate immune system and transcriptionally regulated by bacterial toxins and interferon[292]. As compounds that target those pathways are already in clinical development, some of these could potentially be repurposed for APOL1 associated kidney disease.

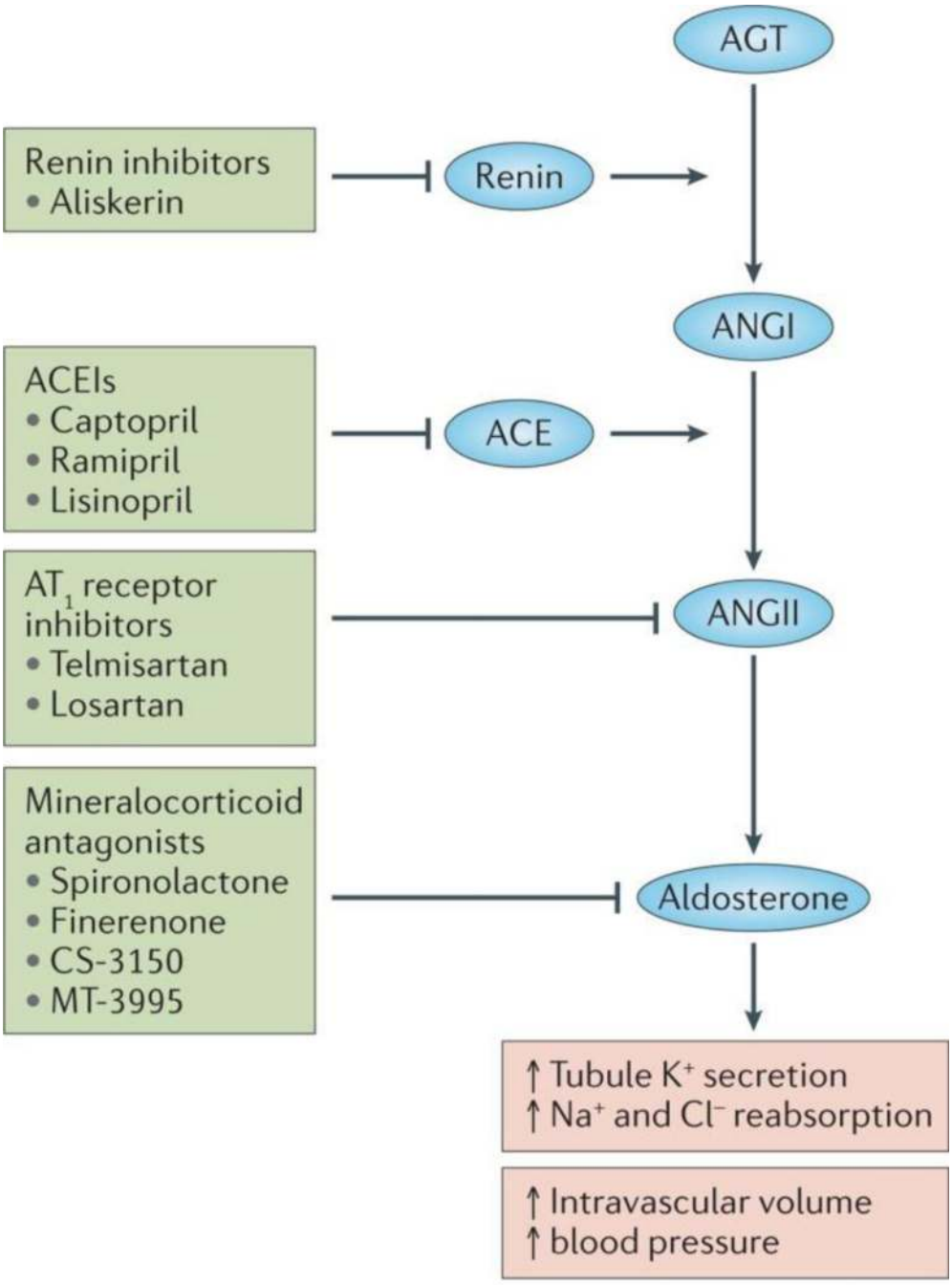


**Figure 1. Targeting the renin angiotensin aldosterone system**

Schematic representation of the renin angiotensin aldosterone system. Renin converts angiotensinogen to Angiotensin I and the angiotensin converting enzyme converts angiotensin I to angiotensin II. Renin inhibitors block renin, ACEI block ACE, AT1R block angiotensin action on the receptor while mineralocorticoid antagonists inhibit aldosterone.

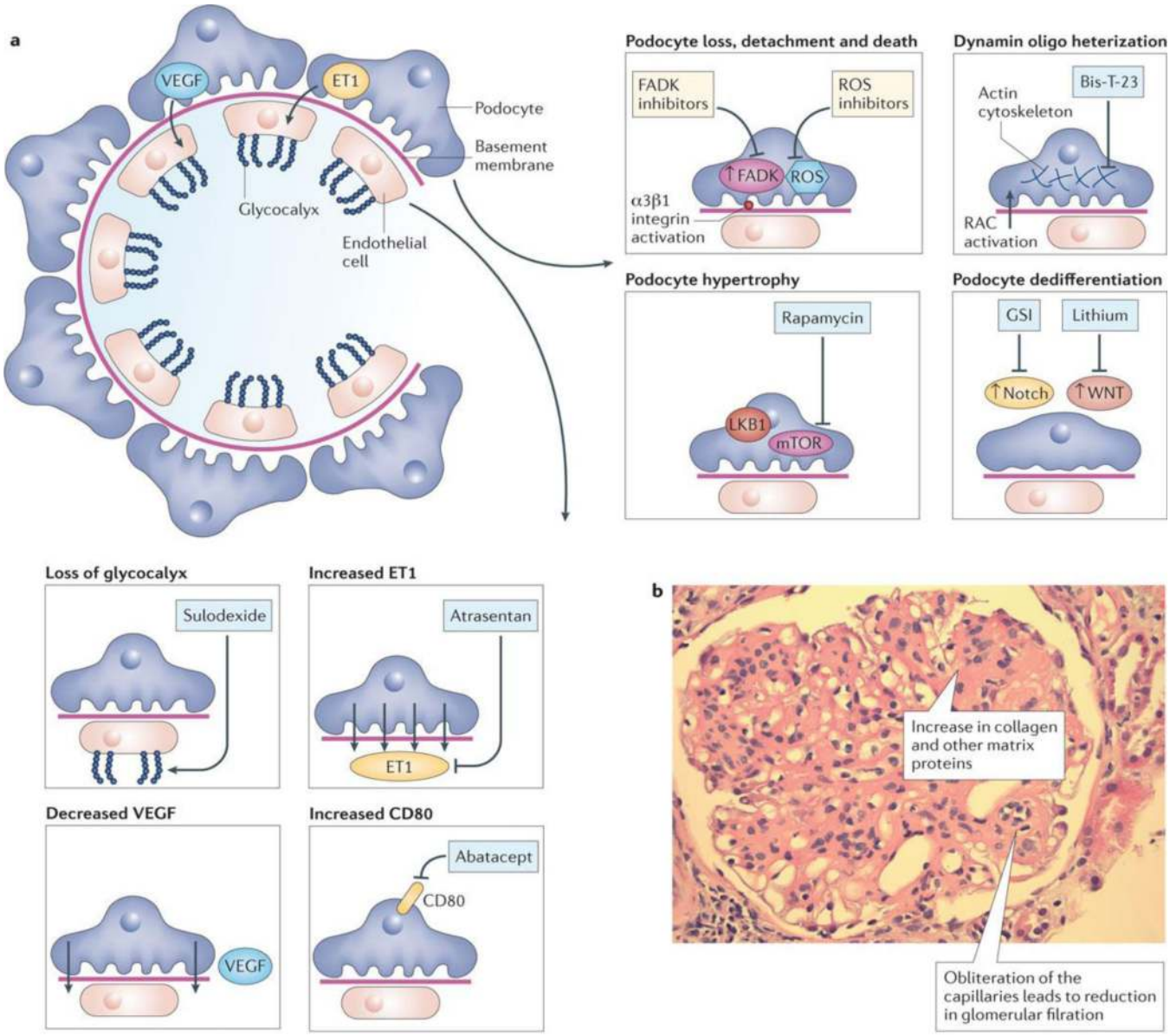


**Figure 2. Targeting the glomerulus in the treatment of CKD**

A. The left of the figure depicts the normal glomerulus with endothelial cells, glycocalyx, basement membrane and podocytes shown. The boxes illustrate the different abnormalities observed in CKD and potential therapeutic strategies and agents to target them. For endothelial cells, these include: loss of glycocalyx targeted by sulodexide, increased endothelin 1A signaling blocked by Atrasertan, decreased VEGF expression and increased B7-1 expression targeted by Abatacept. For podocytes, these include podocyte loss by detachment and apoptosis targeted by ROS inhibitors, FAK inhibitors or integrin inhibitors, increased dynamin oligomerization and cytoskeletal changes targeted by BisT23, Podocyte hypertrophy by increased mTOR and LKB1 targeted by rapamycin, and podocyte dedifferentiation by increased Wnt and Notch signaling targeted by lithium and gamma secretase inhibitors. B. PAS stained kidney sections from a patient with diabetic

glomerulosclerosis. Note the increased pink matrix materials, indicative of glomerulosclerosis and the obliterated glomerular capillaries.

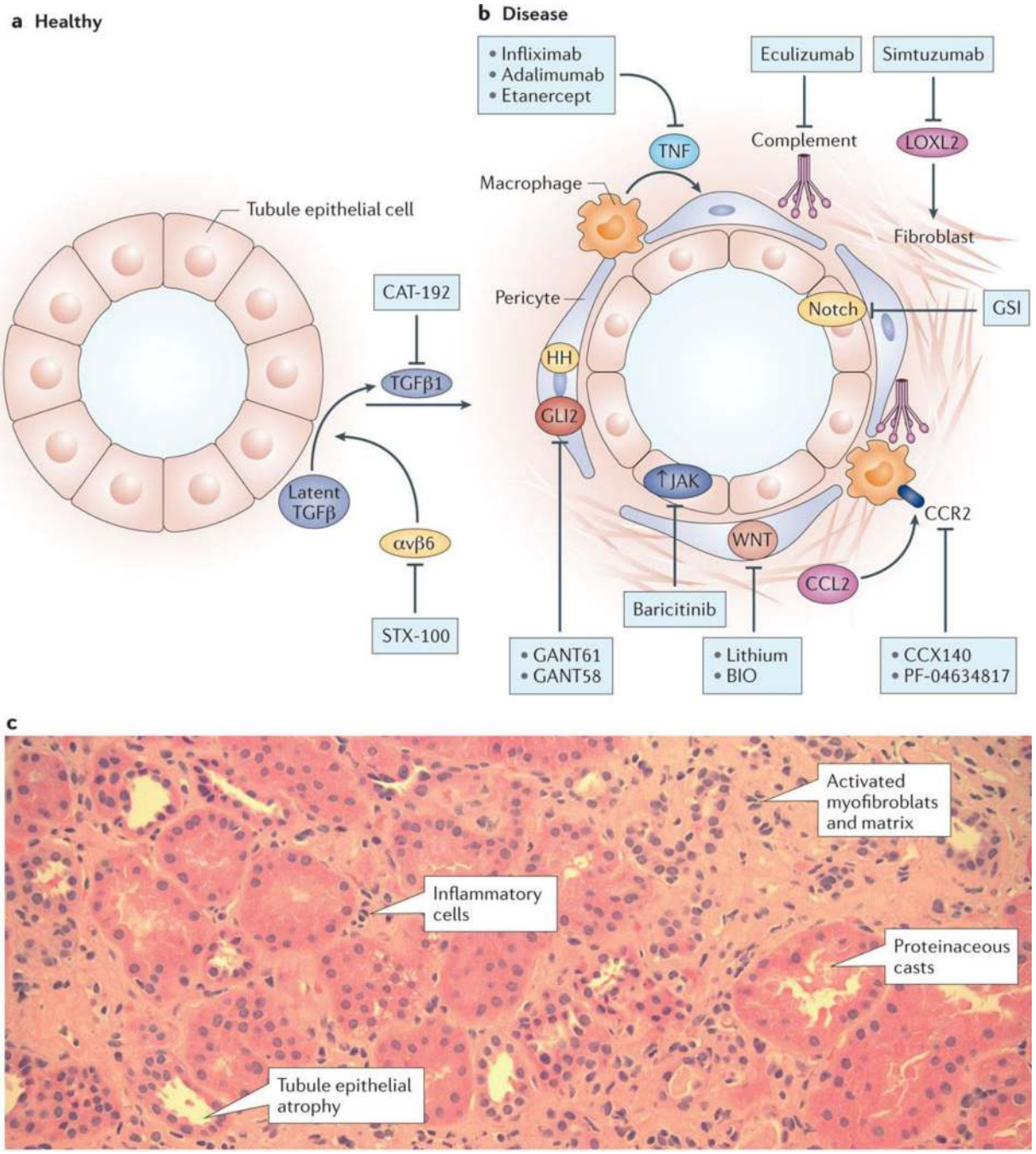


**Figure 3. Targeting tubulointerstitial fibrosis in the treatment of CKD**

A. Normal (healthy) renal tubule cross-section versus B. tubulointerstitium in CKD. Several therapeutic targets are shown including: increased TGFb signaling blocked by neutralizing antibody CAT-192, blocking latent TGFb by inhibiting alphavbeta6 with STX 100 antibody, tubule dedifferentiation and proinflammatory phenotype by increasing JAK and Notch signaling (blocked by gamma secretase inhibitor or baricitinib), increased TNFa and MCP1 levels, increased complement activation, collagen cross-linking by LoxL2, and increased GLi2 and Wnt signaling in fibroblasts leading to activated myofibroblast accumulation. B.

PAS stained human kidney section from a patient with diabetic kidney disease. Note the increased interstitial matrix, the increase in myofibroblasts and inflammatory cells and tubule cell dedifferentiation.

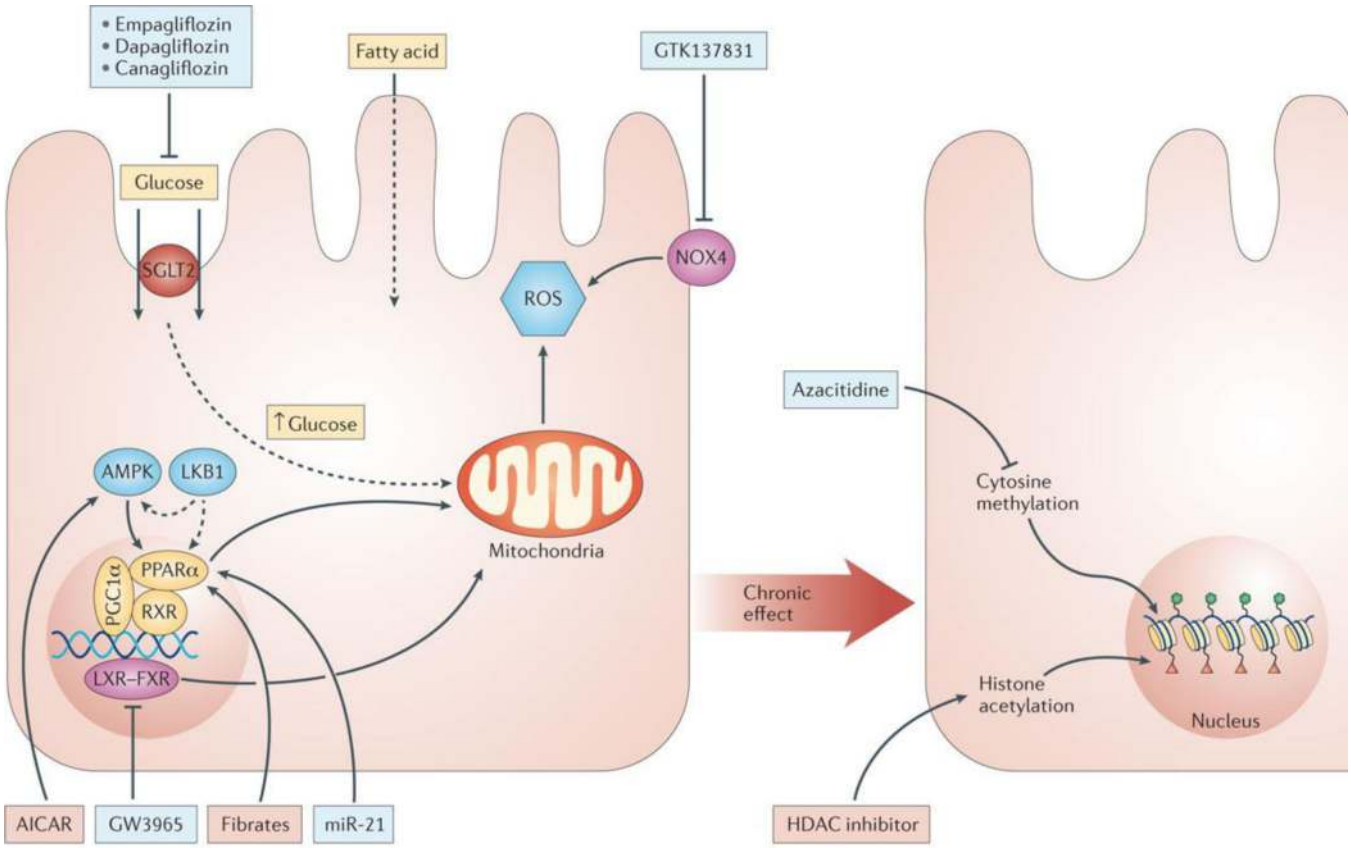


**Figure 4. Metabolic alterations in CKD**

Metabolic dysregulation, particularly poor glucose control, is associated with the development of diabetic nephropathy. Key factors contributing to disease development include increased SGLT2 mediated glucose uptake and increased mitochondrial and plasma membrane ROS generation . In addition, fatty acid oxidation is decreased due to loss of nuclear receptor activity of PPARA, RXR, PPARGC1A, LXR/FXR and their upstream regulator AMPK, LKB1 and miR 21. Effects of metabolic fluctuations are long lasting as they can influence the epigenome; cytosine methylation and histone acetylation. Agents being investigated, which target many of these alterations, including: empagliflozin, dapaglifozin and canagliflozin that block SGLT1, GTK137831 that blocks NOX4, 5Aza that blocks cytosine methylation, HDAC inhibitors that block histone acyltransferases, miR21 and fibrates that activate PPARA, and GW3965 that blocks LXR/FXR, are shown in the red and green boxes.

**Table 1**

Selected ongoing Phase II and Phase III drug development programs for the treatment of kidney disease

| Drug (company) | MOA | Hypothesized benefit | ClinicalTrials.gov identifier (name) | Trial information | Notes | Refs |
|---|---|---|---|---|---|---|
| Atrasentan (AbbVie) | $ET_A$ receptor antagonist | Haemodynamic | NCT01858532 (SONAR) | • Phase III<br>• Recruiting<br>• Enrolment (estimated): *n*=4148<br>• Completion (estimated): July 2018 | Primary outcome measure: time to the first occurrence of a component of the composite creatinine doubling or ESRD | 77,263 |
| Canagliflozin (Janssen) | SGLT2 inhibitor | Haemodynamic | NCT02065791 (CREDENCE) | • Phase III<br>• Recruiting Enrolment (estimated): *n*=3700<br>• Completion (estimated): January 2020 | Primary outcome measure: time to composite end point including ESRD, doubling of serum creatinine, renal or cardiovascular death | 189 |
| Pyridorin (NephroGenex) | Vitamin B6 analogue reducing advanced glycosylation end product protein modification | Antioxidant | NCT02156843 (PIONEER) | • Phase III<br>• Enrolment (estimated): *n*=600<br>• Completion (estimated): Martch 2018 | Primary outcome measure:time to the composite end point ( ≥100% serum creatinine increase or ESRD) | 290 |
| ASP8232 (Astellas Pharma) | VAP1 inhibitor | Anti-inflammatory | NCT02358096 (ALBUM) | • Phase II<br>• Recruiting<br>• Enrolment (estimated): *n*=110 | Study aims to evaluate ASP8232 as an add-on therapy to an ACEI or an ARB in reducing albuminuria in patients | 139 |

| Drug (company) | MOA | Hypothesized benefit | ClinicalTrials.gov identifier (name) | Trial information | Notes | Refs |
|---|---|---|---|---|---|---|
| | | | | • Completion (estimated): November 2016 | withT2D and CKD | |
| Baricitinib (Eli Lilly) | JAK1 and JAK2 inhibition | Anti-inflammatory | NCT01683409 | • Phase II<br>• Enrolment: *n*=131<br>• Completed | Outcome showed an ~30-40% decrease in UACR | 95,105,291 |
| CCX140 (ChemoCentryx) | CCR2 antagonist | Anti-inflammatory | NCT01447147 | • Phase II<br>• Enrolment: *n*=33<br>• Completed | A significant (24%) reduction in first morning UACR at 12 weeks was observed | 104, 292 |
| CTP-499 (Concert Pharmaceuticals) | Deuterium-containing pentoxifylline metabolite, a multi-subtype PDE inhibitor | Anti-fibrotic | NCT01487109 | • Phase II<br>• Enrolment: *n*=170<br>• Completed | Reportedly no significant change in UACR for patients taking CTP-499 compared with those on placebo at 24 weeks | 293 |
| Finerenone (Bayer) | MBA | Haemodynamic and anti-inflammatory | NCT01874431 | • Phase II<br>• Enrolment: *n*=821<br>• Completed | Awaiting final data. Primary outcome change in UACR from that at baseline to that at 90 days | 294, 295 |
| GKT137831 (Genkyotex) | NOX1 and NOX4 inhibitor | Antioxidant | NCT02010242 | • Phase II<br>• Enrolment (estimated): *n*=200<br>• Completed | Awaiting final data. Primary outcome 12-week change in UACR from baseline | 296 |
| VPI-2690B (Vascular Pharmaceuticals) | Monoclonal antibody to αvβ3 integrin | Inhibition of IGF1 signalling | NCT02251067 | • Phase II | Albuminuria reduction and eGFR preservation | 230,232 |

| Drug (company) | MOA | Hypothesized benefit | ClinicalTrials.gov identifier (name) | Trial information | Notes | Refs |
|---|---|---|---|---|---|---|
| | | | | • Enrolment (estimated): *n*=300<br>• Completion (estimated): August 2017 | during the 50-week trial duration | |
| GS-4997 (Gilead Sciences) | ASK1 inhibitor | Protein kinase inhibitor and anti-inflammatory | NCT02177786 | • Phase II<br>• Active, not recruiting<br>• Enrolment (estimated): *n*=300<br>• Completion (estimated): September 2016 | Change in eGFR from baseline at week 48. UACR reduction | 133 |
| PF-04634817 (Pfizer) | CCR2 and/or CCR5 antagonist | Anti-inflammatory | NCT01712061 | • Phase II<br>• Enrolment: *n*=226<br>• Completed | Percent reduction from baseline in UACR at week 12 | - |

ACEI, angiotensin-converting enzyme inhibitor; ARB, type 1 angiotensin II receptor blocker: ASK1, apoptosis signal-regulating kinase 1; CCR2. CC-chemokine receptor 2; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; $ET_A$ receptor, endothelin A receptor; IGF1, insulin-like growth factor 1; JAK, Janus kinase; MOA, mechanism of action; MRA, mineralocorticoid receptor antagonist; NOX, NADPH oxidase; PDE, phosphodiesterase; SGLT2, sodium–glucose co-transporter 2; T2D, type 2 diabetes; UACR, urine albumin/creatinine ratio; VAP1, vascular adhesion protein 1.