



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Anti-fibrotic treatments: A review of clinical evidence

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Anti-fibrotic treatments: A review of clinical evidence / Allinovi M.; De Chiara L.; Angelotti M.L.; Becherucci F.; Romagnani P.. - In: MATRIX BIOLOGY. - ISSN 0945-053X. - ELETTRONICO. - 68-69:(2018), pp. 333-354. [10.1016/j.matbio.2018.02.017]

Availability:

This version is available at: 2158/1204809 since: 2024-06-10T08:07:23Z

Published version:

DOI: 10.1016/j.matbio.2018.02.017

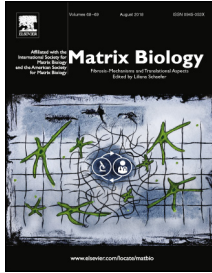
Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)



Anti-fibrotic treatments: A review of clinical evidence



Marco Allinovi^{a,b,c}, Letizia De Chiara^{a,c}, Maria Lucia Angelotti^{a,c},
Francesca Becherucci^b and Paola Romagnani^{a,b,c}

a - Department of Biomedical Experimental and Clinical Sciences “Mario Serio”, University of Florence, Florence, Italy

b - Nephrology and Dialysis Unit, Meyer Children’s University Hospital, Florence, Italy

c - Excellence Center DENOTHE, University of Florence, Florence, Italy

Correspondence to Paola Romagnani: Excellence Centre DENOTHE, University of Florence, Viale Pieraccini 6, 50139 Firenze, Italy. paola.romagnani@unifi.it
<https://doi.org/10.1016/j.matbio.2018.02.017>

Abstract

Renal fibrosis is a condition characterized by excessive extracellular matrix accumulation in the kidney. Representing the final common result of a variety of injuries, it can lead to chronic kidney disease and end-stage renal disease. Although major efforts have been made in understanding the process of renal fibrosis, attempts to halt its progression have been successful only in a laboratory setting with limited success in clinical practice. Here, we review the current knowledge on the process of renal fibrogenesis and the emerging anti-fibrotic drugs that have shown encouraging results in experimental models and were subsequently tested in clinical trials. We also propose possible explanations that may account for clinical trial failures and poor translation outcomes. Finally, we discuss alternative therapeutic options and future directions in which anti-fibrotic treatments may be coupled with drugs that can enhance endogenous tissue regeneration.

© 2018 Elsevier B.V. All rights reserved.

Introduction

Renal fibrosis is a dynamic and converging process that consists of different overlapping phases of the essential process of wound healing and that leads to the formation of excessive fibrous connective tissue in the kidney during a reparative or reactive process [1]. Indeed, following an initial insult, the affected kidney undergoes a series of events in an attempt to repair and recover from the damage. Initially, fibrosis maintains the three dimensional stability and functionality of the remnant nephrons, but in severe and/or chronic renal injury, fibrosis has been recognized to sustain pathological responses in the functioning units of the kidney. Major cellular events in renal fibrosis include infiltration of inflammatory cells; fibroblast activation and expansion; production and deposition of extracellular matrix (ECM) components; tubular atrophy and microvascular rarefaction (Fig. 1). Fibrosis can occur in the tubular interstitium, and is then referred as

“tubulointerstitial fibrosis” or in the glomerulus, where it is defined as “glomerulosclerosis”. In many aspects, major fibrogenic mechanisms are shared by different tissue compartments in the kidney. The established lesions of renal fibrosis have been considered to be permanent, but a reversal of glomerulosclerosis in patients with diabetic nephropathy has been previously reported [2], showing that human kidney has the potential to obtain a substantial architectural remodeling of the glomerular and tubular structures toward healing. Based on these observations, many efforts have been made to definitely understand the underlying pathways responsible for fibrogenesis [3], thus achieving remarkable insights in the identification of critical players and of molecular mechanisms that lead to the transcriptional activation of genes involved in the fibrotic process. Renal fibrosis is consistently associated with chronic kidney disease (CKD) which, with a very high prevalence among the population, poses one of the most serious health problems in current medicine as well as a serious economic burden to society [4].

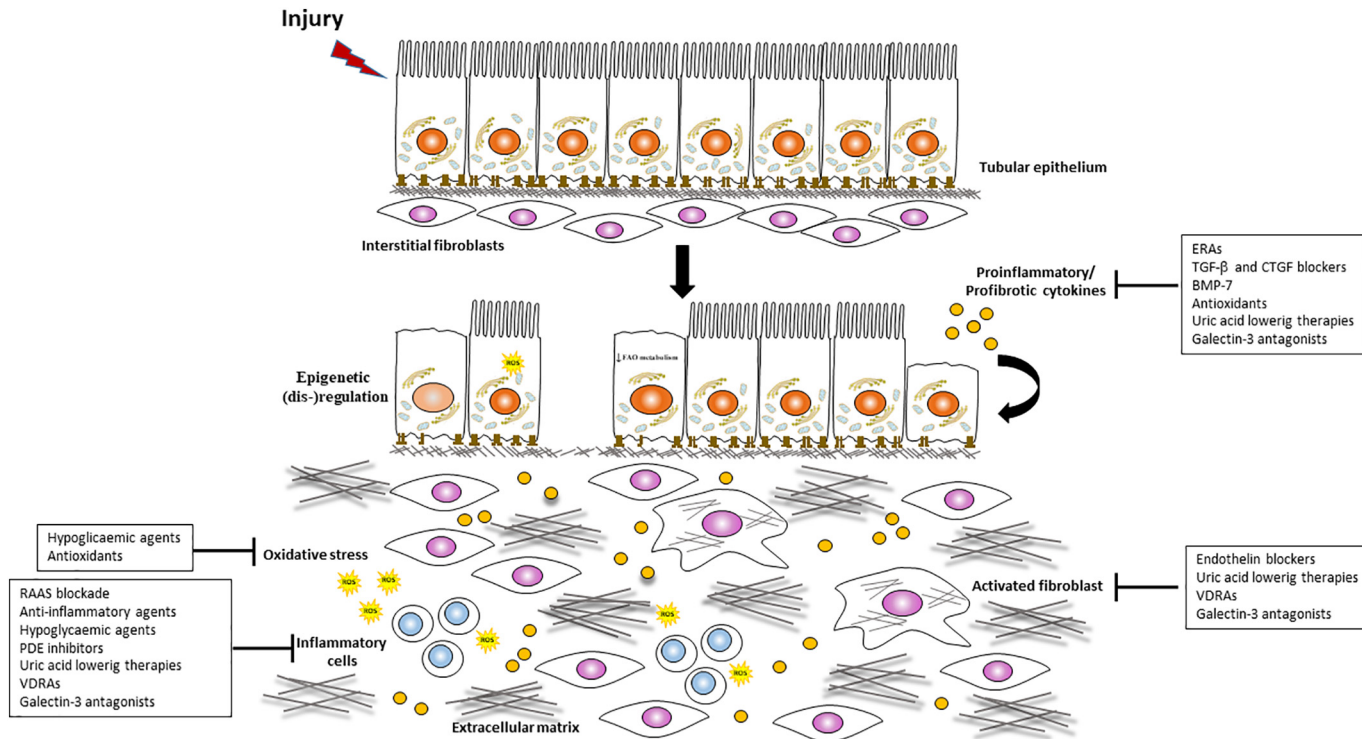


Fig. 1. Schematic representation of fibrosis progression. Summary of the different players involved in the process of fibrosis. The pharmacological interventions (boxes on the side) employed so far are represented in relation to the respective putative targets.

Therefore, blocking fibrosis and its progression appears as an attractive possibility to delay CKD progression.

Here, we review ongoing and concluded clinical trials focusing particularly on compounds with either a direct or an indirect anti-fibrotic effect (Table 1). We also review prematurely terminated clinical trials highlighting the adverse effects or the reasons for discontinuation (Table 2). Finally, we discuss the potential pitfalls and shortcomings in translational research and propose some potential solutions for future research and development.

Current anti-fibrotic strategy

Renin-angiotensin-aldosterone system (RAAS) is a recognized player in the pathogenesis and progression of renal fibrosis. Particularly, the renin-angiotensin system can directly control the production of the key pro-fibrotic mediators transforming growth factor (TGF)- β and reactive oxygen species (ROS) [5]. In experimental models, blocking the RAAS system not only interferes with the progression of renal failure [6], but it stimulates the regeneration of renal tissue [7–10]. During the last two decades, many large-scale randomized clinical trials confirmed the importance of inhibiting RAAS, along with lowering glomerular hyperfiltration and consequently proteinuria and blood pressure to preserve renal function and reduce mortality, in patients with CKD [11–15]. Currently, the standard therapy to treat CKD patients is to block RAAS using angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs), with an individually tailored anti-proteinuric treatment [16]. The most up-to-date guidelines recommended using RAAS blockers in patients with diabetic kidney disease (DKD) and non-diabetic kidney disease, particularly in patients with proteinuria. Single RAAS blockers cannot provide full blockade of the RAAS cascade, suggesting that dual therapy may have more benefit. Although this combinatory treatment of RAAS inhibitors showed higher efficiency compared to monotherapy, it is also associated with higher incidence of adverse events [17–19]. Indeed, combination therapy should be preferred to monotherapy, but should be administered with more caution in those patients with decreased renal function [20–22].

Despite the large use of RAAS inhibitors to treat glomerulosclerosis progression, further drugs are needed, particularly in patients with tubulointerstitial fibrosis and established fibrotic injury. For this reason, it is pivotal to explore new drugs and approaches that favourite renal fibrosis resolution.

Novel anti-fibrotic drugs

Several are the compounds proposed as possible anti-fibrotic drugs, due to their direct or indirect effect

on fibrosis (Table 1). Recently, a classification of the stages of renal fibrosis has been proposed on the basis of the molecular and cellular mechanisms underlying the process [1]. Taking advantage of this classification and in an effort to provide a comprehensive and easy-to-follow overview on the drugs currently under consideration, the following paragraph has been divided accordingly into the suggested overlapping phases of renal fibrosis: *priming*, *activation*, *execution* and *progression*. This sequence of events consists mainly of infiltration of inflammatory cells, fibroblast activation and expansion, ECM deposition, tubular injury and finally microvascular rarefaction (Fig. 1). Such a division is arbitrary, as in reality renal fibrogenesis is a dynamic process in which many of these events occur concomitantly, but this may help readers unfamiliar with the topic to fully understand this complex process.

Priming phase

Immediately after an injury, the inflammatory cells infiltrate the damaged area and following activation trigger the production of pro-fibrotic cytokines and growth factors by releasing ROS in the local microenvironment. This causes fibroblast activation and primes the activated fibroblasts to continuously sustain ECM deposition, thus making inflammation a primer that gives rise to tissue fibrogenesis.

Infiltration of inflammatory cells. As explained above, renal fibrosis is almost always preceded by the infiltration of inflammatory cells, including lymphocytes, monocytes/macrophages, dendritic cells and mast cells, attracted by the pro-inflammatory local microenvironment. Although inflammation is an integral part of the host defence mechanisms in response to injury, persistent inflammation always correlates with fibrosis progression in patients with both DKD and non-diabetic kidney disease [23]. Once infiltrated, inflammatory cells become activated and start to produce damaging molecules such as ROS and growth factors [24]. As a result, patients with high levels of inflammatory markers in their blood or urine are at higher risk of renal function decline, suggesting that chronic non-resolving inflammation is likely to play an important role in the development of renal fibrotic response. Treatment of subclinical inflammation with anti-inflammatory and immunosuppressive drugs may prevent progression of fibrosis and preserve renal function. The question of whether these interventions protect the kidney is undeniably intriguing. In the past years many novel anti-inflammatory drugs were discovered [25,26], showing that these agents may have secondary anti-fibrotic effects in CKD patients especially when used in conjunction with RAAS blockade agents.

Table 1. Novel anti-fibrotic drugs in clinical trials

| Phase | Group | Drug | RAAS blockade association | Kidney disease | Outcome | Status | Reference |
|-----------------|--|---------------------------------|---------------------------|---------------------------------|--|---------|----------------------------------|
| Priming phase | NFκB blockers | ACTHar gel | | DKD | Slowed the rate down of GFR decrease, reduced proteinuria | | 28 |
| | | ACTHar gel Bindarit | | DKD DKD | Reduced albuminuria | Ongoing | NCT01028287 29 NCT01109212 |
| | TNF-α blockers JAK-STAT inhibitors Chemokine antagonists | Adalimumab | | Primary FSGS | No effects on proteinuria reduction | | 33, 34 |
| | | Baricitinib | | DKD | Reduced albuminuria, no changes in kidney function | | 39 |
| | | Emapticap pegol PF-04634817 | | DKD | Reduced albuminuria No effects on proteinuria reduction or on kidney function | | 43 NCT01712061 |
| | Leukocyte migration inhibitors | CCX140-B | Yes | DKD | Reduced albuminuria | | 46 |
| | | Colchicine | | Renal transplant | Prevent the development of interstitial fibrosis | | 54 |
| | PPAR antagonists | ASP8232 | Yes | DKD, CKD | | Ongoing | NCT02358096 |
| | | Fenofibrate | | DKD | Reduced albuminuria, slowed the rate down of GFR decrease | | 68, 69 |
| | | Rosiglitazone | | DKD, non diabetic nephropathies | Reduced albuminuria and proteinuria | | 70, 71 |
| | NOX inhibitors | GKT137831 Selonsertib | | DKD DKD | No effects on albuminuria reduction | Ongoing | 75, NCT02010242 76 |
| | Nrf2 activator | Bardoxolone | | DKD, CKD | Slowed the rate down of GFR decrease, increased albuminuria | | 85, 93 |
| | | Bardoxolone | | Alport | Slowed the rate down of GFR decrease | | 86, 92 |
| | | Bardoxolone | | DKD, CKD | | Ongoing | NCT02316821 |
| | Activation phase | Endothelin receptor antagonists | Bardoxolone | | Alport | | Ongoing |
| Atrasentan | | | Yes | DKD | Reduced albuminuria | | 98, 99 |
| Atrasentan | | | Yes | DKD | | Ongoing | NCT01424319 |
| Avosentan | | | Yes | DKD | Reduced albuminuria | | 96 |
| Execution phase | TGF-β blockers | Sparsentan | Yes | FSGS | Reduced proteinuria | | 101 |
| | | TAK-044 | | CKD | No effects on GFR decline | | 95 |
| | | Fresolimumab | | FSGS | Slowed the rate down of GFR decrease | | 109 |
| | | Fresolimumab | | FSGS | No effect on proteinuria reduction | | 110 |
| | | Pirfenidone | | FSGS | Slowed the rate down of GFR decrease, no effects on proteinuria | | 113 |
| | CTGF blockers BMP-7 agonists | Pirfenidone | | DKD | No changes in kidney function | | 114 |
| | | Pirfenidone | | CKD | | Ongoing | NCT02408744 |
| | | Pirfenidone | | DKD | | Ongoing | NCT02689778 |
| | | FG-3019 | | DKD | Reduced microalbuminuria | | 115 |
| | | THR-184 | | CKD | No effects on GFR decline, reduced incidence of AKI | | NCT01830920 |

| | | | | | | |
|-------------------|-------------------------------|----------------|----------|--|--|-------------------------|
| Progression phase | Uric acid lowering drugs | Allopurinol | | CKD, DKD | No effect on proteinuria, blood pressure or serum creatinine | 121, 122, 123, 124 |
| | | Febuxostat | | CKD | Reduced albuminuria, no changes in kidney function | 125 |
| | SGLT-2 inhibitors | Febuxostat | | DKD | No effects on proteinuria or GFR decline | 126 |
| | | Empagliflozin | | CKD, DKD | Reduced albuminuria and slowed the rate down of GFR decrease | 128 |
| | | Canagliflozin | Yes | CKD, DKD | | Ongoing NCT02065791 |
| | DPP-4 inhibitors | Saxagliptin | | CKD, DKD | Reduced albuminuria | 135 |
| | | Saxagliptin | | CKD, DKD | | Ongoing NCT02462369 |
| | | Sitagliptin | Yes | CKD, DKD | Reduced albuminuria | 136 |
| | | Linagliptin | Yes | CKD, DKD | No effects on albuminuria | 144 |
| | | Linagliptin | | CKD, DKD | | Ongoing NCT02545738 |
| | GLP-1R inhibitors | Exenatide | | CKD, DKD | Reduced albuminuria | 137 |
| | | Exenatide | Yes | CKD, DKD | No effects on proteinuria or on GFR decline | 146 |
| | | Exenatide | Yes | CKD, DKD | | Ongoing NCT03029351 |
| | | Liraglutide | Yes | CKD, DKD | Reduced albuminuria | 138, 140, 141, 142, 143 |
| | | Liraglutide | | CKD, DKD | Reduced albuminuria | 139 |
| | | Liraglutide | Yes | CKD, DKD | No effects on GFR decline | 145 |
| | | Liraglutide | Yes | CKD, DKD | | Ongoing NCT02545738 |
| | Vitamin D receptor activators | Paricalcitol | Yes | DKD | Reduced albuminuria | 148 |
| | | vitamin D3 | Yes | CKD, DKD | Reduced proteinuria | 152, 153 |
| | Galectin-3 antagonists | GCS-100 | | CKD | Slowed the rate down of GFR decrease | NCT01843790 |
| | PDE inhibitors | Pentoxifylline | Yes | CKD, DKD | Reduced albuminuria | 163, 166 |
| | | Pentoxifylline | Yes | CKD, DKD | | Ongoing NCT03006952 |
| | | Pentoxifylline | Yes | CKD, DKD | No effect on albuminuria, slowed the rate down of GFR decrease | 168 |
| | | Pentoxifylline | | CKD, DKD | Reduced albuminuria | 164 |
| | | Pentoxifylline | | CKD, DKD | Slowed the rate down of GFR decrease | NCT01382303 |
| | | Pentoxifylline | | AKI | | Ongoing NCT02951299 |
| | | Pentoxifylline | Yes | CKD, DKD | Reduced proteinuria, slowed the rate down of GFR decrease | 165, 167 |
| Pentoxifylline | | Yes | CKD, DKD | No effects on proteinuria or GFR decline | 169, 170 | |
| Pentoxifylline | | Yes | CKD | No effect on albuminuria, slowed the rate down of GFR decrease | 162 | |
| Pentoxifylline | | Yes | CKD | Reduced albuminuria | 161 | |
| CTP-499 | | | DKD | Slowed the rate down of GFR decrease, no effect on albuminuria | 171 | |
| PF-00489791 | | | DKD | Reduced albuminuria | 172 | |

AKI, acute kidney injury; CKD, chronic kidney disease; CTGF, connective tissue growth factor; DKD, diabetic kidney disease; DPP-4, dipeptidyl peptidase 4; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; GLP-1R, glucagon-like peptide 1 receptor; JAK, Janus kinase; NFκB, nuclear factor-kappaB; NOX, NADPH oxidase; PDE, phosphodiesterase; PPAR, peroxisome proliferator-activated receptors; SGLT-2, sodium-glucose co-transporter-2; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor-β; TNFα, tumor necrosis factor-α.

Table 2. Novel anti-fibrotic drugs tested in clinical trials prematurely interrupted

| Phase | Group | Drug | Target | RAAS blockade association | Kidney disease | Status and adverse effects | Effects | Reference |
|--------------------------------|-----------------------|------------------------|---------------|---------------------------|------------------|--|---|----------------------|
| Priming phase | NFκB blockers | Bindarit | MCP-1 | | DKD, CKD | Discontinuation of clinical development of the drug | | 29 |
| | Chemokine antagonists | MLN1202 | CCR2 | | DKD, CKD | Prematurely stopped for unknown reasons | | NCT02410499 |
| | | BMS-813160 | CCR2/CCR5 | | DKD | Discontinuation of clinical development of the drug | | NCT01752985 |
| Activation and execution phase | Bardoxolone | Nrf2 | | Yes | DKD, CKD | Prematurely terminated for high rate of cardiovascular events | Did not reduce the risk of ESRD or death | 89 |
| | | Gevokizumab Atrasentan | IL-1β ET-1 | Yes | DKD DKD, CKD | Prematurely terminated | Prematurely stopped due to lack of efficacy | [211] NCT01858532 |
| | Integrin blockers | Avosentan | ET-1 | Yes | DKD | Trial stopped because of fluid retention | Reduced albuminuria | 97 |
| | | STX-100 | α5β6 integrin | | Renal transplant | Prematurely stopped for unknown reasons | | NCT00878761 |
| | TGF-β blockers | LY2382770 | TGF-β | | DKD, CKD | | Prematurely stopped due to lack of efficacy | 111 |
| | CTGF blockers | FG-3019 | CTGF | | | FSGS | Prematurely stopped for unknown reasons | |
| FG-3019 | | CTGF | | yes | DKD, CKD | Prematurely stopped for suboptimal study design | | NCT00913393 |
| Progression phase | Galectin-3 antagonist | GCS-100 | galectin-3 | | DKD | Discontinuation of clinical development of the drug because the Company was required to conduct additional chemical characterization | Mild increase of GFR | 154 |

AKI, acute kidney injury; CKD, chronic kidney disease; CTGF, connective tissue growth factor; TGF-β, transforming growth factor; DKD, diabetic kidney disease; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; PDE, phosphodiesterase; NFκB, nuclear factor-kappaB; MCP-1, monocyte chemoattractant protein-1; ET-1, endothelin-1.

NF- κ B blockers. Nuclear factor-kappaB (NF- κ B) comprises a family of transcription factors which have a central role in the expression of genes involved in cell mobilization, cell proliferation and cell differentiation, and, hence, in inflammation, repair and fibrosis processes [27]. ACTHar gel is a highly purified preparation of full-length adrenocorticotrophic hormone (ACTH). Previous studies demonstrate that ACTH inhibits NF- κ B activity and suppresses pro-inflammatory cytokine production. ACTHar gel in patients with advanced DKD stabilizes renal function and reduces proteinuria for up to 6 months after treatment [28]. These promising results led to a clinical trial on patients with DKD and nephrotic range proteinuria (NCT01028287). Bindarit, a new indole compound, downregulates the activation of specific NF- κ B dimers, reducing monocyte chemotactic protein (MCP) synthesis. A small phase 2 trial in patients with DKD (NCT01109212) reported that bindarit administration reduced albuminuria, but full results have not been published yet [29].

TNF- α blockers. Tumor necrosis factor- α (TNF- α) is a pro-inflammatory cytokine produced by a wide range of cells that can activate NF- κ B among others [30]. Elevated TNF- α levels are described in experimental models of renal diseases [31,32]. These findings support a role of TNF- α in mediating proteinuria and renal fibrosis and justify the use of anti TNF- α agents (such as adalimumab and etanercept). However, a phase 1 study [33] and the subsequent phase 2 study [34] in patients with primary focal segmental glomerulosclerosis (FSGS) showed no effects in proteinuria reduction.

JAK-STAT inhibitors. Prominently involved in the inflammatory response are the Janus kinases (JAK) and the signal transducer and activator of transcription (STAT) families of transcription factors. They are thought to mediate renal fibrosis in humans and animal models of different chronic kidney diseases [35,36] and their inhibition showed therapeutic potential in experimental models of fibrotic kidney diseases [37,38]. Considering that several pharmacological inhibitors of JAK2 are available and well tolerated, targeting JAK to treat renal fibrosis deserves careful consideration. Baricitinib, a JAK1/JAK2 inhibitor, is a promising drug for DKD. A phase 2 clinical trial showed that baricitinib resulted in a reduction of both albuminuria and renal inflammation. Despite this, no changes in kidney function at six months were detected [39].

Chemokine antagonists. Renal fibrosis is usually associated with interstitial leukocyte cell infiltrates, whose recruiting involves local expression of chemokines, that interact with respective chemokine receptors on the leukocyte's outer surface [40]. Blockade of chemokine receptors appeared as an effective therapeutic approach in reducing interstitial leukocyte accumulation and the subsequent renal fibrosis in different murine models [41,42]. Thus, specific chemokine receptor antagonists may represent an

attractive therapeutic concept also in humans. Emapticap pegol (NOX-E36) is a direct inhibitor of MCP-1 (CCL2), a chemokine produced by many cell types. The binding of MCP-1 to its receptor, chemokine receptor 2 (CCR2), stimulates the migration and translocation of monocytes. However, a phase 2 study in DKD patients showed that emapticap demonstrated non-significant but persistent reduction of albuminuria [43]. Several lines of evidence supports a role for CCR2-positive monocyte/macrophages in the pathogenesis of FSGS and type 2 DKD, and inhibition of CCR2 presents a potential therapeutic treatment, as supported by experimental animal models [44]. Although the dual CCR2/CCR5 antagonist, cenicriviroc, displayed anti-inflammatory and anti-fibrotic activity in a range of animal kidney fibrosis models [45], a phase 2 trial of PF-04634817, a CCR2/CCR5 antagonist, failed (NCT01712061). However, the CCR2 inhibitor, CCX140-B, showed renoprotective effects when combined with ACE inhibitors or ARBs in patients with DKD [46].

Leukocyte migration inhibitors. Colchicine accumulates in neutrophils and inhibits their adhesion and recruitment, in addition to disrupting intracellular traffic of additional inflammatory and fibrosis mediators [47]. For instance, it reduced TGF- β expression, apoptotic cell death and interstitial fibrosis in different animal models of renal disease [48–52]. Moreover, colchicine has also been used to treat many inflammatory disorders prone to fibrosis development [53,54]. Clinical trials to evaluate the effect of colchicine on diabetic and non-diabetic patients would be strongly advocated [47]. VAP-1 is a membrane-bound glycoprotein, expressed in the endothelium, which regulates leukocyte migration. A phase 2 trial is evaluating the VAP-1 inhibitor, ASP8232, given in association with RAAS blocking agents, on a primary endpoint of albuminuria in type 2 diabetes patients with CKD (NCT02358096).

Oxidative stress and mitochondrial dysfunction. Oxidative stress and mitochondrial dysfunction have been postulated to promote fibrosis. Indeed, infiltrated activated-macrophages produce ROS [55] which stimulates fibroblast activation and the release of pro-fibrotic cytokines, such as TGF- β , a key mediator of fibrosis [56]. In turn, TGF- β has been suggested to impair mitochondrial biogenesis of tubular epithelial cells (TECs) [57] leading to epithelial cell injury. Notably, an approach for improving the metabolic state of the kidney has been to promote activation of antioxidant transcription factors [58]. Antioxidant therapy in pre-dialysis CKD patients was suggested to prevent progression to end-stage renal disease and improve creatinine clearance [59].

PPAR antagonists. The peroxisome proliferator-activated receptors (PPARs), members of the super-family of ligand regulated transcription factors, control

mitochondrial function and biogenesis, fatty acid oxidation (FAO) and mitochondrial ROS generation, and consequently epithelial functions by mediating appropriate changes in gene expressions. PPAR maintains normal epithelial phenotype and antagonizes renal fibrogenesis, possibly via antagonizing oxidative stress and inflammation [60,61]. The activation of PPAR showed to ameliorate renal interstitial fibrosis in animal models [62–65], although the mechanisms are poorly understood. PPAR agonists are mainly used for lowering triglycerides and blood glucose levels. Fenofibrate and rosiglitazone, which are PPAR- α and PPAR- γ agonists respectively, exert a renoprotective effect via the downregulation of inflammatory cytokines and reduction of oxidative stress. Although the effects of PPAR- α agonists have not been fully investigated, and an increase in serum creatinine has been described in some patients [66], fenofibrate reduced albuminuria and slowed the rate down of glomerular filtration rate (GFR) decrease in two large randomized controlled trials of patients with type 2 diabetes [67–69]. Studies in experimental models and clinical studies suggested that glitazones may have favourable effects on renal disease progression, reducing albuminuria in diabetic and non-diabetic nephropathies [70,71], although it may increase the risk of cardiovascular events [72]. Since rosiglitazone is already employed for the treatment of diabetes mellitus, a deeper evaluation in clinical trials of its anti-fibrotic effect and safety profile in CKD patients is mandatory.

NOX inhibitors. NADPH oxidases (NOXs) are enzymes that produce ROS as primary products and may participate in renal fibrosis through their ability to modify lipids and proteins, damage DNA and activate transcriptional programmes [73]. NOX4, NOX2 and NOX5 are expressed in the kidney and are overexpressed in renal diseases. GKT136901 and GKT137831 are two structurally related compounds with preferential inhibition of NOX1 and NOX4 that have been evaluated in a wide range of disease models [74]. In a phase 2 trial in patients with type 2 diabetes and kidney disease, GKT137831 demonstrated an excellent safety profile but did not show any effect on albuminuria (NCT02010242) [75]. Activation of apoptosis signal-regulating kinase 1 (ASK1) has been shown to drive renal inflammation, apoptosis, oxidative stress, and fibrosis by the downstream activation of mitogen-activated protein kinases (MAPK) p38 and c-Jun N terminal kinase (JNK). Recent studies have shown that selective ASK1 inhibitors substantially reduced renal fibrosis and halted the disease progression in mouse models of DKD and HIV-associated nephropathy. Importantly, patients with type 2 DKD are currently being recruited for a phase 2 clinical trial investigating the effects of selonsertib (GS-4997), an ASK1 inhibitor [76].

Bardoxolone. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a central mediator of cellular responses to oxidative stress and showed a renoprotective role in

the development of tubulointerstitial fibrosis in different animal models [77–79]. Upregulators of the Nrf2 signaling pathway showed anti-fibrotic activities in experimental models [80–84], suggesting that Nrf2 might be a potential therapeutic target for renal fibrosis therapy. Bardoxolone methyl (BARD) is a synthetic antioxidant inflammation modulator, which induces the expression of antioxidant and cytoprotective transcription factor Nrf2, reduces the pro-inflammatory activity of the IKK-b/NF-kB pathway, increases the production of antioxidant and reductive molecules, and decreases the overall oxidative stress. Initially, BARD showed potential renoprotective actions in patients with CKD and type 2 diabetes mellitus, but also an increase in albuminuria [85]. A phase 2 trial demonstrated that improved kidney function was accompanied with very few adverse events [86,87], but a subsequent study showed an high frequency of adverse events in rats with overt type 2 diabetes treated by BARD [88]. A large phase 3 study was halted due to detrimental cardiovascular events and death in a number of patients [89,90]. Conceivably, these were most likely due to the intervention on Nrf2 signaling, a pathway which is nearly omnipresent throughout the human body. Furthermore, an editorial highlighted that the efficacy attributed to BARD in improving kidney function (based on creatinine values) was not produced by an anti-inflammatory effect on the kidney, but was probably a false positive effect of dramatic weight loss in that cohort [91]. A phase 2 trial in patients with DKD and low cardiovascular risk demonstrated an improved kidney function as measured by standard inulin clearance and reported mild adverse events [92]. An abstract recently presented at the American Society of Nephrology meeting, reported that a phase 2 trial in patients affected by Alport disease demonstrated improved kidney function, as measured by GFR, but an increase in albuminuria, with mild to moderate adverse events [93]. A subsequent phase 3 study (NCT03019185) is currently recruiting patients.

At present, no definitive conclusions can be drawn about the role of antioxidant drugs in preventing renal fibrosis and appropriately powered studies are needed to reliably assess their effects.

Activation phase

Following the initial priming events, accumulation of inflammatory mediators in the microenvironment post sustained renal injury, triggers the activation of ECM-producing cells, primarily fibroblasts.

Fibroblast activation. A central-driving event of renal fibrogenesis is fibroblast activation. During steady-state conditions, renal interstitial fibroblasts produce basal level of matrix components to maintain tissue homeostasis. Upon stimulation by a pro-fibrotic microenvironment, fibroblasts acquire an activated phenotype and

begin to produce large amount of ECM components (execution phase, see following paragraph).

Endothelin blockers. Endothelin-1 (ET-1) is a well-known pro-fibrotic factor and in the kidney is associated with fibroblasts expansion, via stimulation of endogenous TGF- β production. ET-1 stimulates the activation of two receptor subtypes: endothelin receptor type A and B (ETAR, ETBR), leading to podocyte and mesangial dysfunction, renal inflammation, oxidative stress, and consequently to proteinuria and glomerulosclerosis [94]. TAK-044, an ET receptor antagonist, had no significant effect on the glomerular filtration rate but tended to increase renal plasma flow, in patients with CKD [95]. Avosentan, in combination with RAAS blockade, reduced albuminuria in patients affected by DKD, with adverse effects reported in a small percentage of patients only [96], although in a phase 3 study it induced significant fluid overload and congestive heart failure [97]. Atrasentan, a selective ETAR antagonist, was shown to reduce albuminuria in patients with DKD treated with ACEIs or ARBs [98,99]. Currently, a phase 2 trial on atrasentan in type 2 diabetes is underway (NCT01424319).

In the past few years, a series of randomized controlled trials focused on endothelin receptor antagonists (ERAs), provided negative or inconclusive data, leading to termination due to safety concerns or lack of efficacy (NCT01858532) [97,100]. In fact, although ERAs reduce albuminuria in DKD, fluid overload and body weight increase due to fluid retention limit their use. The safety of these agents needs to be demonstrated in phase 3 trials, and then we cautiously await the results of future trials. There are complex interactions between the ET-1 and RAAS systems along with similarities in their renal physiological and pathophysiological actions that provide theoretical rationale for a combined inhibition. Accordingly, a recent phase 2 trial in patients with primary FSGS showed a greater reduction of proteinuria with sparsentan, which simultaneously blocks angiotensin II and ET-1, than ARBs [101].

Execution phase

Progressively, the activated fibroblasts secrete excessive amount of collagens and proteoglycans raising the stiffness of the organ and triggering a vicious circle that results in further fibroblast activation [102]. Increased stiffness and matrix deposition is likely needed to compensate for lost epithelial cells (see progression phase).

Matrix deposition. The deposition of ECM proteins, by activated fibroblasts, is mainly controlled at the level of gene transcription in response to various cytokines, prominent among which are TGF- β and CTGF [56].

TGF- β blockers. A prominent role in renal fibrosis is played by the TGF- β superfamily. This family of

cytokines acts primarily through the activation of Smad2 and Smad3 transcription factors [103], which represents the “canonical” pathway and through the activation of a “non-canonical” signal pathway independent from Smad activation. TGF- β is a well-recognized player in the progression of renal fibrosis [56] triggering deposition of ECM proteins such as collagen and fibronectin and stimulating mesangial hypertrophy [104]. While previous studies demonstrated that TGF- β antagonism by various anti-TGF- β antibodies prevents fibrosis in mouse models [105–108], clinical approaches based on these findings remain questionable. A phase 1 trial tested fresolimumab (human monoclonal antibody against TGF- β) in steroid-resistant FSGS [109], but the following phase 2 study failed to show any effect on proteinuria reduction [110]. An alternative TGF- β antibody, LY2382770, tested in combination with RAAS blockade, was prescribed in a phase 2 study in patients with DKD, but the study was terminated prematurely due to a lack of efficacy [111]. Pirfenidone, a drug with anti-fibrotic, antioxidant, and anti-inflammatory properties, interferes with the expression, secretion and the effect of TGF- β , although the specific mechanism is unknown. In animal models of FSGS, pirfenidone improved kidney function and proteinuria and reduced kidney scarring [112]. In clinical trials on FSGS or DKD patients, pirfenidone showed conflicting results failing to prevent proteinuria [113] or GFR decline [114], particularly in patients with renal dysfunction [112]. There are two clinical trials currently recruiting patients with DKD (NCT02689778) and CKD (NCT02408744).

CTGF blockers. Connective tissue growth factor (CTGF, also known as CCN2) is a pro-fibrotic factor directly regulated by TGF- β . An anti-CTGF antibody, FG-3019, was tested in two phase 1 trials in DKD (NCT00102297; NCT00754143). FG-3019 was well tolerated and led to a reduction of microalbuminuria [115]. Two following phase 2 trials (NCT00913393; NCT00782561) in subjects with type 2 diabetes and persistent proteinuria, however, were prematurely terminated for unknown reasons.

Integrin blockers. The $\alpha 5\beta 6$ integrin, which generates a localized activation of TGF- β , is involved in cell adhesion during remodeling of ECM in fibrosis. A phase 2 study in renal transplant patients with interstitial fibrosis and tubular atrophy with STX-100, a humanized anti- $\alpha 5\beta 6$ integrin antibody, (NCT00878761) was blocked prior to patient enrolment for unknown reasons.

BMP-7. Another member of TGF- β superfamily, the bone morphogenetic protein (BMP)-7, is critical for determining the final nephron number and size of the developing kidneys, and in preclinical studies, prevented TGF- β -induced kidney fibrosis [116]. The anti-fibrotic effects of BMP-7, however, remain controversial. A multicentre phase 2 study with THR-184 (NCT01830920), a BMP-7 receptor agonist, showed a reduction in the incidence of acute kidney injury (AKI),

especially in patients with underlying CKD. Other BMP-7 agonists are currently undergoing clinical testing in order to assess their ability to inhibit fibrosis progression and facilitate reversal of established fibrotic lesions through potential regenerative mechanisms [117,118].

A striking observation is the fact that, of all the compounds described above, those specifically designed to target known drivers of fibrosis yielded the most disappointing results while non-direct fibrotic agents showed more positive results. Surprisingly, blocking the expression/activity of TGF- β and CTGF, both very prominently involved in renal fibrosis, failed to ameliorate renal function in any meaningful manner. This important remark, would suggest that remission of fibrosis alone may not be sufficient to prevent kidney function decline.

Progression phase

Although the excessive accumulation of matrix components represents a key event in the development of fibrosis, it is most likely not the only driving force that stimulates its progression. As discussed in later paragraphs, fibrosis represents the normal response to wound healing and may not be the actual cause of renal failure. Beyond the importance of matrix deposition process, many cellular and molecular events prominently participate to the progression of fibrosis and the decline of kidney function. For this reason, the events that characterize this phase often determine the outcome of renal fibrogenesis.

Tubular injury. Several factors can induce tubular cell damage and, depending on the degree of injury, TECs display various range of responses, including proliferation, growth arrest, and ultimately death. Death is not the only outcome of tubular cell damage as viable progenitors and epithelial cells can be recovered from the urine of CKD patients [119], suggesting that cellular detachment contributes to epithelial cells loss [119] along with cellular death. Importantly, injured tubules are often characterized by tubular death/atrophy surrounded by interstitial fibrosis.

Uric acid lowering drugs. Hyperuricemia is independently associated with glomerulosclerosis and tubular atrophy. Uric acid can induce inflammation, endothelin-1 expression and fibroblast expansion through the activation of the TLR4/NF- κ B signaling pathway. In animal models uric acid lowering therapies showed promising results in reducing CKD progression [120]. Nevertheless, four randomized clinical trials with sparsentan in patients with CKD and DKD highlighted no effect on proteinuria, blood pressure or serum creatinine [121–124]. Another compound, febuxostat showed significant difference in the albuminuria levels compared to allopurinol in

CKD patients, but no significant changes were found in the levels of serum creatinine and GFR [125]. On the contrary, in overweight or obese adults with hyperuricemia and type 2 DKD, febuxostat had no significant effects on reduction of urinary TGF- β /creatinine ratio and albuminuria, and on GFR decline [126].

Hypoglycaemic agents. Emerging evidence suggests that some hypoglycaemic agents may have renoprotective effects independent of their glucose-lowering effects [127]. For instance, sodium-glucose co-transporter-2 (SGLT-2) inhibitors are a new class of anti-hyperglycaemic drugs that act by inhibiting SGLT-2 mediated glucose reabsorption in renal proximal tubules. These compounds were recently approved for type 2 diabetes treatment, but so far their use has been limited for the treatment of renal damage. Empagliflozin, a SGLT-2 inhibitor, coupled with standard RAAS blockade therapy, showed to decrease albuminuria and DKD progression with additional effects that cannot be directly explained by improved glucose control [128,129]. A recent study, presented at the 2017 American Society of Nephrology congress, showed that the suggested mechanisms include restoration of the tubuloglomerular feedback mechanism, lowering the glomerular hyperfiltration, suppression of the renal oxidative stress and angiotensinogen expression and finally reduction of the inflammatory and fibrotic marker expression [130]. However, a recent preclinical study showed no renoprotective effects in non-diabetic CKD [131]. A clinical trial assessing the anti-albuminuric effects of canagliflozin, another SGLT-2 inhibitor, in combination with RAAS blockade therapy, in patients with DKD is ongoing (NCT02065791). An evaluation of renoprotective effects in non-diabetic CKD in clinical trials is now mandatory. Among the most commonly used oral hypoglycaemic agents, the incretin-based agents like the agonists of glucagon-like peptide 1 receptor (GLP-1R) and the inhibitors of dipeptidyl peptidase 4 (DPP-4), an enzyme that degrades GLP-1, are novel blood-glucose-lowering drugs with a vast range of additional off-target effects. In several preclinical studies, these agents showed a renoprotective effect by reducing inflammation, fibrosis and blood pressure providing additional rationale for using DPP-4 inhibitors and GLP-1R agonists as anti-fibrotic drugs [132–134]. Indeed, clinical trials on DPP-4 inhibitors and GLP-1 agonists yielded for both classes conflicting results, with some of them showing to reduce albuminuria [135–143], while others had no tangible effect on albuminuria [144] or GFR [145,146]. Importantly, where a reduction in albuminuria was observed the effect was most likely independent from changes in glucose control. Currently, clinical trials evaluating linagliptin (NCT02376075, NCT01897532) and saxagliptin (NCT02462369) in the case of DPP-4 inhibitors and liraglutide (NCT02545738) and exenatide (NCT03029351) for the GLP-1 agonists are still on going. Simultaneous use of SGLT-2 inhibitors

combined with DPP-4 inhibitors, GLP-1R agonists, and especially the ACEI/ARBs may represent an effective strategy to slow down the progression of DKD [147].

Vitamin D. Proximal tubules are the site of active vitamin D synthesis [148]. Therefore, tubular atrophy could cause vitamin D deficiency. Vitamin D insufficiency is associated with inflammation and fibrosis, but it remains uncertain whether these anomalies are readily reversible with supplementation.

Vitamin D receptor activators (VDRA), calcitriol and paricalcitol, are available for the treatment of vitamin D deficiency. Beneficial effects of VDRA have been attributed to reduction of proteinuria by a direct protective effect on podocytes, subsequently resulting in reduced inflammation and fibrotic processes, as shown in experimental studies [149–151]. Although results from previous studies with VDRA in monotherapy have been conflicting, recent clinical trials confirmed the anti-proteinuric and anti-fibrotic capacity of VDRA [152,153], especially during RAAS blockade and/or sodium restriction [148]. This would suggest that the combination of RAAS blockade, dietary sodium restriction and VDRA may be a promising intervention to further retard GFR decline in CKD.

Galectin-3 antagonists. Galectin-3, a lectin expressed in the distal tubules and the collecting ducts, is upregulated in renal fibrosis and is produced by tubular cells, endothelium, immune cells, and myofibroblasts. A phase 1 study (NCT01717248) and the subsequent phase 2 study (NCT01843790) investigated in CKD patients the safety of GCS-100, a galectin-3 antagonist. GCS-100 reduced GFR decline and circulating levels of galectin-3. The same company sponsored three further phase 2 trials of GCS-100 (NCT02155673, NCT02333955, NCT02312050). One of these studies withdrawn before enrolment [154].

Microvascular rarefaction. The renal microcirculation is a plastic but precisely organized functional network. In the advanced stage of CKD, microvascular rarefaction is frequently associated to renal fibrosis. The mechanisms that account for capillary deficiency are multiple and often unclear. As many pathogenic processes occur simultaneously, abnormalities in the microcirculation may represent a trigger event rather than a mere consequence of fibrosis [155,156]. However, as this debate is beyond the scope of this review we chose to treat microvascular rarefaction simply as a result of fibrosis development.

PDE inhibitors. Phosphodiesterase (PDE) inhibitors represent a class of drugs traditionally used to treat erectile dysfunction and pulmonary hypertension. However, PDE-5 inhibitors demonstrated to reduce proteinuria, renal TGF- β expression and glomerulosclerosis in experimental models [157,158]. Early treatment with a PDE-5 inhibitor reduced histologic damage and proteinuria and preserved renal capillary

integrity, while delayed treatment failed to demonstrate such protection [159]. Indeed, recent evidence suggests that PDE type 5 inhibitors may have unexpected renoprotective effects [160], probably due to their vasodilation and anti-fibrotic properties. Pentoxifylline (PTX), a phosphodiesterase inhibitor already in clinical use, exhibits prominent anti-inflammatory, anti-proliferative and anti-fibrotic activities. There are several clinical trials on renoprotective potential of PTX, and two trials are currently recruiting patients (NCT01377285, NCT02951299). PTX was reported to delay GFR loss in patients with CKD stage 3–4 both in monotherapy (NCT01382303) and when associated with RAAS blockade [161,162]. Studies in DKD, highlighted that PTX can significantly provide additive anti-proteinuric effects which are not linked to the decrease in blood pressure or improvement in glycaemic control [163–166]. Moreover, a lower decline in GFR was observed in two clinical trials in type 2 diabetes patients [167,168]. Two studies showed conflicting results, and no effect on proteinuria was detected in patients with DKD [169,170]. The majority of published studies have been limited by small sample size and short observation periods, and mild gastrointestinal adverse effects were described in a relatively high percentage of patients. From a phase 2 trial in type 2 diabetes patients, the PTX derivative CTP-499 failed to meet the primary endpoint (change in albuminuria) [171], but a promising effect on serum creatinine was observed. In addition, PF-00489791, a long-acting PDE type 5 inhibitor, in a phase 2 clinical trial in type 2 diabetes patients, showed significant reduction in albuminuria [172]. However, current evidence suggests that PTX, especially when used in combination with RAAS blockade, may effectively slow the progression of kidney disease.

In conclusion, although numerous anti-fibrotic drugs have been tested in clinical trials, there are very few that have moved from the bench to the bedside. This may suggest that a multi-pharmacological approach involving several anti-inflammatory and anti-fibrotic molecules may need to be employed.

Problems and pitfalls

Several reasons may explain why many promising new compounds have failed so far. In the following paragraph, we discuss a series of possible explanations that may potentially account for clinical trial failures including: (1) clinical heterogeneity, (2) genetic heterogeneity, and (3) epigenetic-related heterogeneity predisposition.

Clinical heterogeneity

To optimize its effects, an ideal therapy aimed at slowing down the progression of renal fibrosis should include a multi-faceted and diversified therapeutic

approach, which should act on the specific phase of progression and underlying aetiopathogenetic mechanism. Therapy that may be advantageous at one phase of this progression pathway could be deleterious or ineffective during other phases. In this context, patients at early stages of CKD and patients with subclinical markers of renal fibrosis should be considered for clinical trials, instead of cohorts of patients with multiple comorbidities and different underlying pathogenic processes. However, most of the clinical trials on anti-fibrotic therapies included either a non-homogeneous population of patients with CKD, non-biopsy proved DKD, or FSGS, and this might have modified the anti-fibrotic drug effects. Both FSGS [173] and DKD [174,175] are complex and multi-faceted conditions that can also be described as syndromes with varying different underlying pathophysiological mechanisms, clinical manifestations, rate of progression, and responses to therapy. Whether fibrosis itself actually causes kidney failure or whether fibrosis represents an epiphenomenon of the underlying pathogenic processes remains an open question. However, fibrosis is a reliable predictor of progression of kidney disease and should be therefore characterized and used as the end point of studies which analyse potential treatment targets. Many uncertainties still remain about how to best measure renal fibrosis [176]. Currently, fibrosis can only be assessed specifically by renal biopsy. Potential sampling errors due to the focal nature of renal fibrosis in many diseases and the invasive nature of the renal biopsy procedure limit this approach. Kidney fibrosis, especially when slight or moderate is poorly captured by GFR or albuminuria, which are generally considered as primary end points in almost all anti-fibrotic CKD trials. None of these parameters accurately reflect fibrosis. Several biomarkers of renal fibrosis have been identified, but their utility is limited, none is yet in clinical use and only few of them are considered as intermediate end points in clinical trials [177–181]. Sensitive early and non-invasive biomarkers of fibrosis are needed, which should replace the invasive renal biopsy procedure for assessing and staging histological fibrotic changes, revealing the underlying biological processes, predicting progression of nephropathy and correlating with anti-fibrotic therapeutic efficacy.

Genetic heterogeneity

One possible explanation for poor clinical trial results may also be represented by genetic heterogeneity. Recently, genome-wide association studies (GWASs) made possible to analyse the association of disease traits with single-nucleotide polymorphism (SNP) frequencies suggesting that SNPs may be key factors in the development of fibrosis and CKD progression [182]. This implies that, although the clinical signs of CKD development are the same, the pace of CKD may differ between

individuals as a consequence of differences in the genetic landscape. Almost all risk variants associated with CKD development are localized in noncoding regions of the genome, hypothesizing that these polymorphisms could potentially influence the expression of close genes by altering transcription factor binding. Particularly striking is the case of the *UMOD* gene that is close to two genes associated with fatty acid metabolism, *ACSM5* and *ACSM2* [183], implying that fatty acid oxidation (FAO) may be dysregulated in patients bearing SNPs in this gene. The *UMOD* gene was linked to variable risk for CKD, fibrosis and hypertension in humans. Another unique example is represented by the two coding sequence variants (G1 and G2) of the *APOL1* gene which are strongly associated with FSGS as well as nephroangiosclerosis [184,185].

In summary, whole-genome sequencing (WGS) studies highlighted that fibrosis can be linked to an array of genetic polymorphisms that may critically contribute to the individual progression rates of CKD [186]. Future therapeutic directions should aim at personalized medicine which would take into account individual genetic predispositions for patients' stratification and treatment (Fig. 2) [187].

Epigenetic-related heterogeneity

Another potential confounding factor in clinical trial setting may be represented by the heterogeneity of the recruited patients related to epigenetics. The term epigenetic summarizes all those heritable processes that affect gene expression without causing changing in DNA sequence [188]. There are various levels of epigenetic remodeling, a first level is represented by DNA methylation, while post translational modifications of histone proteins represent a second level. Accumulating evidence shows that alterations in the epigenetic landscape of renal cells at all levels play an important role in the pathogenesis of fibrosis, by regulating fibroblast activation and matrix deposition [189–194]. For example, sustained TGF- β expression irreversibly imprints interstitial fibroblasts and causes hypermethylation of the *RASAL1* promoter [189]. Along with promoting DNA methylation, augmented levels of TGF- β expression induce alteration in histone marks, in mesangial [192] and renal epithelial cells [193]. Upon TGF- β treatment, SET7/9 histone methyltransferase is upregulated and recruited at the promoter of pro-fibrotic genes, enhancing the methylation levels of the lysine 4 on the histone H3 (H3K4me) [192]. Recently, Mitochonic Acid 35 (MA-35), a new indole derivate, has been shown to attenuate renal fibrosis by blocking mono-methylation of H3K4 (H3K4me1) [194], which marks an active state of chromatin [195]. This is particularly interesting as MA-35 has been reported to have a strong effect on mitochondrial biogenesis and rescued mitochondrial disease-derived fibroblasts [196].

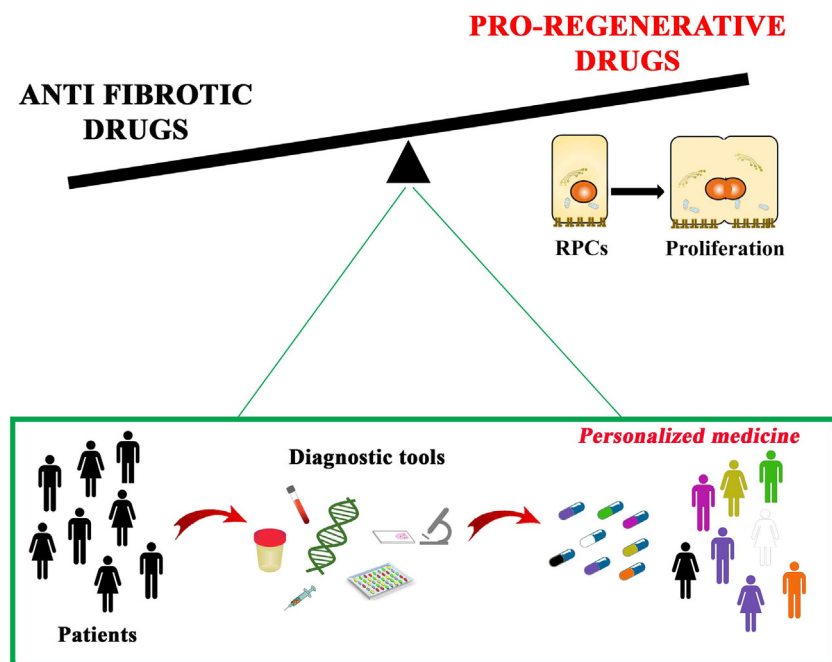


Fig. 2. Future directions and strategies. Emerging evidence suggests that single-nucleotide polymorphisms may be key determinants of CKD progression. Future strategies should aim at favouring endogenous regeneration of the nephrons while taking into account the individual genetic variability.

The role of epigenetic modifications and remodeling in renal fibrogenesis is still poorly understood but some evidence suggests it may significantly impact on the individual heterogeneity of CKD progression.

Alternative strategies and future perspectives

While many anti-fibrotics have failed to realise their potential in clinical trials, showing no or limited ability to ameliorate progressive renal disease, there are still a few candidate compounds awaiting the outcome of phase 3 clinical trials which may yet yield some promising results. Remarkably, the compounds showing a certain degree of success to date, have been those that act during the early phases, prior to the establishment of clinically observed fibrosis. This would suggest that blocking pro-fibrotic pathways or molecular events that drive fibrosis, such as inflammation, once it is already established may not be sufficient to halt its progression and/or reverse it.

Fibrosis is activated after any type of tissue injury, as part of the essential process of wound healing. Evolutionarily, the process of scarring may guarantee the preservation of an intact three-dimensional tissue architecture when the nephrons are lost following damage, supporting the survival of healthy or only partially injured nephrons [197]. Among the first to note such an association was the group of Hishida A. and colleagues who found that scar tissue accumulates around the most injured tubules [198,199]. It is important to note that the mammalian

kidney, unlike other lower organisms, such as fish [200,201], do not undergo nephrogenesis throughout adulthood [202] and its ability to generate new nephrons is limited to a small window during development. In light of these considerations, we should consider whether a therapeutic approach based exclusively on fibrosis removal would be sufficient or even beneficial to favour kidney survival unless it is coupled with appropriate tissue regeneration. Recently, a population of renal progenitor cell (RPCs), a subset of cells endowed with stem cell properties, was identified at the urinary pole of Bowman's capsule and scattered along the tubules in adult human and mouse kidney [203–205]. Experimental evidence suggests that pharmacological enhancement of endogenous regenerative capacity of RPCs can revert kidney diseases. For instance, glomerular regeneration can be enhanced by employing drugs like the Glycogen Synthase Kinase 3 β (GSK3 β) inhibitor that stimulates differentiation of RPCs into podocytes and results in a reduced formation of glomerular scars thus influencing the course of CKD [203]. Particularly, in the context of FSGS, disease remission or progression is determined by the amount of regenerated podocytes and podocyte regeneration is associated with proteinuria remission [203]. This is particularly important as proteinuria was found to impair RPC regeneration by blocking differentiation of RPCs toward podocyte lineage [206]. In animal models, ACE inhibitors, the class of drugs that showed the most encouraging results in clinical practice, reduced proteinuria and promoted the regression of

glomerulosclerosis along with increasing the number of podocytes [7], indicating that they stimulate some level of endogenous regeneration [8–10]. Remarkably, ACE inhibitors appear to promote regeneration on numerous levels, not only by increasing the number of podocytes [7, 10], but also by acting on renal vasculature [8] and moderating progenitor cell activation [9]. In the context of DKD, leptin replacement, but not RAAS blockade, has been shown to restore lost podocytes, thus promoting repair of the diabetic kidney, further suggesting that restoration of podocyte number is indeed critical [207] for regeneration.

Therapeutic strategies to promote regeneration may be extended to tubulointerstitial injury. Indeed, our group recently demonstrated that the regenerative capacity of the renal tubules is limited and observed a residual TEC loss after AKI, implying irreversible nephron loss [204]. Critically, we described that renal response to AKI involves two crucial mechanisms: 1) survival and proliferation of tubular progenitor cells that support partial TEC regeneration and 2) endocycle-mediated hypertrophy of remnant TECs to sustain the renal function despite significant loss of renal mass. Importantly, endocycle is a cell cycle variant in which cells pass through successive rounds of S and G phase without cytokinesis resulting in cells with one polyploid nucleus [208]. The biological rationale for increasing genome content appears most commonly to be to increase cell size as well as facilitate amplified cell metabolism. This implies that, physiologically, endocycling cells sustain a temporary functional recovery of the organ that is not accompanied by structural recovery (that should be sustained by tubular progenitors) leading in the long term to nephron loss, fibrosis development and ultimately CKD. The treatment of mice with HDAC (Histone deacetylase) inhibitors, compounds previously reported to increase TEC proliferation, following AKI, stimulated the proliferation of endogenous progenitor cells and resulted in a sustained recovery of GFR, a better reconstitution of tubular integrity and a relevant reduction of fibrosis [204]. These results, along with those of other groups [209,210], add strength to the idea that compounds capable of improving RPC regenerative potential may significantly reduce renal fibrosis and provide convincing evidence that it is possible to modulate the intrinsic regenerative potential of the renal tissue.

Conclusions

Over the last years, many clinical trials have been undertaken aiming at blocking and/or reversing CKD development. Many of these trials failed, possibly for reasons related to the high clinical, genetic and epigenetic heterogeneity of recruited patients, and likely, because removal of fibrosis alone is not sufficient to restore renal functionality in the absence of restoration of lost nephron tissue. Consistently,

promising results were achieved when fibrosis was targeted at the early stages and on multiple levels, before the scar tissue had replaced the lost nephrons. This may suggest that a multi-pharmacological approach should involve several targets on different pathways, phases of progression and underlying aetiopathogenetic mechanisms of fibrosis as well as promoting regeneration. Indeed, experimental evidence supports the possibility that at least some of the drugs with anti-fibrotic effects, including RAAS blockers, may act by stimulating endogenous tissue regeneration. In addition, new potential drugs were identified in animal models supporting the view that promoting kidney regeneration may represent an attractive strategy to treat kidney disorders. Future clinical studies are needed to address the question of whether this approach may be amenable to treat CKD patients and also enhance the efficacy of anti-fibrotic treatments.

Acknowledgements

This article is supported by the European Research Council under the Consolidator Grant RENOIR (ERC-2014-CoG), grant number 648274.

Received 27 November 2017;

Received in revised form 19 February 2018;

Accepted 20 February 2018

Available online 11 April 2018

Keywords:

Renal fibrosis;

Anti-fibrotic therapy;

Clinical trials;

Nephron regeneration

Marco Allinovi and Letizia De Chiara contributed equally to this work.

References

- [1] Y. Liu, Cellular and molecular mechanisms of renal fibrosis, *Nat. Rev. Nephrol.* 7 (12) (2011) 684–696.
- [2] P. Fioretto, M.W. Steffes, D.E. Sutherland, F.C. Goetz, M. Mauer, Reversal of lesions of diabetic nephropathy after pancreas transplantation, *N. Engl. J. Med.* 339 (2) (1998) 69–75.
- [3] D. Tampe, M. Zeisberg, Potential approaches to reverse or repair renal fibrosis, *Nat. Rev. Nephrol.* 10 (4) (2014) 226–237.
- [4] V. Jha, G. Garcia-Garcia, K. Iseki, Z. Li, S. Naicker, B. Plattner, R. Saran, A.Y. Wang, C.W. Yang, Chronic kidney disease: global dimension and perspectives, *Lancet* 382 (9888) (2013) 260–272.
- [5] D. Macconi, G. Remuzzi, A. Benigni, Key fibrogenic mediators: old players. Renin-angiotensin system, *Kidney Int. Suppl.* (2011) 4 (1) (2014) 58–64.

- [6] A. Remuzzi, E. Gagliardini, C. Donadoni, A. Fassi, F. Sangalli, M.S. Lepre, G. Remuzzi, A. Benigni, Effect of angiotensin II antagonism on the regression of kidney disease in the rat, *Kidney Int.* 62 (3) (2002) 885–894.
- [7] A. Remuzzi, E. Gagliardini, F. Sangalli, M. Bonomelli, M. Piccinelli, A. Benigni, G. Remuzzi, ACE inhibition reduces glomerulosclerosis and regenerates glomerular tissue in a model of progressive renal disease, *Kidney Int.* 69 (7) (2006) 1124–1130.
- [8] A. Remuzzi, F. Sangalli, D. Macconi, S. Tomasoni, I. Cattaneo, P. Rizzo, B. Bonandrini, E. Bresciani, L. Longaretti, E. Gagliardini, S. Conti, A. Benigni, G. Remuzzi, Regression of renal disease by angiotensin II antagonism is caused by regeneration of kidney vasculature, *J. Am. Soc. Nephrol.* 27 (3) (2016) 699–705.
- [9] A. Benigni, M. Morigi, P. Rizzo, E. Gagliardini, C. Rota, M. Abbate, S. Ghezzi, A. Remuzzi, G. Remuzzi, Inhibiting angiotensin-converting enzyme promotes renal repair by limiting progenitor cell proliferation and restoring the glomerular architecture, *Am. J. Pathol.* 179 (2) (2011) 628–638.
- [10] J. Zhang, D. Yanez, A. Floege, J. Lichtnekert, R.D. Krofft, Z. H. Liu, J.W. Pippin, S.J. Shankland, ACE-inhibition increases podocyte number in experimental glomerular disease independent of proliferation, *J. Renin-Angiotensin-Aldosterone Syst.* 16 (2) (2015) 234–248.
- [11] E.J. Lewis, L.G. Hunsicker, W.R. Clarke, T. Berl, M.A. Pohl, J.B. Lewis, E. Ritz, R.C. Atkins, R. Rohde, I. Raz, G. Collaborative Study, Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes, *N. Engl. J. Med.* 345 (12) (2001) 851–860.
- [12] B.M. Brenner, M.E. Cooper, D. de Zeeuw, W.F. Keane, W.E. Mitch, H.H. Parving, G. Remuzzi, S.M. Snapinn, Z. Zhang, S. Shahinfar, R.S. Investigators, Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy, *N. Engl. J. Med.* 345 (12) (2001) 861–869.
- [13] P. Ruggenenti, A. Perna, G. Gherardi, G. Garini, C. Zoccali, M. Salvadori, F. Scolari, F.P. Schena, G. Remuzzi, Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria, *Lancet* 354 (9176) (1999) 359–364.
- [14] G. Remuzzi, C. Chiurciu, P. Ruggenenti, Proteinuria predicting outcome in renal disease: nondiabetic nephropathies (REIN), *Kidney Int. Suppl.* 92 (2004) S90–6.
- [15] G.F. Strippoli, M. Craig, J.J. Deeks, F.P. Schena, J.C. Craig, Effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists on mortality and renal outcomes in diabetic nephropathy: systematic review, *BMJ* 329 (7470) (2004) 828.
- [16] P. Ruggenenti, E. Peticucci, P. Cravedi, V. Gambarà, M. Costantini, S.K. Sharma, A. Perna, G. Remuzzi, Role of remission clinics in the longitudinal treatment of CKD, *J. Am. Soc. Nephrol.* 19 (6) (2008) 1213–1224.
- [17] F. Zhang, H. Liu, D. Liu, Y. Liu, H. Li, X. Tan, F. Liu, Y. Peng, H. Zhang, Effects of RAAS inhibitors in patients with kidney disease, *Curr. Hypertens. Rep.* 19 (9) (2017) 72.
- [18] L.F. Fried, N. Emanuele, J.H. Zhang, M. Brophy, T.A. Conner, W. Duckworth, D.J. Leehey, P.A. McCullough, T. O'Connor, P.M. Palevsky, R.F. Reilly, S.L. Seliger, S.R. Warren, S. Watnick, P. Peduzzi, P. Guarino, V.N.-D. Investigators, Combined angiotensin inhibition for the treatment of diabetic nephropathy, *N. Engl. J. Med.* 369 (20) (2013) 1892–1903.
- [19] C. Werner, J. Poss, M. Bohm, Optimal antagonism of the renin-angiotensin-aldosterone system: do we need dual or triple therapy? *Drugs* 70 (10) (2010) 1215–1230.
- [20] F. Ren, L. Tang, Y. Cai, X. Yuan, W. Huang, L. Luo, J. Zhou, Y. Zheng, Meta-analysis: the efficacy and safety of combined treatment with ARB and ACEI on diabetic nephropathy, *Ren. Fail.* 37 (4) (2015) 548–561.
- [21] T.A. Mavranakas, K. Gariani, P.Y. Martin, Mineralocorticoid receptor blockade in addition to angiotensin converting enzyme inhibitor or angiotensin II receptor blocker treatment: an emerging paradigm in diabetic nephropathy: a systematic review, *Eur. J. Intern. Med.* 25 (2) (2014) 173–176.
- [22] F. Angeli, G. Reboldi, G. Mazzotta, C. Poltronieri, P. Verdecchia, Safety and efficacy of aliskiren in the treatment of hypertension: a systematic overview, *Expert Opin. Drug Saf.* 11 (4) (2012) 659–670.
- [23] J.F. Navarro-Gonzalez, C. Mora-Fernandez, M. Muros de Fuentes, J. Garcia-Perez, Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy, *Nat. Rev. Nephrol.* 7 (6) (2011) 327–340.
- [24] J.S. Duffield, Macrophages and immunologic inflammation of the kidney, *Semin. Nephrol.* 30 (3) (2010) 234–254.
- [25] M.V. Perez-Gomez, M.D. Sanchez-Nino, A.B. Sanz, B. Zheng, C. Martin-Cleary, M. Ruiz-Ortega, A. Ortiz, B. Fernandez-Fernandez, Targeting inflammation in diabetic kidney disease: early clinical trials, *Expert Opin. Investig. Drugs* 25 (9) (2016) 1045–1058.
- [26] H.J. Heerspink, D. De Zeeuw, Novel anti-inflammatory drugs for the treatment of diabetic kidney disease, *Diabetologia* 59 (8) (2016) 1621–1623.
- [27] S. Mitchell, J. Vargas, A. Hoffmann, Signaling via the NF-kappaB system, *Wiley Interdiscip. Rev. Syst. Biol. Med.* 8 (3) (2016) 227–241.
- [28] J.A. Tumlin, C.M. Galphin, B.H. Rovin, Advanced diabetic nephropathy with nephrotic range proteinuria: a pilot study of the long-term efficacy of subcutaneous ACTH gel on proteinuria, progression of CKD, and urinary levels of VEGF and MCP-1, *J. Diabetes Res.* 2013 (2013) 489869.
- [29] P. Ruggenenti, Effects of MCP-1 inhibition by bindarit therapy in type 2 diabetes subjects with micro- or macroalbuminuria, *J. Am. Soc. Nephrol.* 21 (Suppl. 1) (2010) 44A.
- [30] S. Schutze, K. Wiegmann, T. Machleidt, M. Kronke, TNF-induced activation of NF-kappa B, *Immunobiology* 193 (2–4) (1995) 193–203.
- [31] M.G. Suranyi, A. Guasch, B.M. Hall, B.D. Myers, Elevated levels of tumor necrosis factor-alpha in the nephrotic syndrome in humans, *Am. J. Kidney Dis.* 21 (3) (1993) 251–259.
- [32] B. Huang, Y. Cheng, K. Usa, Y. Liu, M.A. Baker, D.L. Mattson, Y. He, N. Wang, M. Liang, Renal tumor necrosis factor alpha contributes to hypertension in dahl salt-sensitive rats, *Sci. Rep.* 6 (2016) 21960.
- [33] M.S. Joy, D.S. Gipson, L. Powell, J. MacHardy, J.C. Jettette, S. Vento, C. Pan, V. Savin, A. Eddy, A.B. Fogo, J.B. Kopp, D. Cattran, H. Trachtman, Phase 1 trial of adalimumab in Focal Segmental Glomerulosclerosis (FSGS): II. Report of the FONT (Novel Therapies for Resistant FSGS) study group, *Am. J. Kidney Dis.* 55 (1) (2010) 50–60.
- [34] H. Trachtman, S. Vento, E. Herreshoff, M. Radeva, J. Gassman, D.T. Stein, V.J. Savin, M. Sharma, J. Reiser, C. Wei, M. Somers, T. Srivastava, D.S. Gipson, Efficacy of galactose and adalimumab in patients with resistant focal

- segmental glomerulosclerosis: report of the font clinical trial group, *BMC Nephrol.* 16 (2015) 111.
- [35] F. Matsui, K.K. Meldrum, The role of the Janus kinase family/signal transducer and activator of transcription signaling pathway in fibrotic renal disease, *J. Surg. Res.* 178 (1) (2012) 339–345.
- [36] M.B. Marrero, A.K. Baner-Berceli, D.M. Stern, D.C. Eaton, Role of the JAK/STAT signaling pathway in diabetic nephropathy, *Am. J. Physiol. Ren. Physiol.* 290 (4) (2006) F762–8.
- [37] F.C. Brosius 3rd, J.C. He, JAK inhibition and progressive kidney disease, *Curr. Opin. Nephrol. Hypertens.* 24 (1) (2015) 88–95.
- [38] M. Pang, L. Ma, R. Gong, E. Tolbert, H. Mao, M. Ponnusamy, Y.E. Chin, H. Yan, L.D. Dworkin, S. Zhuang, A novel STAT3 inhibitor, S3I-201, attenuates renal interstitial fibroblast activation and interstitial fibrosis in obstructive nephropathy, *Kidney Int.* 78 (3) (2010) 257–268.
- [39] F.C. Brosius, K.R. Tuttle, M. Kretzler, JAK inhibition in the treatment of diabetic kidney disease, *Diabetologia* 59 (8) (2016) 1624–1627.
- [40] T.T. Braga, M. Correa-Costa, R.C. Silva, M.C. Cruz, M.I. Hiyane, J.S. da Silva, K.R. Perez, I.M. Cuccovia, N.O. Camara, CCR2 contributes to the recruitment of monocytes and leads to kidney inflammation and fibrosis development, *Inflammopharmacology* 26 (2) (2018) 403–411, <https://doi.org/10.1007/s10787-017-0317-4> [Epub 2017 Feb 6].
- [41] H.J. Anders, V. Vielhauer, M. Frink, Y. Linde, C.D. Cohen, S.M. Blattner, M. Kretzler, F. Strutz, M. Mack, H.J. Grone, J. Onuffer, R. Horuk, P.J. Nelson, D. Schlondorff, A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation, *J. Clin. Invest.* 109 (2) (2002) 251–259.
- [42] V. Vielhauer, E. Berning, V. Eis, M. Kretzler, S. Segerer, F. Strutz, R. Horuk, H.J. Grone, D. Schlondorff, H.J. Anders, CCR1 blockade reduces interstitial inflammation and fibrosis in mice with glomerulosclerosis and nephrotic syndrome, *Kidney Int.* 66 (6) (2004) 2264–2278.
- [43] J. Menne, D. Eulberg, D. Beyer, M. Baumann, F. Saudek, Z. Valkusz, A. Wiecek, H. Haller, G. Emapticap Study, C-C motif-ligand 2 inhibition with emapticap pegol (NOX-E36) in type 2 diabetic patients with albuminuria, *Nephrol. Dial. Transplant.* 32 (2) (2017) 307–315.
- [44] K. Kitagawa, T. Wada, K. Furuichi, H. Hashimoto, Y. Ishiwata, M. Asano, M. Takeya, W.A. Kuziel, K. Matsushima, N. Mukaida, H. Yokoyama, Blockade of CCR2 ameliorates progressive fibrosis in kidney, *Am. J. Pathol.* 165 (1) (2004) 237–246.
- [45] E. Lefebvre, G. Moyle, R. Reshef, L.P. Richman, M. Thompson, F. Hong, H.L. Chou, T. Hashiguchi, C. Plato, D. Poulin, T. Richards, H. Yoneyama, H. Jenkins, G. Wolfgang, S.L. Friedman, Antifibrotic effects of the dual CCR2/CCR5 antagonist cenicriviroc in animal models of liver and kidney fibrosis, *PLoS One* 11 (6) (2016) e0158156.
- [46] D. de Zeeuw, P. Bekker, E. Henkel, C. Hasslacher, I. Gouni-Berthold, H. Mehling, A. Potarca, V. Tesar, H.J. Heerspink, T.J. Schall, C.B.D.N.S. Group, The effect of CCR2 inhibitor CCX140-B on residual albuminuria in patients with type 2 diabetes and nephropathy: a randomised trial, *Lancet Diabetes Endocrinol.* 3 (9) (2015) 687–696.
- [47] Y. Solak, D. Siritopol, A. Yildiz, M.I. Yilmaz, A. Ortiz, A. Covic, M. Kanbay, Colchicine in renal medicine: new virtues of an ancient friend, *Blood Purif.* 43 (1–3) (2017) 125–135.
- [48] C. McClurkin Jr., S.H. Phan, C.H. Hsu, S.R. Patel, J.K. Spicker, A.M. Kshirsagar, W.Y. Yuan, R.C. Wiggins, Moderate protection of renal function and reduction of fibrosis by colchicine in a model of anti-GBM disease in the rabbit, *J. Am. Soc. Nephrol.* 1 (3) (1990) 257–265.
- [49] C. Li, C.W. Yang, H.J. Ahn, W.Y. Kim, C.W. Park, J.H. Park, M.J. Lee, J.H. Yang, Y.S. Kim, B.K. Bang, Colchicine decreases apoptotic cell death in chronic cyclosporine nephrotoxicity, *J. Lab. Clin. Med.* 139 (6) (2002) 364–371.
- [50] U. Disel, S. Paydas, A. Dogan, G. Gulfiliz, S. Yavuz, Effect of colchicine on cyclosporine nephrotoxicity, reduction of TGF-beta overexpression, apoptosis, and oxidative damage: an experimental animal study, *Transplant. Proc.* 36 (5) (2004) 1372–1376.
- [51] T. Guan, B. Gao, G. Chen, X. Chen, M. Janssen, L. Uttarwar, A.J. Ingram, J.C. Krepinsky, Colchicine attenuates renal injury in a model of hypertensive chronic kidney disease, *Am. J. Physiol. Ren. Physiol.* 305 (10) (2013) F1466–76.
- [52] S. Kim, E.S. Jung, J. Lee, N.J. Heo, K.Y. Na, J.S. Han, Effects of colchicine on renal fibrosis and apoptosis in obstructed kidneys, *Korean J. Intern. Med.* (2017) <https://doi.org/10.3904/kjim.2016.131> [Epub ahead of print].
- [53] G. Cocco, D.C. Chu, S. Pandolfi, Colchicine in clinical medicine. A guide for internists, *Eur. J. Intern. Med.* 21 (6) (2010) 503–508.
- [54] B.H. Ozdemir, F.N. Ozdemir, S. Sezer, A. Sar, M. Haberal, Does colchicine have an antifibrotic effect on development of interstitial fibrosis in renal allografts of recipients with familial Mediterranean fever? *Transplant. Proc.* 38 (2) (2006) 473–476.
- [55] Y. Wang, D.C. Harris, Macrophages in renal disease, *J. Am. Soc. Nephrol.* 22 (1) (2011) 21–27.
- [56] I. Loeffler, G. Wolf, Transforming growth factor-beta and the progression of renal disease, *Nephrol. Dial. Transplant.* 29 (Suppl. 1) (2014) i37–i45.
- [57] G. Casalena, I. Daehn, E. Bottinger, Transforming growth factor-beta, bioenergetics, and mitochondria in renal disease, *Semin. Nephrol.* 32 (3) (2012) 295–303.
- [58] D. Impellizzeri, E. Esposito, J. Attley, S. Cuzzocrea, Targeting inflammation: new therapeutic approaches in chronic kidney disease (CKD), *Pharmacol. Res.* 81 (2014) 91–102.
- [59] M. Jun, V. Venkataraman, M. Razavian, B. Cooper, S. Zoungas, T. Ninomiya, A.C. Webster, V. Perkovic, Antioxidants for chronic kidney disease, *Cochrane Database Syst. Rev.* 10 (2012) CD008176.
- [60] M. Zhao, Y. Chen, G. Ding, Y. Xu, M. Bai, Y. Zhang, Z. Jia, S. Huang, A. Zhang, Renal tubular epithelium-targeted peroxisome proliferator-activated receptor-gamma maintains the epithelial phenotype and antagonizes renal fibrogenesis, *Oncotarget* 7 (40) (2016) 64690–64701.
- [61] S. Li, N. Mariappan, J. Megyesi, B. Shank, K. Kannan, S. Theus, P.M. Price, J.S. Duffield, D. Portilla, Proximal tubule PPARalpha attenuates renal fibrosis and inflammation caused by unilateral ureteral obstruction, *Am. J. Physiol. Ren. Physiol.* 305 (5) (2013) F618–27.
- [62] J. Lu, J. Shi, B. Gui, G. Yao, L. Wang, Y. Ou, D. Zhu, L. Ma, H. Ge, R. Fu, Activation of PPAR-gamma inhibits PDGF-induced proliferation of mouse renal fibroblasts, *Eur. J. Pharmacol.* 789 (2016) 222–228.
- [63] E.N. Kim, J.H. Lim, M.Y. Kim, H.W. Kim, C.W. Park, Y.S. Chang, B.S. Choi, PPARalpha agonist, fenofibrate, ameliorates age-related renal injury, *Exp. Gerontol.* 81 (2016) 42–50.

- [64] P. Balakumar, S. Kadian, N. Mahadevan, Are PPAR alpha agonists a rational therapeutic strategy for preventing abnormalities of the diabetic kidney? *Pharmacol. Res.* 65 (4) (2012) 430–436.
- [65] T. Kawai, T. Masaki, S. Doi, T. Arakawa, Y. Yokoyama, T. Doi, N. Kohno, N. Yorioka, PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta, *Lab. Invest.* 89 (1) (2009) 47–58.
- [66] D.E. Bonds, T.E. Craven, J. Buse, J.R. Crouse, R. Cuddihy, M. Elam, H.N. Ginsberg, K. Kirchner, S. Marcovina, J.C. Mychaleckyj, P.J. O'Connor, J.A. Sperl-Hillen, Fenofibrate-associated changes in renal function and relationship to clinical outcomes among individuals with type 2 diabetes: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) experience, *Diabetologia* 55 (6) (2012) 1641–1650.
- [67] Y.M. Smulders, A.E. van Eeden, C.D. Stehouwer, R.N. Weijers, E.H. Slaats, J. Silberbusch, Can reduction in hypertriglyceridaemia slow progression of microalbuminuria in patients with non-insulin-dependent diabetes mellitus? *Eur. J. Clin. Investig.* 27 (12) (1997) 997–1002.
- [68] J.C. Ansquer, C. Foucher, S. Rattier, M.R. Taskinen, G. Steiner, D. Investigators, Fenofibrate reduces progression to microalbuminuria over 3 years in a placebo-controlled study in type 2 diabetes: results from the Diabetes Atherosclerosis Intervention Study (DAIS), *Am. J. Kidney Dis.* 45 (3) (2005) 485–493.
- [69] T.M. Davis, R. Ting, J.D. Best, M.W. Donoghoe, P.L. Drury, D.R. Sullivan, A.J. Jenkins, R.L. O'Connell, M.J. Whiting, P. P. Glasziou, R.J. Simes, Y.A. Kesaniemi, V.J. GebSKI, R.S. Scott, A.C. Keech, I. Fenofibrate, i. Event Lowering in Diabetes Study, Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study, *Diabetologia* 54 (2) (2011) 280–290.
- [70] J.M. Lachin, G. Viberti, B. Zinman, S.M. Haffner, R.P. Aftring, G. Paul, B.G. Kravitz, W.H. Herman, R.R. Holman, S.E. Kahn, A.S. Group, Renal function in type 2 diabetes with rosiglitazone, metformin, and glyburide monotherapy, *Clin. J. Am. Soc. Nephrol.* 6 (5) (2011) 1032–1040.
- [71] D. Bolignano, C. Zoccali, Glitazones in chronic kidney disease: potential and concerns, *Nutr. Metab. Cardiovasc. Dis.* 22 (3) (2012) 167–175.
- [72] A. Abbas, J. Blandon, J. Rude, A. Elfar, D. Mukherjee, PPAR-gamma agonist in treatment of diabetes: cardiovascular safety considerations, *Cardiovasc. Hematol. Agents Med. Chem.* 10 (2) (2012) 124–134.
- [73] C.E. Holterman, N.C. Read, C.R. Kennedy, Nox and renal disease, *Clin. Sci. (Lond.)* 128 (8) (2015) 465–481.
- [74] G. Teixeira, C. Szyndralewicz, S. Molango, S. Carneseccchi, F. Heitz, P. Wiesel, J.M. Wood, Therapeutic potential of NADPH oxidase 1/4 inhibitors, *Br. J. Pharmacol.* 174 (12) (2017) 1647–1669.
- [75] Genkyotex, Genkyotex Announces Investigator Initiated Phase 2 Clinical Trial to Evaluate 48 Week Treatment With GKT831 in Patients With Type 1 Diabetes and Kidney Disease, 2017.
- [76] J.H. Lin, J.J. Zhang, S.L. Lin, G.M. Chertow, Design of a phase 2 clinical trial of an ASK1 inhibitor, GS-4997, in patients with diabetic kidney disease, *Nephron* 129 (1) (2015) 29–33.
- [77] W. Kong, J. Fu, N. Liu, C. Jiao, G. Guo, J. Luan, H. Wang, L. Yao, L. Wang, M. Yamamoto, J. Pi, H. Zhou, Nrf2 deficiency promotes the progression from acute tubular damage to chronic renal fibrosis following unilateral ureteral obstruction, *Nephrol. Dial. Transplant.* (2017) <https://doi.org/10.1093/ndt/gfx299> [Epub ahead of print].
- [78] M.A. Aminzadeh, S.B. Nicholas, K.C. Norris, N.D. Vaziri, Role of impaired Nrf2 activation in the pathogenesis of oxidative stress and inflammation in chronic tubulointerstitial nephropathy, *Nephrol. Dial. Transplant.* 28 (8) (2013) 2038–2045.
- [79] H.J. Kim, N.D. Vaziri, Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure, *Am. J. Physiol. Ren. Physiol.* 298 (3) (2010) F662–71.
- [80] S. Ruiz, P.E. Pergola, R.A. Zager, N.D. Vaziri, Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease, *Kidney Int.* 83 (6) (2013) 1029–1041.
- [81] S. Chung, S. Kim, M. Kim, E.S. Koh, H.E. Yoon, H.S. Kim, C.W. Park, Y.S. Chang, S.J. Shin, T-type calcium channel blocker attenuates unilateral ureteral obstruction-induced renal interstitial fibrosis by activating the Nrf2 antioxidant pathway, *Am. J. Transl. Res.* 8 (11) (2016) 4574–4585.
- [82] T. Qin, S. Yin, J. Yang, Q. Zhang, Y. Liu, F. Huang, W. Cao, Sinomenine attenuates renal fibrosis through Nrf2-mediated inhibition of oxidative stress and TGFbeta signaling, *Toxicol. Appl. Pharmacol.* 304 (2016) 1–8.
- [83] R. Kanlaya, S. Khamchun, C. Kapincharanon, V. Thongboonkerd, Protective effect of epigallocatechin-3-gallate (EGCG) via Nrf2 pathway against oxalate-induced epithelial mesenchymal transition (EMT) of renal tubular cells, *Sci. Rep.* 6 (2016) 30233.
- [84] P.Y. Tsai, S.M. Ka, T.K. Chao, J.M. Chang, S.H. Lin, C.Y. Li, M.T. Kuo, P. Chen, A. Chen, Antroquinolone reduces oxidative stress by enhancing the Nrf2 signaling pathway and inhibits inflammation and sclerosis in focal segmental glomerulosclerosis mice, *Free Radic. Biol. Med.* 50 (11) (2011) 1503–1516.
- [85] S.A. Reisman, G.M. Chertow, S. Hebbar, N.D. Vaziri, K.W. Ward, C.J. Meyer, Bardoxolone methyl decreases megalin and activates nrf2 in the kidney, *J. Am. Soc. Nephrol.* 23 (10) (2012) 1663–1673.
- [86] P.E. Pergola, M. Krauth, J.W. Huff, D.A. Ferguson, S. Ruiz, C.J. Meyer, D.G. Warnock, Effect of bardoxolone methyl on kidney function in patients with T2D and Stage 3b–4 CKD, *Am. J. Nephrol.* 33 (5) (2011) 469–476.
- [87] P.E. Pergola, P. Raskin, R.D. Toto, C.J. Meyer, J.W. Huff, E.B. Grossman, M. Krauth, S. Ruiz, P. Audhya, H. Christ-Schmidt, J. Wittes, D.G. Warnock, B.S. Investigators, Bardoxolone methyl and kidney function in CKD with type 2 diabetes, *N. Engl. J. Med.* 365 (4) (2011) 327–336.
- [88] C. Zoja, D. Corna, V. Nava, M. Locatelli, M. Abbate, F. Gaspari, F. Carrara, F. Sangalli, G. Remuzzi, A. Benigni, Analogs of bardoxolone methyl worsen diabetic nephropathy in rats with additional adverse effects, *Am. J. Physiol. Ren. Physiol.* 304 (6) (2013) F808–19.
- [89] D. de Zeeuw, T. Akizawa, P. Audhya, G.L. Bakris, M. Chin, H. Christ-Schmidt, A. Goldsberry, M. Houser, M. Krauth, H. J. Lambers Heerspink, J.J. McMurray, C.J. Meyer, H.H. Parving, G. Remuzzi, R.D. Toto, N.D. Vaziri, C. Wanner, J. Wittes, D. Wrolstad, G.M. Chertow, B.T. Investigators, Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease, *N. Engl. J. Med.* 369 (26) (2013) 2492–2503.
- [90] M.P. Chin, D. Wrolstad, G.L. Bakris, G.M. Chertow, D. de Zeeuw, A. Goldsberry, P.G. Linde, P.A. McCullough, J.J. McMurray, J. Wittes, C.J. Meyer, Risk factors for heart

- failure in patients with type 2 diabetes mellitus and stage 4 chronic kidney disease treated with bardoxolone methyl, *J. Card. Fail.* 20 (12) (2014) 953–958.
- [91] J.A. Tayek, K. Kalantar-Zadeh, The extinguished BEACON of bardoxolone: not a Monday morning quarterback story, *Am. J. Nephrol.* 37 (3) (2013) 208–211.
- [92] R. S, M. Nangaku, T. Akizawa, Bardoxolone methyl improved GFR measured by standard inulin clearance: the TSUBAKI Study, American Society of Nephrology American Society of Nephrology (ASN) Kidney Week 2017 New Orleans, 2017.
- [93] P. P, G.A. Block, L. Inker, McCullough PA, M. Chin, C.J. Meyer, M.N. Rheault, C.E. Kashtan, D.G. Warnock, Initial data report from “CARDINAL”: A phase 2/3 study of bardoxolone methyl in patients with Alport syndrome, American Society of Nephrology (ASN) Kidney Week 2017 Annual Meeting, New Orleans, 2017.
- [94] W. Neuhofer, D. Pittrow, Endothelin receptor selectivity in chronic kidney disease: rationale and review of recent evidence, *Eur. J. Clin. Investig.* 39 (Suppl. 2) (2009) 50–67.
- [95] N. Dhaun, C.J. Ferro, A.P. Davenport, W.G. Haynes, J. Goddard, D.J. Webb, Haemodynamic and renal effects of endothelin receptor antagonism in patients with chronic kidney disease, *Nephrol. Dial. Transplant.* 22 (11) (2007) 3228–3234.
- [96] R.R. Wenzel, T. Littke, S. Kuranoff, C. Jurgens, H. Bruck, E. Ritz, T. Philipp, A. Mitchell, S.P.P.E.A.E.i.D.N.S. Investigators, Avosentan reduces albumin excretion in diabetics with macroalbuminuria, *J. Am. Soc. Nephrol.* 20 (3) (2009) 655–664.
- [97] J.F. Mann, D. Green, K. Jamerson, L.M. Ruilope, S.J. Kuranoff, T. Littke, G. Viberti, A.S. Group, Avosentan for overt diabetic nephropathy, *J. Am. Soc. Nephrol.* 21 (3) (2010) 527–535.
- [98] D. de Zeeuw, B. Coll, D. Andress, J.J. Brennan, H. Tang, M. Houser, R. Correa-Rotter, D. Kohan, H.J. Lambers Heerspink, H. Makino, V. Perkovic, Y. Pritchett, G. Remuzzi, S.W. Tobe, R. Toto, G. Viberti, H.H. Parving, The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy, *J. Am. Soc. Nephrol.* 25 (5) (2014) 1083–1093.
- [99] D.E. Kohan, Y. Pritchett, M. Molitch, S. Wen, T. Garimella, P. Audhya, D.L. Andress, Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy, *J. Am. Soc. Nephrol.* 22 (4) (2011) 763–772.
- [100] J. Egido, J. Rojas-Rivera, S. Mas, M. Ruiz-Ortega, A.B. Sanz, E. Gonzalez Parra, C. Gomez-Guerrero, Atrasentan for the treatment of diabetic nephropathy, *Expert Opin. Investig. Drugs* 26 (6) (2017) 741–750.
- [101] R. Komers, D.S. Gipson, P. Nelson, S. Adler, T. Srivastava, V.K. Derebail, K.E. Meyers, P. Pergola, M.E. MacNally, J.L. Hunt, A. Shih, H. Trachtman, Efficacy and safety of sparsentan compared with irbesartan in patients with primary focal segmental glomerulosclerosis: randomized, controlled trial design (DUET), *Kidney Int. Rep.* 2 (4) (2017) 654–664.
- [102] M. Edeling, G. Ragi, S. Huang, H. Pavenstadt, K. Susztak, Developmental signalling pathways in renal fibrosis: the roles of Notch, Wnt and Hedgehog, *Nat. Rev. Nephrol.* 12 (7) (2016) 426–439.
- [103] R. Derynck, Y.E. Zhang, Smad-dependent and Smad-independent pathways in TGF-beta family signalling, *Nature* 425 (6958) (2003) 577–584.
- [104] N.E. Castro, M. Kato, J.T. Park, R. Natarajan, Transforming growth factor beta1 (TGF-beta1) enhances expression of profibrotic genes through a novel signaling cascade and microRNAs in renal mesangial cells, *J. Biol. Chem.* 289 (42) (2014) 29001–29013.
- [105] S. McGaraughty, R.A. Davis-Taber, C.Z. Zhu, T.B. Cole, A. L. Nikkel, M. Chhaya, K.J. Doyle, L.M. Olson, G.M. Preston, C.M. Grinnell, K.M. Salte, A.M. Giamis, Y. Luo, V. Sun, A.D. Goodearl, M. Gopalakrishnan, S.E. Lacy, Targeting anti-TGF-beta therapy to fibrotic kidneys with a dual specificity antibody approach, *J. Am. Soc. Nephrol.* 28 (12) (2017) 3616–3626, <https://doi.org/10.1681/ASN.2017010013> [Epub 2017 Aug 21].
- [106] P.G. Wilson, J.C. Thompson, M.H. Yoder, R. Charnigo, L.R. Tannock, Prevention of renal apoB retention is protective against diabetic nephropathy: role of TGF-beta inhibition, *J. Lipid Res.* 58 (12) (2017) 2264–2274, <https://doi.org/10.1194/jlr.M078204> [Epub 2017 Sep 14].
- [107] X. Liang, H.W. Schnaper, T. Matsusaka, I. Pastan, S. Ledbetter, T. Hayashida, Anti-TGF-beta antibody, 1D11, ameliorates glomerular fibrosis in mouse models after the onset of proteinuria, *PLoS One* 11 (5) (2016) e0155534.
- [108] A. Benigni, C. Zoja, D. Corna, C. Zatelli, S. Conti, M. Campana, E. Gagliardini, D. Rottoli, C. Zanchi, M. Abbate, S. Ledbetter, G. Remuzzi, Add-on anti-TGF-beta antibody to ACE inhibitor arrests progressive diabetic nephropathy in the rat, *J. Am. Soc. Nephrol.* 14 (7) (2003) 1816–1824.
- [109] H. Trachtman, F.C. Fervenza, D.S. Gipson, P. Heering, D. R. Jayne, H. Peters, S. Rota, G. Remuzzi, L.C. Rump, L.K. Sellin, J.P. Heaton, J.B. Streisand, M.L. Hard, S.R. Ledbetter, F. Vincenti, A phase 1, single-dose study of fresolimumab, an anti-TGF-beta antibody, in treatment-resistant primary focal segmental glomerulosclerosis, *Kidney Int.* 79 (11) (2011) 1236–1243.
- [110] F. F, F. Vincenti, K.N. Campbell, M. Diaz, L. Gesualdo, P. Nelson, M. Praga, J. Radhakrishnan, L. Sellin, A. Singh, D. Thornley-Brown, F.V. Veronese, B. Accomando, S. Engstrand, S. Ledbetter, J. Lin, J. Neylan, J. Tumlin, Focal Segmental Glomerulosclerosis Study Group, A phase 2, double-blind, placebo-controlled, randomized study of fresolimumab in patients with steroid-resistant primary focal segmental glomerulosclerosis, *Kidney Int. Rep.* (2017) 800–810.
- [111] J. Voelker, P.H. Berg, M. Sheetz, K. Duffin, T. Shen, B. Moser, T. Greene, S.S. Blumenthal, I. Rychlik, Y. Yagil, P. Zaoui, J.B. Lewis, Anti-TGF-beta1 antibody therapy in patients with diabetic nephropathy, *J. Am. Soc. Nephrol.* 28 (3) (2017) 953–962.
- [112] M.E. Cho, J.B. Kopp, Pirfenidone: an anti-fibrotic therapy for progressive kidney disease, *Expert Opin. Investig. Drugs* 19 (2) (2010) 275–283.
- [113] M.E. Cho, D.C. Smith, M.H. Branton, S.R. Penzak, J.B. Kopp, Pirfenidone slows renal function decline in patients with focal segmental glomerulosclerosis, *Clin. J. Am. Soc. Nephrol.* 2 (5) (2007) 906–913.
- [114] K. Sharma, J.H. Ix, A.V. Mathew, M. Cho, A. Pflueger, S.R. Dunn, B. Francos, S. Sharma, B. Falkner, T.A. McGowan, M. Donohue, S. Ramachandrarao, R. Xu, F.C. Fervenza, J.B. Kopp, Pirfenidone for diabetic nephropathy, *J. Am. Soc. Nephrol.* 22 (6) (2011) 1144–1151.
- [115] S.G. Adler, S. Schwartz, M.E. Williams, C. Arauz-Pacheco, W.K. Bolton, T. Lee, D. Li, T.B. Neff, P.R. Urquilla, K.L. Sewell, Phase 1 study of anti-CTGF monoclonal antibody in patients with diabetes and microalbuminuria, *Clin. J. Am. Soc. Nephrol.* 5 (8) (2010) 1420–1428.

- [116] R.X. Li, W.H. Yiu, S.C. Tang, Role of bone morphogenetic protein-7 in renal fibrosis, *Front. Physiol.* 6 (2015) 114.
- [117] I.H. Ali, D.P. Brazil, Bone morphogenetic proteins and their antagonists: current and emerging clinical uses, *Br. J. Pharmacol.* 171 (15) (2014) 3620–3632.
- [118] J.W. Lowery, B. Brookshire, V. Rosen, A survey of strategies to modulate the bone morphogenetic protein signaling pathway: current and future perspectives, *Stem Cells Int.* 2016 (2016) 7290686.
- [119] E. Lazzeri, E. Ronconi, M.L. Angelotti, A. Peired, B. Mazzinghi, F. Becherucci, S. Conti, G. Sansavini, A. Sisti, F. Ravaglia, D. Lombardi, A. Provenzano, A. Manonelles, J. M. Cruzado, S. Giglio, R.M. Roperto, M. Materassi, L. Lasagni, P. Romagnani, Human urine-derived renal progenitors for personalized modeling of genetic kidney disorders, *J. Am. Soc. Nephrol.* 26 (8) (2015) 1961–1974.
- [120] T. Kosugi, T. Nakayama, M. Heinig, L. Zhang, Y. Yuzawa, L. G. Sanchez-Lozada, C. Roncal, R.J. Johnson, T. Nakagawa, Effect of lowering uric acid on renal disease in the type 2 diabetic db/db mice, *Am. J. Physiol. Ren. Physiol.* 297 (2) (2009) F481–8.
- [121] M. Goicoechea, S.G. de Vinuesa, U. Verdalles, C. Ruiz-Caro, J. Ampuero, A. Rincon, D. Arroyo, J. Luno, Effect of allopurinol in chronic kidney disease progression and cardiovascular risk, *Clin. J. Am. Soc. Nephrol.* 5 (8) (2010) 1388–1393.
- [122] A. Momeni, S. Shahidi, S. Seirafian, S. Taheri, S. Kheiri, Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients, *Iran. J. Kidney Dis.* 4 (2) (2010) 128–132.
- [123] M.P. Kao, D.S. Ang, S.J. Gandy, M.A. Nadir, J.G. Houston, C.C. Lang, A.D. Struthers, Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease, *J. Am. Soc. Nephrol.* 22 (7) (2011) 1382–1389.
- [124] Y.P. Siu, K.T. Leung, M.K. Tong, T.H. Kwan, Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level, *Am. J. Kidney Dis.* 47 (1) (2006) 51–59.
- [125] S. Kim, H.J. Kim, H.S. Ahn, W. Oh, K.H. Han, T.H. Um, C.R. Cho, S.Y. Han, Renoprotective effects of febuxostat compared with allopurinol in patients with hyperuricemia: a systematic review and meta-analysis, *Kidney Res. Clin. Pract.* 36 (3) (2017) 274–281.
- [126] S. Beddhu, R. Filipowicz, B. Wang, G. Wei, X. Chen, A.C. Roy, S.L. DuVall, H. Farrukh, A.N. Habib, T. Bjordahl, D.L. Simmons, M. Munger, G. Stoddard, D.E. Kohan, T. Greene, Y. Huang, A randomized controlled trial of the effects of febuxostat therapy on adipokines and markers of kidney fibrosis in asymptomatic hyperuricemic patients with diabetic nephropathy, *Can. J. Kidney Health Dis.* 3 (2016) (2054358116675343).
- [127] L. Gnudi, J. Karalliedde, Beat it early: putative renoprotective haemodynamic effects of oral hypoglycaemic agents, *Nephrol. Dial. Transplant.* 31 (7) (2016) 1036–1043.
- [128] C. Wanner, S.E. Inzucchi, J.M. Lachin, D. Fitchett, M. von Eynatten, M. Mattheus, O.E. Johansen, H.J. Woerle, U.C. Broedl, B. Zinman, E.-R.O. Investigators, Empagliflozin and progression of kidney disease in type 2 diabetes, *N. Engl. J. Med.* 375 (4) (2016) 323–334.
- [129] D.Z.I. Cherney, B. Zinman, S.E. Inzucchi, A. Koitka-Weber, M. Mattheus, M. von Eynatten, C. Wanner, Effects of empagliflozin on the urinary albumin-to-creatinine ratio in patients with type 2 diabetes and established cardiovascular disease: an exploratory analysis from the EMPA-REG OUTCOME randomised, placebo-controlled trial, *Lancet Diabetes Endocrinol.* 5 (8) (2017) 610–621.
- [130] S. P. C. Dekkers, G.D. Laverman, R.T. Gansevoort, H.J. Lambers Heerspink, Effect of the SGLT-2 inhibitor dapagliflozin on glomerular and tubular injury markers, American Society of Nephrology (ASN) Kidney Week 2017 Annual Meeting, New Orleans, 2017.
- [131] Q. Ma, S. Steiger, H.J. Anders, Sodium glucose transporter-2 inhibition has no renoprotective effects on non-diabetic chronic kidney disease, *Physiol. Rep.* 5 (7) (2017).
- [132] M.H. Muskiet, M.M. Smits, L.M. Morsink, M. Diamant, The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? *Nat. Rev. Nephrol.* 10 (2) (2014) 88–103.
- [133] M. Gangadharan Komala, S. Gross, A. Zaky, C. Pollock, U. Panchapakesan, Saxagliptin reduces renal tubulointerstitial inflammation, hypertrophy and fibrosis in diabetes, *Nephrology (Carlton)* 21 (5) (2016) 423–431.
- [134] S. Shi, S.P. Srivastava, M. Kanasaki, J. He, M. Kitada, T. Nagai, K. Nitta, S. Takagi, K. Kanasaki, D. Koya, Interactions of DPP-4 and integrin beta1 influences endothelial-to-mesenchymal transition, *Kidney Int.* 88 (3) (2015) 479–489.
- [135] J.A. Udell, D.L. Bhatt, E. Braunwald, M.A. Cavender, O. Mosenson, P.G. Steg, J.A. Davidson, J.C. Nicolau, R. Corbalan, B. Hirshberg, R. Frederich, K. Im, A.A. Umez-Eronini, P. He, D.K. McGuire, L.A. Leiter, I. Raz, B.M. Scirica, S.-T.S. Committee Investigators, Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes and moderate or severe renal impairment: observations from the SAVOR-TIMI 53 Trial, *Diabetes Care* 38 (4) (2015) 696–705.
- [136] I. Goldshtein, A. Karasik, C. Melzer-Cohen, S.S. Engel, S. Yu, O. Sharon, K. Brodovicz, N. Gadir, H.L. Katzeff, L. Radican, G. Chodick, V. Shalev, K. Tunceli, Urinary albumin excretion with sitagliptin compared to sulfonylurea as add on to metformin in type 2 diabetes patients with albuminuria: a real-world evidence study, *J. Diabetes Complicat.* 30 (7) (2016) 1354–1359.
- [137] H. Zhang, X. Zhang, C. Hu, W. Lu, Exenatide reduces urinary transforming growth factor-beta1 and type IV collagen excretion in patients with type 2 diabetes and microalbuminuria, *Kidney Blood Press. Res.* 35 (6) (2012) 483–488.
- [138] S. Imamura, K. Hirai, A. Hirai, The glucagon-like peptide-1 receptor agonist, liraglutide, attenuates the progression of overt diabetic nephropathy in type 2 diabetic patients, *Tohoku J. Exp. Med.* 231 (1) (2013) 57–61.
- [139] M. Zavattaro, M. Caputo, M.T. Sama, C. Mele, L. Chasseur, P. Marzullo, L. Pagano, M.G. Mauri, M.C. Ponziani, G. Aimaretti, F. Prodham, One-year treatment with liraglutide improved renal function in patients with type 2 diabetes: a pilot prospective study, *Endocrine* 50 (3) (2015) 620–626.
- [140] V.G. Athyros, N. Katsiki, N. Tentolouris, Editorial: do some glucagon-like-peptide-1 receptor agonists (GLP-1 RA) reduce macrovascular complications of type 2 diabetes mellitus? A commentary on the liraglutide effect and action in diabetes: evaluation of cardiovascular outcome results (LEADER) trial, *Curr. Vasc. Pharmacol.* 14 (5) (2016) 469–473.
- [141] J.F.E. Mann, D.D. Orsted, K. Brown-Frandsen, S.P. Marso, N.R. Poulter, S. Rasmussen, K. Tornoe, B. Zinman, J.B. Buse, L.S. Committee Investigators, Liraglutide and renal outcomes in type 2 diabetes, *N. Engl. J. Med.* 377 (9) (2017) 839–848.
- [142] T. Hiramatsu, A. Ozeki, H. Ishikawa, S. Furuta, Long term effects of liraglutide in Japanese patients with type 2

- diabetes among the subgroups with different renal functions: results of 2-year prospective study, *Drug Res. (Stuttg.)* 67 (11) (2017) 640–646.
- [143] B.J. von Scholten, F. Persson, S. Rosenlund, P. Hovind, J. Faber, T.W. Hansen, P. Rossing, The effect of liraglutide on renal function: a randomized clinical trial, *Diabetes Obes. Metab.* 19 (2) (2017) 239–247.
- [144] P.H. Groop, M.E. Cooper, V. Perkovic, B. Hocher, K. Kanasaki, M. Haneda, G. Scherthner, K. Sharma, R.C. Stanton, R. Toto, J. Cescutti, M. Gordat, T. Meinicke, A. Koitka-Weber, S. Thiemann, M. von Eynatten, Linagliptin and its effects on hyperglycaemia and albuminuria in patients with type 2 diabetes and renal dysfunction: the randomized MARLINA-T2D trial, *Diabetes Obes. Metab.* 19 (11) (2017) 1610–1619.
- [145] M.J. Davies, S.C. Bain, S.L. Atkin, P. Rossing, D. Scott, M. S. Shamkhalova, H. Bosch-Traberg, A. Syren, G.E. Umpierrez, Efficacy and safety of liraglutide versus placebo as add-on to glucose-lowering therapy in patients with type 2 diabetes and moderate renal impairment (LIRA-RENAL): a randomized clinical trial, *Diabetes Care* 39 (2) (2016) 222–230.
- [146] M. Pawaskar, K.R. Tuttle, Q. Li, J.H. Best, P.W. Anderson, Observational study of kidney function and albuminuria in patients with type 2 diabetes treated with exenatide BID versus insulin glargine, *Ann. Pharmacother.* 48 (5) (2014) 571–576.
- [147] H. Zou, B. Zhou, G. Xu, SGLT2 inhibitors: a novel choice for the combination therapy in diabetic kidney disease, *Cardiovasc. Diabetol.* 16 (1) (2017) 65.
- [148] D. de Zeeuw, R. Agarwal, M. Amdahl, P. Audhya, D. Coyne, T. Garimella, H.H. Parving, Y. Pritchett, G. Remuzzi, E. Ritz, D. Andress, Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial, *Lancet* 376 (9752) (2010) 1543–1551.
- [149] C.R. Doorenbos, J. van den Born, G. Navis, M.H. de Borst, Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism, *Nat. Rev. Nephrol.* 5 (12) (2009) 691–700.
- [150] S. Lucisano, M. Buemi, A. Passantino, C. Aloisi, V. Cernaro, D. Santoro, New insights on the role of vitamin D in the progression of renal damage, *Kidney Blood Press. Res.* 37 (6) (2013) 667–678.
- [151] K. Mirkovic, A.S. Frenay, J. van den Born, H. van Goor, G. Navis, M.H. de Borst, N. Consortium, Sodium restriction potentiates the renoprotective effects of combined vitamin D receptor activation and angiotensin-converting enzyme inhibition in established proteinuric nephropathy, *Nephrol. Dial. Transplant.* 32 (8) (2017) 1293–1301.
- [152] M.H. de Borst, R. Hajhosseiny, H. Tamez, J. Wenger, R. Thadhani, D.J. Goldsmith, Active vitamin D treatment for reduction of residual proteinuria: a systematic review, *J. Am. Soc. Nephrol.* 24 (11) (2013) 1863–1871.
- [153] J. Zhao, J. Dong, H. Wang, H. Shang, D. Zhang, L. Liao, Efficacy and safety of vitamin D3 in patients with diabetic nephropathy: a meta-analysis of randomized controlled trials, *Chin. Med. J.* 127 (15) (2014) 2837–2843.
- [154] P.E. Pergola, Weekly Doses of GCS-100, a Galectin-3 Antagonist, Resulted in Significant Improvement in eGFR in Patients With CKD in a Randomized, 2014.
- [155] A. Bobik, The structural basis of hypertension: vascular remodelling, rarefaction and angiogenesis/arteriogenesis, *J. Hypertens.* 23 (8) (2005) 1473–1475.
- [156] S. Xavier, R. Vasko, K. Matsumoto, J.A. Zullo, R. Chen, J. Maizel, P.N. Chander, M.S. Goligorsky, Curtailing endothelial TGF-beta signaling is sufficient to reduce endothelial-mesenchymal transition and fibrosis in CKD, *J. Am. Soc. Nephrol.* 26 (4) (2015) 817–829.
- [157] L. Fang, T. Radovits, G. Szabo, M.M. Mozes, L. Rosivall, G. Kokeny, Selective phosphodiesterase-5 (PDE-5) inhibitor vardenafil ameliorates renal damage in type 1 diabetic rats by restoring cyclic 3',5' guanosine monophosphate (cGMP) level in podocytes, *Nephrol. Dial. Transplant.* 28 (7) (2013) 1751–1761.
- [158] Y. Kuno, M. Iyoda, T. Shibata, Y. Hirai, T. Akizawa, Sildenafil, a phosphodiesterase type 5 inhibitor, attenuates diabetic nephropathy in non-insulin-dependent Otsuka Long-Evans Tokushima Fatty rats, *Br. J. Pharmacol.* 162 (6) (2011) 1389–1400.
- [159] B. Rodriguez-Iturbe, A. Ferrebuz, V. Vanegas, Y. Quiroz, F. Espinoza, H. Pons, N.D. Vaziri, Early treatment with cGMP phosphodiesterase inhibitor ameliorates progression of renal damage, *Kidney Int.* 68 (5) (2005) 2131–2142.
- [160] B. Afsar, A. Ortiz, A. Covic, A. Gaipov, T. Esen, D. Goldsmith, M. Kanbay, Phosphodiesterase type 5 inhibitors and kidney disease, *Int. Urol. Nephrol.* 47 (9) (2015) 1521–1528.
- [161] S.L. Lin, Y.M. Chen, W.C. Chiang, K.D. Wu, T.J. Tsai, Effect of pentoxifylline in addition to losartan on proteinuria and GFR in CKD: a 12-month randomized trial, *Am. J. Kidney Dis.* 52 (3) (2008) 464–474.
- [162] R.M. Perkins, M.C. Aboudara, A.L. Uy, S.W. Olson, H.M. Cushner, C.M. Yuan, Effect of pentoxifylline on GFR decline in CKD: a pilot, double-blind, randomized, placebo-controlled trial, *Am. J. Kidney Dis.* 53 (4) (2009) 606–616.
- [163] S.J. Han, H.J. Kim, D.J. Kim, S.S. Sheen, C.H. Chung, C.W. Ahn, S.H. Kim, Y.W. Cho, S.W. Park, S.K. Kim, C.S. Kim, K. W. Kim, K.W. Lee, Effects of pentoxifylline on proteinuria and glucose control in patients with type 2 diabetes: a prospective randomized double-blind multicenter study, *Diabetol. Metab. Syndr.* 7 (2015) 64.
- [164] M. Rodriguez-Moran, F. Guerrero-Romero, Pentoxifylline is as effective as captopril in the reduction of microalbuminuria in non-hypertensive type 2 diabetic patients—a randomized, equivalent trial, *Clin. Nephrol.* 64 (2) (2005) 91–97.
- [165] A. Ghorbani, B. Omidvar, S.S. Beladi-Mousavi, E. Lak, S. Vaziri, The effect of pentoxifylline on reduction of proteinuria among patients with type 2 diabetes under blockade of angiotensin system: a double blind and randomized clinical trial, *Nefrologia* 32 (6) (2012) 790–796.
- [166] J. Roozbeh, M.A. Banihashemi, M. Ghezlou, R. Afshariani, S. Salari, M. Moini, M.M. Sagheb, Captopril and combination therapy of captopril and pentoxifylline in reducing proteinuria in diabetic nephropathy, *Ren. Fail.* 32 (2) (2010) 172–178.
- [167] J.F. Navarro-Gonzalez, C. Mora-Fernandez, M. Muros de Fuentes, J. Chahin, M.L. Mendez, E. Gallego, M. Macia, N. del Castillo, A. Rivero, M.A. Getino, P. Garcia, A. Jarque, J. Garcia, Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial, *J. Am. Soc. Nephrol.* 26 (1) (2015) 220–229.
- [168] M. Goicoechea, S. Garcia de Vinuesa, B. Quiroga, U. Verdalles, D. Barraca, C. Yuste, N. Panizo, E. Verde, M.A. Munoz, J. Luno, Effects of pentoxifylline on inflammatory parameters in chronic kidney disease patients: a randomized trial, *J. Nephrol.* 25 (6) (2012) 969–975.

- [169] S. Shahidi, M. Hoseinbalam, B. Iraj, M. Akbari, Effect of pentoxifylline on microalbuminuria in diabetic patients: a randomized controlled trial, *Int. J. Nephrol.* 2015 (2015) 259592.
- [170] C.J. Diskin, T.J. Stokes, L.M. Dansby, L. Radcliff, T.B. Carter, Will the addition of pentoxifylline reduce proteinuria in patients with diabetic glomerulosclerosis refractory to maximal doses of both an angiotensin-converting enzyme inhibitor and an angiotensin receptor blocker? *J. Nephrol.* 20 (4) (2007) 410–416.
- [171] S. D., B. Singh, S.E. Pergola, J.E. Shipley, L. Wu, L.A. Sabounjian, P.B. Graham, Effect of CTP-499 on renal function in patients with type 2 diabetes and kidney disease, *Am. J. Kidney Dis.* 63 (5) (2014) B120.
- [172] W. Scheele, S. Diamond, J. Gale, V. Clerin, N. Tamimi, V. Le, R. Walley, F. Grover-Paez, C. Perros-Huguet, T. Rolph, M. El Nahas, Phosphodiesterase type 5 inhibition reduces albuminuria in subjects with overt diabetic nephropathy, *J. Am. Soc. Nephrol.* 27 (11) (2016) 3459–3468.
- [173] J.S. Kim, B.G. Han, S.O. Choi, S.K. Cha, Secondary focal segmental glomerulosclerosis: from podocyte injury to glomerulosclerosis, *Biomed. Res. Int.* 2016 (2016) 1630365.
- [174] C. Canadian Diabetes Association Clinical Practice Guidelines Expert, P. McFarlane, R.E. Gilbert, L. MacCallum, P. Senior, Chronic kidney disease in diabetes, *Can. J. Diabetes* 37 (Suppl. 1) (2013) S129–36.
- [175] G.B. Piccoli, G. Grassi, G. Cabiddu, M. Nazha, S. Roggero, I. Capizzi, A. De Pascale, A.M. Priola, C. Di Vico, S. Maxia, V. Loi, A.M. Asunis, A. Pani, A. Veltri, Diabetic kidney disease: a syndrome rather than a single disease, *Rev. Diabet. Stud.* 12 (1–2) (2015) 87–109.
- [176] A.B. Farris, C.E. Alpers, What is the best way to measure renal fibrosis?: a pathologist's perspective, *Kidney Int. Suppl.* (2011) 4 (1) (2014) 9–15.
- [177] S. Hwang, J. Park, J. Kim, H.R. Jang, G.Y. Kwon, W. Huh, Y.G. Kim, D.J. Kim, H.Y. Oh, J.E. Lee, Tissue expression of tubular injury markers is associated with renal function decline in diabetic nephropathy, *J. Diabetes Complicat.* 31 (12) (2017) 1704–1709.
- [178] E.G.D. Stribos, S.H. Nielsen, S. Brix, M.A. Karsdal, M.A. Seelen, H. van Goor, S.J.L. Bakker, P. Olinga, H.A.M. Mutsaers, F. Genovese, Non-invasive quantification of collagen turnover in renal transplant recipients, *PLoS One* 12 (4) (2017) e0175898.
- [179] J.H. Ix, R. Katz, N. Bansal, M. Foster, D.E. Weiner, R. Tracy, V. Jotwani, J. Hughes-Austin, D. McKay, F. Gabbai, C.Y. Hsu, A. Bostom, A.S. Levey, M.G. Shlipak, Urine fibrosis markers and risk of allograft failure in kidney transplant recipients: a case-cohort ancillary study of the FAVORIT trial, *Am. J. Kidney Dis.* 69 (3) (2017) 410–419.
- [180] F. Genovese, A.A. Manresa, D.J. Leeming, M.A. Karsdal, P. Boor, The extracellular matrix in the kidney: a source of novel non-invasive biomarkers of kidney fibrosis? *Fibrogenesis Tissue Repair* 7 (1) (2014) 4.
- [181] M.G. Wong, C.A. Pollock, Biomarkers in kidney fibrosis: are they useful? *Kidney Int. Suppl.* (2011) 4 (1) (2014) 79–83.
- [182] A.Y. Chu, A. Tin, P. Schlosser, Y.A. Ko, C. Qiu, C. Yao, R. Joehanes, M.E. Grams, L. Liang, C.A. Gluck, C. Liu, J. Coresh, S.J. Hwang, D. Levy, E. Boerwinkle, J.S. Pankow, Q. Yang, M. Fornage, C.S. Fox, K. Susztak, A. Kottgen, Epigenome-wide association studies identify DNA methylation associated with kidney function, *Nat. Commun.* 8 (1) (2017) 1286.
- [183] N. Ledo, Y.A. Ko, A.S. Park, H.M. Kang, S.Y. Han, P. Choi, K. Susztak, Functional genomic annotation of genetic risk loci highlights inflammation and epithelial biology networks in CKD, *J. Am. Soc. Nephrol.* 26 (3) (2015) 692–714.
- [184] P. Romagnani, S. Giglio, M.L. Angelotti, A. Provenzano, F. Becherucci, B. Mazzinghi, S. Muller, K. Amann, M. Weidenbusch, S. Romoli, E. Lazzeri, H.J. Anders, Next generation sequencing and functional analysis of patient urine renal progenitor-derived podocytes to unravel the diagnosis underlying refractory lupus nephritis, *Nephrol. Dial. Transplant.* 31 (9) (2016) 1541–1545.
- [185] X. Lan, A. Jhaveri, K. Cheng, H. Wen, M.A. Saleem, P.W. Mathieson, J. Mikulak, S. Aviram, A. Malhotra, K. Skorecki, P.C. Singhal, APOL1 risk variants enhance podocyte necrosis through compromising lysosomal membrane permeability, *Am. J. Physiol. Ren. Physiol.* 307 (3) (2014) F326–36.
- [186] B. Tampe, M. Zeisberg, Contribution of genetics and epigenetics to progression of kidney fibrosis, *Nephrol. Dial. Transplant.* 29 (Suppl. 4) (2014) iv72–9.
- [187] M. Zeisberg, E.M. Zeisberg, Precision renal medicine: a roadmap towards targeted kidney fibrosis therapies, *Fibrogenesis Tissue Repair* 8 (2015) 16.
- [188] P. Tessarz, T. Kouzarides, Histone core modifications regulating nucleosome structure and dynamics, *Nat. Rev. Mol. Cell Biol.* 15 (11) (2014) 703–708.
- [189] W. Bechtel, S. McGoohan, E.M. Zeisberg, G.A. Muller, H. Kalbacher, D.J. Salant, C.A. Muller, R. Kalluri, M. Zeisberg, Methylation determines fibroblast activation and fibrogenesis in the kidney, *Nat. Med.* 16 (5) (2010) 544–550.
- [190] B. Tampe, D. Tampe, C.A. Muller, H. Sugimoto, V. LeBleu, X. Xu, G.A. Muller, E.M. Zeisberg, R. Kalluri, M. Zeisberg, Tet3-mediated hydroxymethylation of epigenetically silenced genes contributes to bone morphogenic protein 7-induced reversal of kidney fibrosis, *J. Am. Soc. Nephrol.* 25 (5) (2014) 905–912.
- [191] B. Tampe, D. Tampe, E.M. Zeisberg, G.A. Muller, W. Bechtel-Walz, M. Koziolok, R. Kalluri, M. Zeisberg, Induction of Tet3-dependent epigenetic remodeling by low-dose hydralazine attenuates progression of chronic kidney disease, *EBioMedicine* 2 (1) (2015) 19–36.
- [192] G. Sun, M.A. Reddy, H. Yuan, L. Lanting, M. Kato, R. Natarajan, Epigenetic histone methylation modulates fibrotic gene expression, *J. Am. Soc. Nephrol.* 21 (12) (2010) 2069–2080.
- [193] K. Sasaki, S. Doi, A. Nakashima, T. Irifuku, K. Yamada, K. Kokoroishi, T. Ueno, T. Doi, E. Hida, K. Arihiro, N. Kohno, T. Masaki, Inhibition of SET domain-containing lysine methyltransferase 7/9 ameliorates renal fibrosis, *J. Am. Soc. Nephrol.* 27 (1) (2016) 203–215.
- [194] H. Shima, K. Sasaki, T. Suzuki, C. Mukawa, T. Obara, Y. Oba, A. Matsuo, T. Kobayashi, E. Mishima, S. Watanabe, Y. Akiyama, K. Kikuchi, T. Matsuhashi, Y. Oikawa, F. Nanto, Y. Akiyama, H.J. Ho, C. Suzuki, D. Saigusa, A. Masamune, Y. Tomioka, T. Masaki, S. Ito, K.I. Hayashi, T. Abe, A novel indole compound MA-35 attenuates renal fibrosis by inhibiting both TNF-alpha and TGF-beta1 pathways, *Sci. Rep.* 7 (1) (2017) 1884.
- [195] C.A. Musselman, M.E. Lalonde, J. Cote, T.G. Kutateladze, Perceiving the epigenetic landscape through histone readers, *Nat. Struct. Mol. Biol.* 19 (12) (2012) 1218–1227.
- [196] T. Matsuhashi, T. Sato, S.I. Kanno, T. Suzuki, A. Matsuo, Y. Oba, M. Kikusato, E. Ogasawara, T. Kudo, K. Suzuki, O. Ohara, H. Shimbo, F. Nanto, H. Yamaguchi, D. Saigusa, Y.

- Mukaiyama, A. Watabe, K. Kikuchi, H. Shima, E. Mishima, Y. Akiyama, Y. Oikawa, H.O. Hsin-Jung, Y. Akiyama, C. Suzuki, M. Uematsu, M. Ogata, N. Kumagai, M. Toyomizu, A. Hozawa, N. Mano, Y. Owada, S. Aiba, T. Yanagisawa, Y. Tomioka, S. Kure, S. Ito, K. Nakada, K.I. Hayashi, H. Osaka, T. Abe, Mitochondrial acid 5 (MA-5) facilitates ATP synthase oligomerization and cell survival in various mitochondrial diseases, *EBioMedicine* 20 (2017) 27–38.
- [197] B. Kaissling, M. Lehir, W. Kriz, Renal epithelial injury and fibrosis, *Biochim. Biophys. Acta* 1832 (7) (2013) 931–939.
- [198] T. Suzuki, M. Kimura, M. Asano, Y. Fujigaki, A. Hishida, Role of atrophic tubules in development of interstitial fibrosis in microembolism-induced renal failure in rat, *Am. J. Pathol.* 158 (1) (2001) 75–85.
- [199] D.F. Sun, Y. Fujigaki, T. Fujimoto, K. Yonemura, A. Hishida, Possible involvement of myofibroblasts in cellular recovery of uranyl acetate-induced acute renal failure in rats, *Am. J. Pathol.* 157 (4) (2000) 1321–1335.
- [200] W. Zhou, R.C. Boucher, F. Bollig, C. Englert, F. Hildebrandt, Characterization of mesonephric development and regeneration using transgenic zebrafish, *Am. J. Physiol. Ren. Physiol.* 299 (5) (2010) F1040–7.
- [201] C.Q. Diep, D. Ma, R.C. Deo, T.M. Holm, R.W. Naylor, N. Arora, R.A. Wingert, F. Bollig, G. Djordjevic, B. Lichman, H. Zhu, T. Ikenaga, F. Ono, C. Englert, C.A. Cowan, N.A. Hukriede, R.I. Handin, A.J. Davidson, Identification of adult nephron progenitors capable of kidney regeneration in zebrafish, *Nature* 470 (7332) (2011) 95–100.
- [202] H.A. Hartman, H.L. Lai, L.T. Patterson, Cessation of renal morphogenesis in mice, *Dev. Biol.* 310 (2) (2007) 379–387.
- [203] L. Lasagni, M.L. Angelotti, E. Ronconi, D. Lombardi, S. Nardi, A. Peired, F. Becherucci, B. Mazzinghi, A. Sisti, S. Romoli, A. Burger, B. Schaefer, A. Buccoliero, E. Lazzeri, P. Romagnani, Podocyte regeneration driven by renal progenitors determines glomerular disease remission and can be pharmacologically enhanced, *Stem Cell Rep.* 5 (2) (2015) 248–263.
- [204] E. Lazzeri, M.L. Angelotti, A. Peired, C. Conte, J.A. Marschner, L. Maggi, B. Mazzinghi, D. Lombardi, M.A. Melica, S. Nardi, E. Ronconi, A. Sisti, G. Antonelli, F. Becherucci, L. De Chiara, R.R. Guevara, A. Burger, B. Schaefer, F. Annunziato, H.J. Anders, L. Lasagni, P. Romagnani, Endocycle-related tubular cell hypertrophy and progenitor proliferation recover renal function after acute kidney injury, *Nat. Commun.* 9 (1) (2018) 1344.
- [205] M.L. Angelotti, E. Ronconi, L. Ballerini, A. Peired, B. Mazzinghi, C. Sagrinati, E. Parente, M. Gacci, M. Carini, M. Rotondi, A.B. Fogo, E. Lazzeri, L. Lasagni, P. Romagnani, Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury, *Stem Cells* 30 (8) (2012) 1714–1725.
- [206] A. Peired, M.L. Angelotti, E. Ronconi, G. la Marca, B. Mazzinghi, A. Sisti, D. Lombardi, E. Giocaliere, M. Della Bona, F. Villanelli, E. Parente, L. Ballerini, C. Sagrinati, N. Wanner, T.B. Huber, H. Liapis, E. Lazzeri, L. Lasagni, P. Romagnani, Proteinuria impairs podocyte regeneration by sequestering retinoic acid, *J. Am. Soc. Nephrol.* 24 (11) (2013) 1756–1768.
- [207] W. Pichaiwong, K.L. Hudkins, T. Wietecha, T.Q. Nguyen, C. Tachaudomdach, W. Li, B. Askari, T. Kobayashi, K.D. O'Brien, J.W. Pippin, S.J. Shankland, C.E. Alpers, Reversibility of structural and functional damage in a model of advanced diabetic nephropathy, *J. Am. Soc. Nephrol.* 24 (7) (2013) 1088–1102.
- [208] B.A. Edgar, N. Zielke, C. Gutierrez, Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth, *Nat. Rev. Mol. Cell Biol.* 15 (3) (2014) 197–210.
- [209] E.D. de Groh, L.M. Swanhart, C.C. Cosentino, R.L. Jackson, W. Dai, C.A. Kitchens, B.W. Day, T.E. Smithgall, N.A. Hukriede, Inhibition of histone deacetylase expands the renal progenitor cell population, *J. Am. Soc. Nephrol.* 21 (5) (2010) 794–802.
- [210] T. Novitskaya, L. McDermott, K.X. Zhang, T. Chiba, P. Paueksakon, N.A. Hukriede, M.P. de Caestecker, A PTBA small molecule enhances recovery and reduces postinjury fibrosis after aristolochic acid-induced kidney injury, *Am. J. Physiol. Ren. Physiol.* 306 (5) (2014) F496–504.
- [211] Genetic Engineering & Biotechnology News, Xoma's gevokizumab fails Phase iii trial, <https://www.genengnews.com/gen-news-highlights/xoma-s-gevokizumab-fails-phase-iii-trial/81251535/> 2015, Accessed date: 22 July 2015.