Anti-fibrotic treatments: A review of clinical evidence

Review

Anti-fibrotic treatments: A review of clinical evidence

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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.

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].

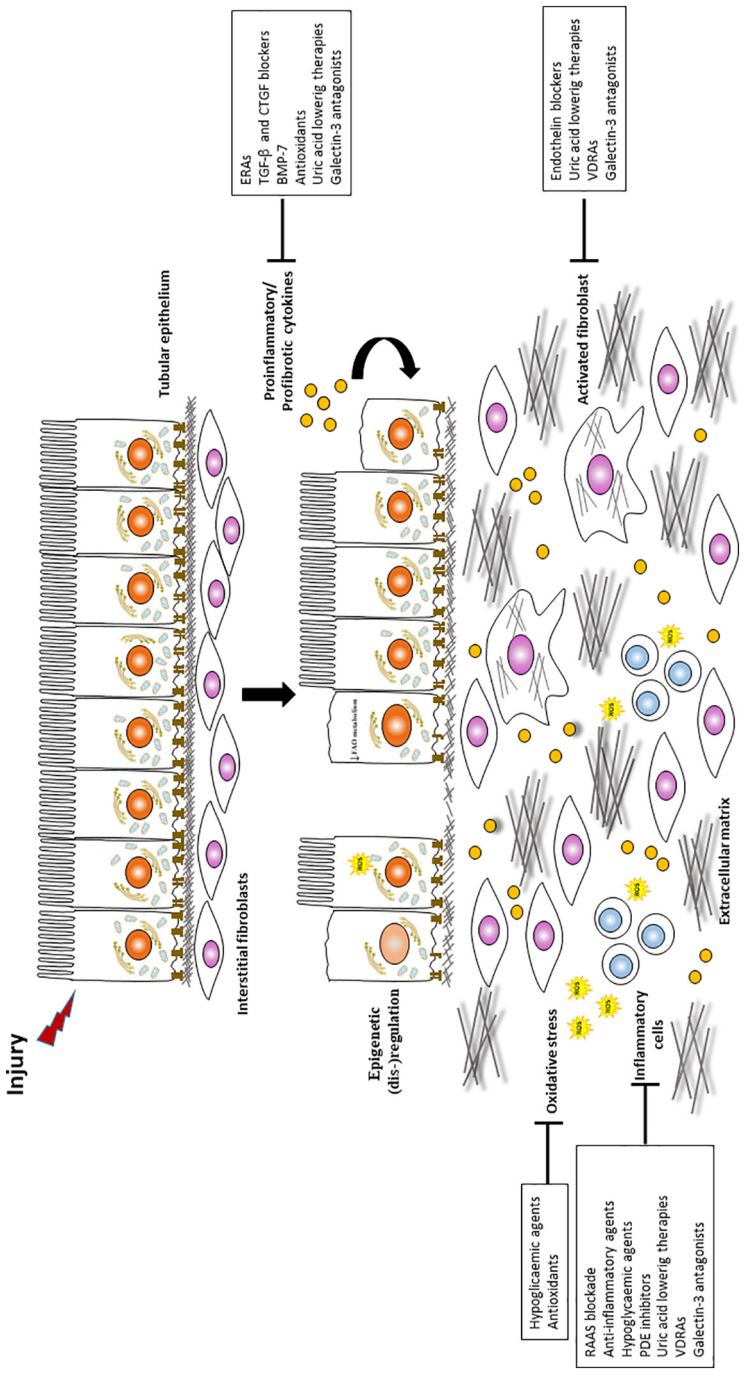

Fig. 1. Schematic representation of fibrosis progression. Summary of the different players involved in the process of fibrosis. The pharmacological interventions (boxes on the side) employed so far are represented in relation to the respective putative targets.

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	NFkB blockers	ACTHar gel		DKD	Slowed the rate down of GFR decrease, reduced proteinuria		28
		ACTHar gel		DKD		Ongoing	NCT01028287
		Bindarit		DKD	Reduced albuminuria		29
							NCT01109212
	TNF-α blockers	Adalimumab		Primary FSGS	No effects on proteinuria reduction		33, 34
	JAK-STAT inhibitors	Baricitinib		DKD	Reduced albuminuria, no changes in kidney function		39
	Chemokine antagonists	Emapticap pegol		DKD	Reduced albuminuria		43
		PF-04634817			No effects on proteinuria reduction or on kidney function		NCT01712061
		CCX140-B	Yes	DKD	Reduced albuminuria		46
	Leukocyte migration inhibitors	Colchicine		Renal transplant	Prevent the development of interstitial fibrosis		54
		ASP8232	Yes	DKD, CKD		Ongoing	NCT02358096
	PPAR antagonists	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		DKD	No effects on albuminuria reduction		75, NCT02010242
		Selonsertib		DKD		Ongoing	76
	Nrf2 activator	Bardoxolone		DKD, CKD Alport	Slowed the rate down of GFR decrease, increased albuminuria		85, 93
		Bardoxolone		DKD	Slowed the rate down of GFR decrease		86, 92
		Bardoxolone		DKD, CKD		Ongoing	NCT02316821
		Bardoxolone		Alport		Ongoing	NCT03019185
Activation phase	Endothelin receptor antagonists	Atrasentan	Yes	DKD	Reduced albuminuria		98, 99
		Atrasentan	Yes	DKD		Ongoing	NCT01424319
		Avosentan	Yes	DKD	Reduced albuminuria		96
		Sparsentan	Yes	FSGS	Reduced proteinuria		101
		TAK-044		CKD	No effects on GFR decline		95
Execution phase	TGF-β blockers	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
		Pirfenidone		DKD	No changes in kidney function		114
		Pirfenidone		CKD		Ongoing	NCT02408744
		Pirfenidone		DKD		Ongoing	NCT02689778
	CTGF blockers	FG-3019		DKD	Reduced microalbuminuria		115
	BMP-7 agonists	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
		Febuxostat		DKD	No effects on proteinuria or GFR decline		126
	SGLT-2 inhibitors	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	NCT02376075, NCT01897532
	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	NCT01377285, 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	NFkB 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
		Bardoxolone	Nrf2	Yes	DKD, CKD	Prematurely terminated for high rate of cardiovascular events	Did not reduce the risk of ESRD or death	89
		Gevokizumab	IL-1β		DKD	Prematurely terminated		[211]
Activation and execution phase	Endothelin receptor antagonists	Atrasentan	ET-1	Yes	DKD, CKD		Prematurely stopped due to lack of efficacy	NCT01858532
		Avosentan	ET-1	Yes	DKD	Trial stopped because of fluid retention	Reduced albuminuria	97
	Integrin blockers	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		NCT00782561
		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 chemotactic protein-1; ET-1, endothelin-1.

NF-κB blockers. Nuclear factor-kappaB (NF-κB) 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 adrenocorticotropic hormone (ACTH). Previous studies demonstrate that ACTH inhibits NF-κB 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-κB 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-κB 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 superfamily 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 α5β6 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-α5β6 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-κB 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 VDRAs 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 VDRAs in monotherapy have been conflicting, recent clinical trials confirmed the anti-proteinuric and anti-fibrotic capacity of VDRAs [152,153], especially during RAAS blockade and/or sodium restriction [148]. This would suggest that the combination of RAAS blockade, dietary sodium restriction and VDRAs 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 summaries all those hereditable 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].

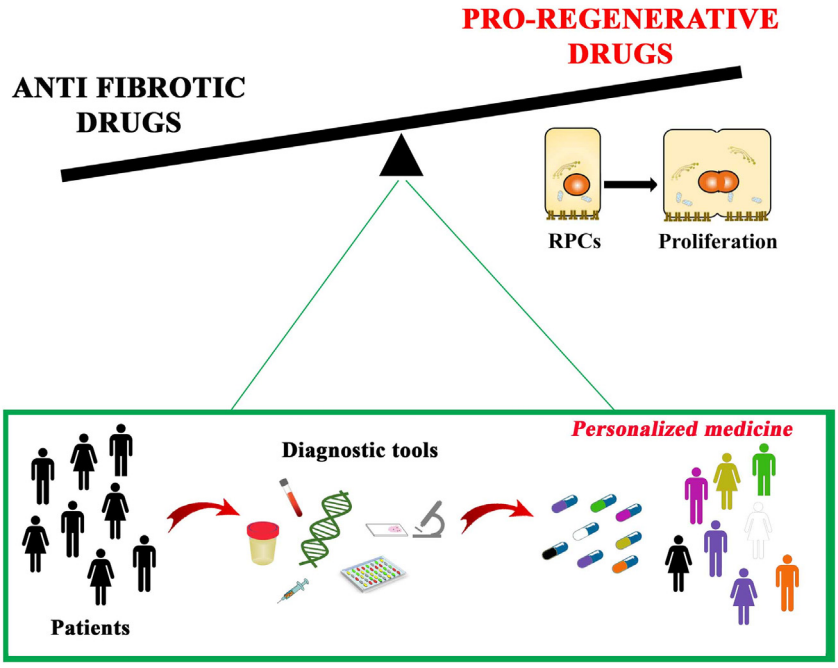

Fig. 2. Future directions and strategies. Emerging evidence suggests that single-nucleotide polymorphisms may be key determinants of CKD progression. Futures strategies should aim at favourite endogenous regeneration of the nephrons while taking into account the individual genetic variability.

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 3β (GSK3β) 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.

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