Rodent models of diabetic kidney disease: human translatability and preclinical validity

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Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD). Except for SGLT2 inhibitors and GLP-1R agonists, there have been few changes in DKD treatment over the past 25 years, when multifactorial intervention was introduced in patients with type 2 diabetes mellitus (T2DM). The unmet clinical need is partly due to the lack of animal models that replicate clinical features of human DKD, which has raised concern about the utility of these models in preclinical drug discovery. In this review, we performed a comprehensive analysis of rodent models of DKD to compare treatment efficacy from preclinical testing with outcome from clinical trials. We also investigated whether rodent models are predictive for clinical outcomes of therapeutic agents in human DKD.

Introduction: diabetic kidney disease

The prevalence of diabetes has reached world-wide epidemic proportions, with 700 million people projected to have diabetes by year 2045 [1]. DKD is a microvascular complication of diabetes and the leading cause of ESRD. Approximately 50% and 25% of patients with ESRD in need of dialysis or kidney transplantation have diabetes in the USA and EU, respectively [2,3].

The development of DKD in T1DM is often divided into five clinical stages (Fig. 1), as described by Mogensen *et al.* [4]. It remains the standard classification of DKD progression, although disease development can vary considerably in patients with T2DM. The progression of DKD includes single-nephron hyperfiltration, often progressive albuminuria, declining total glomerular filtration rate (GFR), and ultimately ESRD. With the onset of diabetes, hyperglycemia induces renal damage directly or indirectly through hemodynamic changes. Although hyperglycemia is a common risk factor for DKD in patients with either T1DM or T2DM, progression to chronic kidney disease (CKD) in patients with T2DM might not be caused by diabetes alone. The global epidemic of T2DM is accompanied by a rise in obesity and aging populations. These risk factors, among others, might be the underlying causes of CKD in a fraction of patients with T2DM without DKD [5].

During the initial stage of DKD, some patients display glomerular hyperfiltration partly because of intraglomerular hypertension. The second stage is known as the 'silent' stage without a clinical phenotype but with histological abnormalities, including thickening of the glomerular basement membrane and mesangial expansion [6,7]. However, the definition is misleading because it masks ongoing single nephron hyperfiltration that leads to glomerular hypertrophy to accommodate the increased glomerular filtration pressure [8]. Single nephron hyperfiltration is further augmented by underlying nephron loss because of age, for example. Loss of podocytes and changes in the glomerular filtration barrier result in albuminuria, as often seen during the third stage of DKD, together with a decline in the total GFR [9]. The fourth stage is characterized by overt proteinuria (>300 mg albumin/24 h) [4]. In these later stages, Kimmelstiel–Wilson nodules (nodular glomerulosclerosis) are seen together with diffuse glomerulosclerosis (>50% of glomeruli are sclerotic) [6], whereas the tubulointerstitium presents with fibrosis and tubular atrophy [6,10]. The final stage is ESRD, where the total GFR is <15 ml/min/1.73 m^2, and

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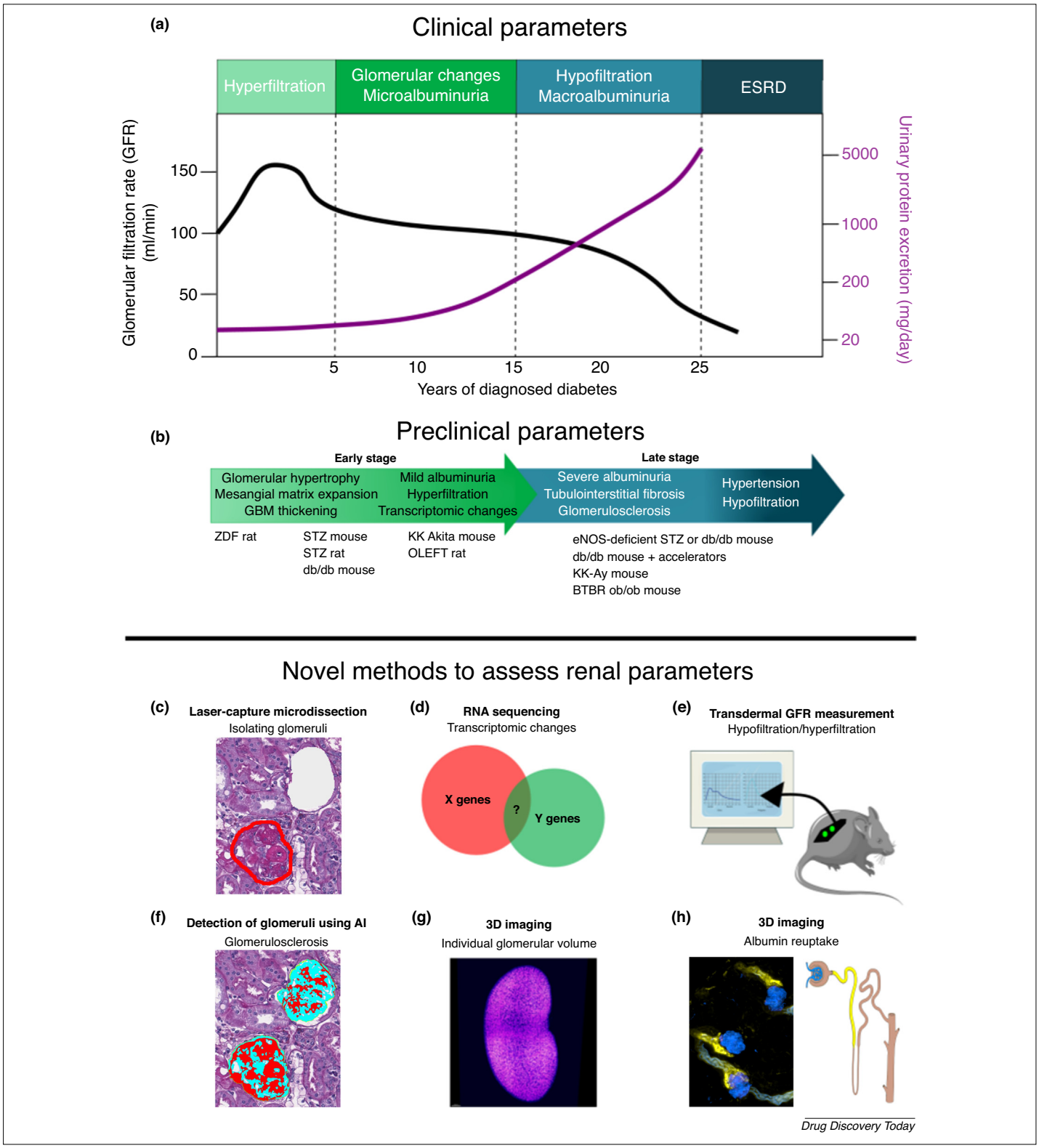

FIGURE 1

Summary of clinical and preclinical parameters and novel methods to assess them in rodent models of diabetic kidney disease (DKD). **(a)** Clinical parameters of DKD. **(b)** Preclinical parameters of DKD with rodent models of DKD divided into early and late-stage DKD. **(c)** Novel methods to assess renal parameters. Laser-capture microdissection can be used to isolate glomeruli for RNA isolation. **(d)** RNA sequencing to quantify transcriptomic changes. **(e)** Using a transdermal device for measurement of glomerular filtration rate (GFR) in rodents. **(f)** Scoring of glomerulosclerosis using machine learning/artificial intelligence (AI). **(g)** 3D imaging to assess the volume of the individual glomeruli. **(h)** Visualization of glomerular and tubular structures using an injected double-stain combined with 3D imaging. Lectin marks blood vessels in glomeruli (blue), whereas the yellow colour is albumin that is filtered in glomeruli and reabsorbed in the proximal tubules. Modified from [93] (a). Abbreviations: eNOS, endothelial nitric oxide synthase; GBM, glomerular basement membrane; STZ, streptozotocin; ZDF, Zucker diabetic fatty rat.

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patients with DKD require renal replacement therapy, including dialysis and kidney transplantation.

The clinical diagnosis of DKD is based on the presence of albuminuria and decline of the estimated GFR (eGFR) along with clinical features, such as diabetic retinopathy and diabetes duration [11]. However, in many patients, the progression of DKD does not follow the classical pattern and the lack of albuminuria or low eGFR might not necessarily preclude structural DKD [8]. Moreover, for patients with T2DM and CKD, a low number of nephrons per body mass usually precedes the onset of diabetes because of, for example, obesity or aging, resulting in progressive CKD at a faster rate in patients with T2DM compared with those with T1DM [8]. In this review, we address mouse and human DKD as primarily diabetes driven, although we acknowledge that clinical trial populations might comprise patients with diverse disease etiologies.

The current understanding of DKD progression has led to many efforts to target several features of the disease, alone or in combination with a renin–angiotensin–aldosterone system (RAAS) inhibitor. In this review, we investigate the translatability of preclinical disease models by comparing the treatment efficacy in rodent models of DKD with corresponding results from clinical studies. Furthermore, we discuss the potential of emerging drug treatments that have shown therapeutic efficacy in rodent models of DKD and are currently being tested in clinical trials.

Rodent models of diabetic kidney disease

Many rodent models are available for investigating the pathophysiology of DKD and testing of novel therapeutic strategies (Table 1). However, most of these models only exhibit early features of human DKD, such as increased total GFR, mild albuminuria, and mesangial expansion (Fig. 1). For example, the most widely used rodent model of DKD, the *db/db* mouse, demonstrates early-stage DKD pathology [12]. The susceptibility to DKD is strain dependent, with C57BL/6 J mice having low susceptibility, whereas C57BLKS/J, KK, and especially DBA/2 J mice are more prone to develop features of DKD [13–15]. The C57BLKS/J mouse strain is a genetic composite between C57BL/6 J and DBA/2 J strains. It also has alleles from other strains, and is often used as the genetic background in the *db/db* mouse model [16]. In addition to the genetic background, multiple approaches exist to accelerate diabetic renal injury. The most common approach is unilateral nephrectomy, where the removal of one kidney increases filtration load on the remaining kidney, resulting in progression of renal pathology [12,17]. Other approaches include high-protein diet regimens and induction of hypertension by renin overexpression or angiotensin II administration [18,19]. The acceleration of disease progression conferred by these approaches could improve the translatability of the models because the pathogenesis of DKD in patients is known to be multifactorial. In particular, renal mass ablation because of unilateral nephrectomy can mimic CKD in patients with T2DM because particularly older patients with diabetes may show nephron loss before onset of T2DM [8]. Knockout of the gene encoding eNOS in the *db/db* model and in streptozotocin (STZ)-treated mice causes features of more advanced DKD, including albuminuria, decreased GFR, and glomerulosclerosis, but without concomitant development of tubulointerstitial fibrosis [20,21] (Fig. 1). The *ob/ob* mouse on a BTBR background has also been associated with more advanced DKD because it has been shown to develop albuminuria along with severe pathological features of DKD [22].

Several advanced methods can be used to assess features of DKD in rodent models, complementing assessment of hyperglycemia, albuminuria, and serum creatinine (Fig. 1). Laser-capture microdissection (LCM) allows the separation of the glomerular structure from the surrounding renal tissue before RNA purification. RNA sequencing is a fast and unbiased technique to detect genes differentially expressed in a model or reversed by a therapeutic agent. Renal and glomerular hypertrophy are features of early DKD, and are usually reported as diameter or volume using 2D histology [23] or unbiased stereology [17]. However, recent developments in 3D imaging and machine learning enable volumetric analysis of individual glomeruli, improving the resolution of glomerular pathological changes in DKD progression [17,24]. Furthermore, visualization of glomerular and tubular structures can be achieved using an injected double-stain combined with 3D imaging [25]. Machine learning has also been used to improve the histopathology scoring of glomerulosclerosis in human renal biopsies [26]. These molecular and cell type-specific measurements can reveal subtle effects and mechanisms of action of drug candidates not evident from whole-body physiology, and are important for refining new treatments. As in humans, evaluation of renal function is important for characterization of kidney disease in the rodent models. Several methods have been developed to measure GFR, including creatinine clearance and fluorescein-isothiocyanate (FITC)-conjugated inulin clearance. Recently, transdermal devices that assess sinistrin clearance without the need for repeated blood sampling have successfully been applied [27], thereby providing a more sensitive and accurate measurement of GFR in animal models [28].

Preclinical studies in rodent models of diabetic kidney disease

A selection of recently completed or currently ongoing clinical trials of novel DKD therapeutics is listed in Table 2. The main renal outcomes of a subset of these clinical trials are summarized in Table 3, with comparison to corresponding data from preclinical studies in rodent models of DKD. Included is the spectrum from smaller exploratory trials, often with albuminuria outcomes, to large cardiovascular outcomes trials (CVOTs) wherein antidiabetic agents were tested for non-inferiority (and for the SGLT2i and GLP-1 analog-classes of drugs, showed superiority and clinically very relevant CV benefits), and the more recent renal outcome trials initiated following promising results in the CVOTs. The latter used CKD outcomes as primary endpoints including eGFR, progression of albuminuria, doubling of serum creatinine, incidence of ESRD (or need for renal replacement therapy), and death from renal causes.

Inhibitors of the renin–angiotensin–aldosterone system

Over the past 25 years, oral RAAS inhibitors, such as angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II type 1 receptor blockers (ARBs), have been the standard of care to control blood pressure (BP), reduce albuminuria, and slow the decline in kidney function in patients with DKD [29]. However, despite the therapeutic benefits of RAAS blockade, DKD remains the leading cause of ESRD.

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TABLE 1

Rodent models of DKD[a]

Model	Strain(s)	Induction	GFR	Albuminuria	Plasma urea	BP	Glomerular pathology	Tubulointerstitial inflammation	Tubulointerstitial fibrosis	OS
T1DM										
STZ mouse	C57BL/6J, 129SV, DBA/2J	STZ [30,69,72,94]	ND	↑	ND	↑	↑	ND	↑	↑
	C57BL/6J	STZ and eNOS$^{-/-}$ [21,30,58,73]	ND	↑	↑	↑/NS	↑/NS	↑	↑/NS	↑
		STZ and ApoE$^{-/-}$ [95,96]	ND	↑	ND	NS	↑	ND	ND	↑
Akita$^{(INS2+/C96Y)}$ mouse	C57BL/6J, KK/Ta	Genetic [59,63,70,74]	↑	↑	↑	↑/NS	↑	↑	↑	↑
NOD mouse	NOD/ShiLtJ	Genetic [97]	↑	↑	ND	↑	↑	ND	ND	ND
OVE26 mouse	FVB	Genetic [98]	ND	↑	ND	ND	↑	↑	ND	↑
STZ rat	Sprague–Dawley (SD), (Munich)-Wistar	STZ [31,40,64,71,81,87,90,92,99–104]	ND	↑/NS	↑/NS	↑/NS	↑	↑	↑	↑
		STZ and UNx [105,106]	ND	↑	↑	NS	↑	↑	↑	↑
Dahl salt-sensitive rat	–	STZ [107]	NS	↑	ND	↑	↑	ND	↑	ND
Spontaneously hypertensive rat	–	STZ [108]	↑	↑	ND	↑	ND	ND	↑	ND
Px-UNx rat	SD	Surgical [109]	NS	↑	↑	ND	↑	ND	↑	ND
Heterozygous (mREN-2) 27 rat	–	STZ [110,111]	↓	↑	ND	↑	↑	ND	↑	ND
T2DM										
db/db mouse	C57BLKS/J, C57BL/6J	Genetic [23,33–35,53,54,56,57,75,79,86,112–115]	ND	↑	NS	↑/NS/↓	↑	↑	↑	↑
	C57BLKS/J	UNx [55,116]	ND	↑	ND	NS	↑	ND	ND	ND
		ReninAAV and UNx [18]	↓	↑	↑	↑	↑	↑	↑	↑
		High-protein diet [19]	↑	↑	↑	ND	↑	ND	ND	ND
		eNOS$^{-/-}$ [20]	↓	↑	ND	↑	↑	ND	ND	ND
ob/ob mouse	BTBR	Genetic [51,60]	ND	↑	↑	↓	↑	ND	ND	ND
		ANG II (arterial hypertension) [51]	ND	↑	ND	↑	↑	ND	ND	ND
KK-Ay mouse	KK	Genetic [50,117–119]	ND	↑	ND	NS	↑	↑	ND	↑
		High-salt diet [43]	ND	↑	ND	↑	↑	↑	↑	↑
High-fat diet mouse	DBA/2J	High fat diet and STZ [120]	ND	↑	ND	NS	↑	↑	ND	ND
Zucker obese rat	–	Genetic [80,121]	ND	↑	ND	↑/NS	↑	↑	↑	↑
Zucker diabetic fatty (ZDF) rat	–	Genetic [84,85,122]	NS	↑/NS	NS	NS	↑	↑	↑	ND
Wistar fatty rat	–	Genetic [123]	ND	↑	ND	NS	↑	ND	ND	ND
ZSF-1 rat	–	Genetic [124]	NS	↑	ND	NS	↑	↑	↑	ND
		UNx [125]	↓	↑	ND	ND	ND	ND	↑	ND
Goto-Kakizaki (GK) rat	–	Genetic [126]	ND	↑	ND	↑	ND	ND	ND	ND

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TABLE 1 (*Continued*)

Model	Strain(s)	Induction	GFR	Albuminuria	Plasma urea	BP	Glomerular pathology	Tubulointerstitial inflammation	Tubulointerstitial fibrosis	OS
T2DN rat	GK substrain	Genetically modified [107,127]	↓	↑	ND	↑	↑	ND	↑	ND
Otsuka Long-Evans Tokushima Fatty (OLEFT) rat	–	Genetic [41,52]	ND	↑	ND	↑	↑	↑	↑	↑
Spontaneously Diabetic Torii fatty rat	–	Genetic [128]	ND	ND	↑	NS	↑	ND	ND	↑
STZ rat	Wistar	STZ and fructose [65]	ND	NS	ND	ND	ND	ND	↑	↑
		STZ and nicotinamide [61]	ND	↑	↑	NS	↑	ND	ND	↑

[a] Abbreviations: ANG, angiotensin; ND, not determined; NS, nonsignificant compared to nondiabetic healthy control group; OS, oxidative stress; Px, pancreatectomy; ReninAAV, renin-coding adeno-associated virus; UNx, unilateral nephrectomy; STZ, streptozotocin; ↑, increased compared to nondiabetic healthy control group; ↓, decreased compared to nondiabetic healthy control group.

Blocking of the RAAS system has been extensively investigated in a range of models of DKD. Treatment with the ACEi enalapril in STZ mice or rats reduces albuminuria and BP, as seen in patients with DKD [30,31]. Moreover, enalapril improves renal histopathology [30,31]. By contrast, no major effect was seen with administration of enalapril in the eNOS-deficient STZ mouse model of advanced DKD. The poor response to enalapril might be attributed to the inability to stimulate eNOS [32]. By contrast, treatment with the ARB telmisartan in eNOS-deficient STZ mice reduced BP and glomerular pathology [30].

Treatment with the ACEi lisinopril in uninephrectomized (UNx) and hypertensive *db/db* mice reduced BP and albuminuria [18]. Likewise, administration of the ARBs losartan and valsartan in *db/db* mice reduced BP and albuminuria [33,34], whereas telmisartan suppressed oxidative stress (OS), macrophage infiltration, and mesangial matrix expansion [35]. GFR was not measured in these rodent studies, but overall, ACEi/ARB-induced improvements in BP, albuminuria, and renal histopathology in preclinical models of early and advanced DKD replicate clinical effects seen in humans.

Mineralocorticoid receptor antagonists

The RAAS component aldosterone is a steroid hormone that activates the mineralocorticoid receptor (MR). The MR is expressed in many tissues, including the kidney, where it is involved in blood volume, sodium, and potassium homeostasis [36]. Pharmacological blockade of the MR antagonizes the action of aldosterone, resulting in reduced sodium reabsorption and increased levels of potassium in the blood. Two Phase III trials (FIDELIO-DKD [37] and FIGARO-DKD [38]) are investigating the efficacy and safety of the MR antagonist (MRA) finerenone in patients with T2DM and DKD, whereas the PRIORITY study, examining the effect of the MRA spironolactone on albuminuria progression, was recently completed [39]. Results from the PRIORITY trial showed no attenuation of progression to microalbuminuria in patients with T2DM. By contrast, data from rodent models [30,40,41] suggest that spironolactone improves DKD renal histopathology and attenuates albuminuria progression (Table 3) independently of glycemic control [30,40,41]. However, spironolactone does not affect serum creatinine levels in STZ eNOS mice [30] or STZ rats. Results from the ongoing ESAX-DN study investigating the effect of the MRA esaxerenone were recently presented and showed increased rate of remission to normoalbuminuria and reduced albuminuria progression [42]. Similarly, esaxerenone reduces albuminuria in high salt-treated T2DM KK-A^{y} mice along with decreasing BP and improving renal histopathology [43]. The apparent lack of clinical translatability in preventing albuminuria by spironolactone is unknown, but might be related to normal kidney function at baseline, a short follow-up time period or to spironolactone being a steroidal MRA [44]. Steroidal MRA increases the risk of hyperkalemia, limiting dose escalation. By contrast, esaxerenone is a second-generation and nonsteroidal MRA.

SGLT2 inhibitors

Sodium-glucose co-transporter 2 (SGLT-2) inhibitors are one of the newest additions to pharmacological interventions in diabetes. SGLT-2 inhibitors improve glycemic control by reducing tubular reabsorption of glucose, thereby increasing urinary glucose excre-

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TABLE 2

Drug classes in ongoing or recently concluded clinical trials for DKD[a]

Drug class/mechanism of action	Compounds	ClinicalTrials.gov Identifier	Phase	Trial status	Completed date (or expected)
Advanced glycation end product (AGE) inhibitor	DW1029M	NCT01935167	II	Completed	February 2015
	Pyridorin	NCT02156843	III	Terminated (strategic considerations)	March 2018
ACEi	Lisinopril, enalapril and perindopril	NCT03502031	IV	Recruiting	*(December 2020)*
Angiotensin II type 1 receptor blocker (ARB)	Losartan and valsartan	NCT03502031	IV	Recruiting	*(December 2020)*
Antifibrotic	Pirfenidone	NCT02689778	III	Active, not recruiting	*(December 2019)*
Anti-inflammatory	Colchicine	NCT02035891	I and II	Active, not recruiting	*(June 2023)*
Antioxidants	*N*-acetylcysteine	NCT00915200	II	Completed	Apr 2016
		NCT01265563	II	Completed	December 2016
Apoptosis signal-regulating kinase-1 (ASK1)/mitogen-activated protein kinase-5 (MAPK5) inhibitor	Selonsertib	NCT02177786	II	Completed	August 2016
C-C chemokine receptor type 2 and 5 (CCR2/CCR5) antagonist	BMS-813160	NCT01752985	II	Terminated (business objectives changed)	June 2015
Chymase inhibitor	Fulacimstat	NCT03412006	II	Active, not recruiting	*(October 2019)*
DPP-IV inhibitor	Linagliptin	NCT01897532	IV	Completed	January 2018
		NCT03433248	IV	Recruiting	*(December 2020)*
Endothelin A (ETA) receptor antagonist	Atrasentan	NCT01858532	III	Terminated (strategic considerations)	March 2018
Farnesoid X receptor (FXR) agonist	LMB763	NCT03804879	II	Recruiting	*(July 2021)*
GLP-1 analog	Liraglutide	NCT01179048	III	Completed	December 2015
	Semaglutide	NCT01720446	III	Completed	March 2016
		NCT03819153	III	Recruiting	*(August 2024)*
Guanylate cyclase (GC) stimulator	IW-1973	NCT03217591	II	Active, not recruiting	*(October 2019)*
MRA	Apararenone	NCT01889277	I and II	Completed	January 2015
		NCT02517320	II	Completed	January 2017
		NCT02676401	II	Completed	August 2017
	Esaxerenone	NCT02345057	II	Completed	July 2016
		NCT02807974	III	Completed	March 2017
		(JapicCTI-173695)[b]	III	Active, not recruiting	*(March 2021)*
	Finerenone	NCT02540993	III	Active, not recruiting	*(May 2020)*
		NCT02545049	III	Active, not recruiting	*(June 2021)*
	Spironolactone	NCT02040441	II and III	Completed	November 2018
NADPH oxidase (NOX) inhibitor	GKT137831	NCT02010242	II	Completed	March 2015
Nrf2 activator	Bardoxolone methyl	NCT02316821	II	Completed	September 2017
		NCT03550443	III	Recruiting	*(March 2022)*
PPAR agonist	Fenofibrate	NCT03869931	III	Recruiting	*(Apr 2021)*
Phosphodiesterase (PDE) inhibitor	CTP-499	NCT01487109	II	Completed	January 2015
	Pentoxifylline	NCT03006952	IV	Completed	September 2015
		NCT03664414	IV	Recruiting	*(December 2021)*
Renin inhibitor	TAK 272 (Imarikiren)	NCT02332824	II	Completed	August 2016

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TABLE 2 (*Continued*)

Drug class/mechanism of action	Compounds	ClinicalTrials.gov Identifier	Phase	Trial status	Completed date (or expected)
SGLT-2 inhibitor	Canagliflozin	NCT01032629	III	Completed	February 2017
		NCT01989754	IV	Completed	February 2017
		NCT02065791	III	Completed	October 2018
		NCT03436693	III	Recruiting	*(Sep 2021)*
	Dapagliflozin	NCT01730534	III	Completed	September 2018
		NCT03573102	IV	Recruiting	*(September 2019)*
	Empagliflozin	NCT01131676	III	Completed	April 2015
		NCT03078101	II	Recruiting	*August 2019*
		NCT03433248	IV	Recruiting	*(December 2020)*
Transforming growth factor alpha (TGF-α) and epidermal growth factor (epiregulin) inhibitor	LY3016859	NCT01774981	I and II	Completed	August 2015
Uric acid reabsorption (URAT-1) inhibitor	Verinurad	NCT03118739	II	Completed	August 2018
Vitamin D receptor agonist	Calcitriol	NCT02410005	II and III	Terminated (Logistic challenges)	September 2015
		NCT03216564	III	Recruiting	*(May 2019)*
	Cholecalciferol with/without omega-3 fatty acids (fish oil)	NCT01684722	NA	Active, not recruiting	*(January 2020)*
Xanthine oxidase (XO) inhibitor	Allopurinol	NCT02017171	III	Active, not recruiting	*(June 2019)*
	Febuxostat	NCT02344602	IV	Completed	September 2015
	TMX049	NCT03449199	II	Active, not recruiting	*(June 2019)*
	Topiroxostat	NCT02327754	II	Completed	December 2016
αVß3 integrin receptor antibody	VPI-2690B	NCT02251067	II	Completed	March 2017

[a] Compounds were selected based on completion date (from 2015 onwards) and the number of participants (≥50). Outcome of clinical and preclinical studies of drug classes in bold are presented in Table 3. Both ACEi and ARB are long-known therapeutics of DKD and are not presented in Table 3.
[b] From www.clinicaltrials.jp.

tion. Four recent clinical trials (EMPA-REG OUTCOME [45], CANVAS [46] and CREDENCE [47], and DECLARE-TIMI 58 [48]) showed that treatment with empaglifozin, canagliflozin, and dapagliflozin, respectively, consistently reduces adverse renal outcomes as well as cardiovascular events. The underlying mechanism is multifactorial because SGLT-2 inhibitors might have renoprotective effects beyond glucose lowering, which could include restoration of the tubuloglomerular feedback mechanism [49], leading to reduction of hyperfiltration and albuminuria. Another contributing mechanism could be increased sodium excretion and concomitant lowered BP, which were reported in all these clinical studies.

In clinical trials, treatment with canagliflozin and empagliflozin resulted in attenuated progression of albuminuria [45,46]. Likewise, SGLT-2 blockade attenuated albuminuria in several rodent models of DKD (Table 3), including KK-Ay and BTBR *ob/ob* mice as well as the OLEFT rat [50–52]. Administration of dapagliflozin to *db/db* mice given high-protein diet did not reduce albuminuria [19]. In the *db/db* mouse, regulation of albuminuria with SGLT-2 inhibitors showed opposing results. Hence, most experimental studies present decreased albuminuria [53–56], although some studies show no effect [57,58]. These results indicate an interlaboratory variability potentially caused by dosing, treatment duration, or environmental factors. Effects of SGLT2 inhibitors on GFR have been investigated in *db/db* mice fed a high-protein diet [19] and in the T1DM Akita mouse model [59] using intravenous (i.v.) injection of FITC-sinistrin. Both models present with hyperfiltration that is attenuated with SGLT-2 inhibitors, potentially because of restoration of the tubuloglomerular feedback mechanism. Treatment with empagliflozin in Akita mice also prevented the increase in BP seen in vehicle-treated Akita mice [59]. In accordance with this, a reduction in BP in patients with T2DM and DKD was demonstrated in all four clinical trials [45–48]. Administration of dapagliflozin to BTBR *ob/ob* mice [60], OLEFT rats [52],and STZ nicotinamide-treated rats [61] resulted in reduced serum creatinine levels in all models, whereas serum creatinine levels were not significantly regulated in dapagliflozin-treated *db/db* mice [54]. Serum creatinine levels were not investigated in DECLARE-TIMI 58, but results from the EMPA-REG OUTCOME trial showed that empagliflozin decreased the risk of doubling of serum creatinine [45].

Furthermore, there is increasing interest in the impact of OS on the development and progression of DKD. The main site of reactive oxygen species (ROS) generation is the proximal tubule be-

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cause of a highly active mitochondrial electron transport chain necessary to support the transport activity, which renders the epithelial cells lining the tubule sensitive to OS [62]. Dapagliflozin and empagliflozin reduced OS in both T1DM [63,64] and T2DM [51,53–55,63,65] models, but biomarkers of OS are rarely reported in clinical trials of DKD. Reduced OS can arise from better glycemic control, blockage of proximal tubular glucose uptake, or another yet unknown mechanism of action.

Despite the beneficial effects of SGLT-2 blockade in *db/db* mice (Table 3), the model is limited by the pathology reflecting early-stage DKD in humans. By contrast, marked glomerulosclerosis and decreased GFR that parallel advanced human DKD have been shown in the KK-Ay mouse, which likewise displays renoprotection by SGLT-2 inhibitors [14,50]. In general, findings in preclinical studies of SGLT-2 inhibitors demonstrate effects comparable to that seen in clinical trials.

Incretin-related therapies

Incretin-based diabetes therapies include glucagon-like peptide 1 receptor agonists (GLP-1RAs) and dipeptidyl peptidase 4 (DPP-4) inhibitors. Both GLP-1 and DPP-4 regulate glucose metabolism, but where GLP-1 stimulates the secretion of insulin from pancreatic beta cells, DPP-4 degrades endogenous GLP-1.

Evidence from recently completed CVOTs (LEADER [66] and SUSTAIN-6 [67]) in patients with T2DM revealed that the GLP-1RAs liraglutide and semaglutide reduced the progression of DKD. Even when correcting for changes in body weight (BW), systolic BP, and hemoglobin A1c (HbA_{1c}) [68], a significantly lowered risk for renal failure was apparent, suggesting that GLP-1RAs have a glucose-independent effect on DKD.

These findings have partly been corroborated in preclinical studies, but, in contrast to the clinical trials, most of the preclinical studies were performed in T1DM experimental models. In the STZ mouse model, liraglutide treatment reduced terminal HbA_{1c} and improved the levels of the antioxidant defense enzymes GPX3 and catalase [69]. By contrast, increased albuminuria levels in STZ mice were not improved by liraglutide [69], as otherwise observed in clinical trials [67,68]. However, liraglutide reduced the urinary albumin:creatinine ratio (ACR) and glomerular hyperfiltration, and improved glomerular pathology, including the mesangial expansion score in KK-Akita mice, without affecting serum creatinine levels [70], corresponding to the lack of effect on the risk of persistent doubling of serum creatinine level in the LEADER trial [68]. Thus, data from the KK-Akita mouse model show treatment benefits comparable to the results of the LEADER trial.

Liraglutide increased eNOS expression and activity in both the STZ rat and the *db/db* mouse, indicating an eNOS-associated beneficial effect of liraglutide [70,71]. However, data in eNOS-knockout models are needed to address this further.

DPP-4 inhibitors have been on the market as glucose-regulating compounds for more than a decade. The DPP-4 inhibitor linagliptin has shown beneficial renal effects, including reduction of albuminuria and improved renal histopathology, in rodent models of DKD. Treatment with the DPP-4 inhibitor linagliptin in STZ mice reduced urinary ACR in addition to mRNA and protein levels of catalase and MnSOD, which protect against OS [72]. Vehicle-treated and linagliptin-treated STZ mice displayed similar blood glucose levels, suggesting a glucose-independent effect of linaglip-

TABLE 3

Renal outcome in clinical and preclinical studies

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD	
Canagliflozin	CANVAS Program (NCT01032629, NCT01989754) [46]	• HbA1c (%), BW and BP • (Hazard ratio, 0.73) progression of albuminuria • (Hazard ratio, 0.60) in composite outcome of a sustained 40% reduction in eGFR, need for renal-replacement therapy, or death from renal causes • (Hazard ratio, 0.70-0.95) eGFR decline	***db/db* mouse** (C57BLKS/J) [53] ↓ BG ↓ mean BP ↓ albuminuria ↓ mesangial expansion	**KK-Ay mouse** [50] ↓ BG ↓ HbA1c (%) ↓ albuminuria ↓ glomerular pathology • did not affect creatinine clearance
	CREDENCE (NCT02065791) [47]	• HbA1c (%), BW and BP • Short-term decrease in eGFR followed by stabilization • (Hazard ratio, 0.70) in composite outcome of ESRD, a doubling of serum creatinine level, or death from renal or cardiovascular causes • (Hazard ratio, 0.80) risk of cardiovascular death, myocardial infarction, or stroke		

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD				
Dapagliflozin	DECLARE-TIMI 58 (NCT01730534) [48]	• HbA1c (%), BW and BP • (Hazard ratio, 0.76) risk of renal composite outcome (≥40% decrease in eGFR rate to 60 ml per minute per 1.73 m2 of body-surface area, new incidence of ESRD, or death from renal or cardiovascular causes)	**Akita mouse** (C57BL/6J) [63] ↓BG ↓ HbA1c (%) ↓ albuminuria ↓ oxidative stress ↓ BUN ↓ renal histopathology	***db/db* mouse** (C57BL/6J, C57BLKS/J) [54,23] ↓BG ↓ HbA1c (%) ↓/↑ albuminuria regulation ↓/↑ glomerular pathology ↓ interstitial fibrosis ↓ macrophage infiltration ↓ oxidative stress ↑ plasma urea ↑ tubular injury • did not affect BP, serum creatinine and creatinine clearance	***db/db* UNx mouse** (C57BLKS/J) [55] ↓ BG ↓albuminuria ↓ glomerular pathology ↓ renal inflammation ↓ oxidative stress • did not affect creatinine clearance	***db/db* high-protein diet mouse** (C57BLKS/J) [19] ↓ BG ↓GFR • did not affect albuminuria	***ob/ob* mouse** (BTBR) [60] ↓ BG ↓ serum creatinine ↓ serum BUN ↓ glomerular pathology
			KK-Ay mouse [50] ↓ BG ↓ HbA1c ↓ albuminuria ↓ creatinine clearance ↓ cell infiltration ↓ glomerular pathology	**OLEFT rat** [52] ↓ BG ↓ HbA1c (%) ↓ albuminuria ↑ creatinine clearance ↓ oxidative stress ↓ renal histopathology ↓ serum creatinine	**STZ and fructose rat** [65] ↓ BG ↓ oxidative stress ↓ tubulointersti-tial fibrosis. • did not lower albuminuria (albuminuria was not increased in model)	**STZ and nicotinamide rat** (Wistar) [61] ↓ BG ↓ albuminuria ↑ creatinine clearance ↓ urea ↓ serum creatinine ↓ oxidative stress ↓ glomerular pathology	

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD				
Empagliflozin	EMPA-REG OUTCOME (NCT01131676) [45]	• HbA1c (%), BW and BP • (Hazard ratio, 0.61) risk for new or worsening DKD • (Hazard ratio, 0.61) risk of progression to macroalbuminuria • (Hazard ratio, 0.56) risk of doubling of serum creatinine • (Hazard ratio, 0.45) risk of need for initiation of RRT • Short-term decrease in eGFR followed by stabilization	**STZ eNOS -/- mouse** (C57BL/6J) [58] • No major effects • did not affect BG, ACR, glomerulosclerosis, tubulointerstitial inflammation and fibrosis	**Akita mouse** (C57BL/6-Ins2) [59] ↓ BG ↓ BP ↓ albuminuria ↓GFR ↓ glomerular pathology	**STZ rat** (Spraque-Dawley) [64] ↓ BG ↓ oxidative stress ↓ macrophage infiltration in glomeruli • did not affect albuminuria	***db/db* mouse** (C57BLKS/J) [56,57] ↓ BG ↓/NS albuminuria regulation ↑ eNOS activation ↓ oxidative stress ↓/NS tubulointerstitial fibrosis regulation • did not improve glomerulosclerosis	***ob/ob* mouse** (BTBR) [51] ↓ BG ↓ albuminuria ↓ glomerular pathology. • did not affect BP (BP is higher in control) or creatinine clearance
			KK-Ay mouse [50] ↓ BG ↓ HbA1c (%) ↓ albuminuria ↓ glomerular pathology. • did not affect creatinine clearance				
Liraglutide	LEADER (NCT01179048) [66,68]	• (Hazard ratio, 0.78) risk of renal outcome (new-onset persistent macroalbuminuria, persistent doubling of serum creatinine level, renal replacement therapy, or death due to renal disease) • (Hazard ratio, 1.07) eGFR decline in patients with a baseline eGFR of 30-59 mL/min/1.73 m^2 • (Hazard ratio, 0.78) death from cardiovascular causes	**STZ mouse** (129SV) [69] ↓ BG (at some time points) ↓ HbA1c (%) ↓ oxidative stress • did not reduce albuminuria	**Akita mouse** (KK/Ta) [70] ↓ GFR ↓ albuminuria ↓ oxidative stress ↓ glomerular pathology • did not change BP, BG, BUN or plasma creatinine	**STZ rat** (Wistar) [71,103] ↓ albuminuria ↓ urea ↓ serum creatinine ↓ renal histopathology ↑ eNOS activity ↓/NS BP regulation ↓ oxidative stress • did not affect BG	***db/db* mouse** (C57BLKS/J) [112] ↓ glomerular pathology ↑ eNOS protein expression • did not lower BG	
Linagliptin	CARMELINA (NCT01897532) [76]	• (Hazard ratio, 0.86) albuminuria progression (i.e., a change from normoalbuminuria to microalbuminuria/macroalbuminuria or a change from microalbuminuria to macroalbuminuria) • did not affect (Hazard ratio, 1.04) renal outcome (first sustained end-stage renal disease, death due to renal failure, or sustained decrease of 40% in eGFR from baseline)	**STZ mouse** (DBA/2J) [72] ↓ albuminuria ↓ oxidative stress	**STZ eNOS -/- mouse** (C57BL/6J) [73] ↓ glomerular pathology ↓ oxidative stress • did not affect BG or albuminuria	**Akita mouse** (KK/Ta) [74] ↓ GFR ↓ albuminuria ↓ oxidative stress ↓ renal histopathology ↓ plasma creatinine • did not affect BP, BG or BUN	***db/db* mouse** (C57BL/6J) [75] ↓ renal histopathology ↓ albuminuria at day 56 (not at end of study) • did not affect BG or HbA1c (%) or BP	

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD		
Xanthine oxidase inhibitors	PERL (NCT02017171) [78]	*Active, not recruiting*	**STZ rat** (Sprague-Dawley) [81] *Febuxostat:* ↓ albuminuria ↓ oxidative stress ↓ macrophage infiltration • did not improve BG	***db/db* mouse** (C57BLKS/J) [79] *Allopurinol:* ↓ BP ↓ BUN ↓ albuminuria ↓ tubulointerstitial injury ↓ macrophage infiltration • did not affect BG, mesangial expansion or oxidative stress	**Zucker obese rat** [80] *Febuxostat:* ↓ albuminuria ↓ renal histopathology • did not affect BG, HbA1c (%), BP or systemic oxidative stress
Spironolactone	PRIORITY (NCT02040441) [39]	• Presented (EASD 2019), not published • Did not affect renal outcome including albuminuria progression.	**STZ eNOS -/- mouse** (C57BL/6J) [30] ↓ albuminuria ↓ BP ↓ renal histopathology • did not affect serum creatinine, BUN or BG	**STZ rat** (Sprague–Dawley) [40] ↓ renal histopathology ↓ urinary protein excretion ↓ oxidative stress • did not affect BG or serum creatinine	**OLEFT rat** [41] ↓ albuminuria ↓ renal histopathology • did not affect BG or BP
Esaxerenone	ESAX-DN (JapicCTI-173695) [42]	• Results from ongoing trial presented (Kidney Week 2019), not published • rate of remission to normoalbuminuria • UACR • progression from microalbuminuria to overt DKD	**High-salt diet KK-A^{y} mouse** [43] ↓ albuminuria ↓ BP ↓ renal histopathology ↓ oxidative stress • did not affect BG		

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status			Rodent models of DKD
Bardoxolone methyl (RTA 402)	BEACON (NCT01351675)[82]	• eGFR decline • albuminuria • (Hazard ratio, 1.44) death from cardiovascular causes	**ZDF rat** [84,85] *Bardoxolone methyl analogue (RTA 405):* ↓/NS BG regulation ↑/NS proteinuria regulation ↑/NS renal histopathology • did not affect BP	**ZDF rat** [84,85] *Bardoxolone methyl analogue (dh404):* • did not affect BG, BP, proteinuria, renal histopathology or GFR	
Fenofibrate	NCT03869931 [88]	*Recruiting*	**STZ rat** (Wistar) [87] ↓ proteinuria ↓ oxidative stress ↓ serum creatinine ↓ BUN ↑ creatinine clearance • did not affect BG	***db/db* mouse** (C57BLKS/J) [86,114] ↓ BG ↓ HbA1c (%) ↓ albuminuria ↑ BUN (comparable level with db/m controls) ↓ renal histopathology ↓ creatinine clearance • did not affect serum creatinine	**ZDF rat** [122] ↓ macrophage infiltration ↓ tubulointerstitial fibrosis and inflammation
Pentoxifylline	PENFOSIDINE (NCT03664414) [129] NCT03006952 [130]	PENFOSIDINE: *Recruiting* NCT03006952: • albuminuria • HbA1c (%) • Did not affect serum creatinine, eGFR, fasting plasma glucose or BP	**STZ rat** (Sprague–Dawley) [90] ↓ albuminuria ↓ Pro-inflammatory cytokines • did not affect BG or HbA1c (%)		

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD				
Colchicine	NCT02035891 [91]	*Active, not recruiting*	**STZ rat** (strain not published) [92] ↓ albuminuria ↓ renal histopathology • did not affect BG, BUN or creatinine clearance				
Pirfenidone	NCT02689778 [131]	*Active, not recruiting*	***db/db* mouse** (C57BLKS/J) [113] ↓ renal histopathology • did not affect BG or albuminuria				
AGE inhibitors	NCT01935167 NCT02156843	NCT01935167 (DW1029M): *Completed, not published* NCT02156843 (Pyridorin): *Terminated, not published*	**STZ mouse** (CD1) [94] *Pyridorin:* ↓ albuminuria ↓ serum creatinine ↓ renal histopathology	**STZ rat** (Sprague-Dawley) [104] *DW1029M:* ↓ albuminuria	**STZ rat** (Sprague-Dawley) [99] *Pyridorin:* ↓ albuminuria ↓ plasma creatinine ↓ glomerular pathology • did not affect BG or HbA1c (%)	**KK-Ay mouse** [117] *Pyridorin:* ↓ HbA1c (%) ↓ albuminuria ↓ oxidative stress • did not affect BP (BP not upregulated)	**Zucker obese rat** [121] *Pyridorin:* ↓ albuminuria ↓ BP ↓ Plasma creatinine
Atrasentan	NCT01858532 [132]	• (Hazard ratio, 0.65) primary composite renal endpoint (doubling of serum creatinine or onset of ESRD). • (Hazard ratio, 0.73) 50% eGFR decline • did not affect cardiovascular composite endpoint (Hazard ratio, 0.88)	**STZ ApoE -/- mouse** [96] ↓ albuminuria • did not affect BG, glomerular pathology or BP (not regulated)	**STZ rat** (Sprague-Dawley) [100] ↓ albuminuria ↓ BP • did not affect BG or oxidative stress	**STZ Dahl salt-sensitive rat** [107] ↓ proteinuria ↓ BP ↓ renal histopathology • did not affect GFR (not regulated)	**KK-Ay mouse** [118] ↓ BG ↓ albuminuria	**T2DN rat** [107] ↑ GFR ↓ renal histopathology • did not affect proteinuria or BP
GKT137831	NCT02010242	*Completed, not published*	**STZ ApoE -/- mouse** (C57BL/6J) [95] ↓ albuminuria ↓ glomerular pathology ↓ oxidative stress • did not affect BG or HbA1c (%)	**OVE26 mouse** [98] ↓ albuminuria ↓ renal histopathology • did not affect BG	***db/db* mouse** (C57BLKS/J) [115] ↓ oxidative stress ↓ plasma creatinine • did not affect BG, BP, HbA1c (%) or albuminuria		

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TABLE 3 (*Continued*)

Drug	Clinical trial	Main renal outcomes or NCT status	Rodent models of DKD	
Vitamin D receptor agonists	NCT02410005 NCT03216564 NCT01684722	NCT02410005: *Completed, not published* NCT03216564: *Recruiting* NCT01684722: *Active, not recruiting*	**STZ rat** (Sprague-Dawley) [101,102] ↓/NS serum creatinine regulation ↓/NS urea regulation ↓ proteinuria ↓ renal histopathology • did not affect BG	**KK-Ay mouse** [119] ↓ albuminuria ↓histopathology • did not affect HbA1c (%), serum creatinine or BP.

To generate this overview, we searched PubMed for papers published until 15 July 2019, using the search terms ``diabetic nephropathy'' and, ``mouse'' or ``rat,'' and the names of therapeutic agents. In all the clinical trials listed, patients with T2D were included except for the PERL trial (NCT02017171), which included patients with T1D (highlighted in light green). As the aim of this review is to compare renal outcome of therapeutic agents between clinical and preclinical studies in recently concluded trials or ongoing studies, ACEi and ARBs are not presented in Table 3.

Abbreviations: BG, blood glucose; BP, blood pressure; BW, body weight; NCT, ClinicalTrials.gov Identifier; ↑, increased compared to untreated diabetic control group; ↓, decreased compared to untreated diabetic control group.

tin [72]. Treatment with linagliptin alone in eNOS-deficient STZ mice reduced glomerulosclerosis and renal OS, whereas the combination of linagliptin with telmisartan also reduced urinary ACR [73]. Treatment with linagliptin in the KK-Akita mouse model lowered albuminuria and GFR, and improved mesangial matrix expansion, tubulointerstitial fibrosis, and OS [74]. In the *db/db* mouse model, administration of linagliptin improved glomerulosclerosis and interstitial fibrosis without changing blood glucose or HbA_{1c} (%) levels [75]. Thus, the improved renal histopathology with DPP-4 inhibition appears to be independent of blood glucose control (Table 3).

Despite these preclinical results, there have been few data from clinical studies to confirm a protective effect of DPP-4 inhibition on DKD. The CARMELINA clinical trial investigated the cardiovascular outcome of treatment with linagliptin in patients with T2DM and high cardiovascular and renal risk. Linagliptin only slightly prevented the progression of albuminuria, but did not change the renal endpoints of sustained ESRD or 40% decreased eGFR from baseline [76] (Table 3).

The lack of effect by linagliptin on renal endpoints in human disease might result from the inclusion of patients with high renal risk, whereas the preclinical models, apart from the eNOS-deficient STZ mouse model, represent early stages of DKD. Moreover, the preclinical studies were primarily conducted in T1DM rodent models, whereas the clinical trials were performed in patients with T2DM.

Xanthine oxidase inhibitors

Hyperuricemia is associated with progression of DKD, and xanthine oxidase inhibitors, such as allopurinol and febuxostat, lower uric acid levels. The efficacy of xanthine oxidase inhibitors to attenuate the eGFR decline in CKD was proven in a small randomized trial including 113 participants [77]. In the recently completed PERL trial, allopurinol was tested in patients with T1DM and mild to moderate kidney disease [78]. Lowering of uric acid levels with allopurinol showed no impact on DKD progression.

Administration of allopurinol in *db/db* mice reduced albuminuria, BP, macrophage infiltration, and tubulointerstitial injury, but did not prevent mesangial expansion or OS in the kidney [79]. In agreement, treatment of Zucker obese rats with febuxostat attenuated albuminuria and macrophage infiltration, and improved glomerulosclerosis and interstitial fibrosis [80]. Positive effects of febuxostat on kidney injury were also demonstrated in the STZ-induced diabetic rat model, which presents with reduced albuminuria, macrophage infiltration, and OS [81]. Allopurinol remains to be characterized in rodent models of T1DM, leaving it unresolved to what degree preclinical data on this drug class translate to the clinic.

Bardoxolone methyl

Bardoxolone methyl acts by activating the nuclear factor erythroid-derived 2-related factor 2 (Nrf2) pathway, leading to upregulation of the antioxidant response and suppression of inflammation. In the BEACON trial, patients with T2DM and an eGFR of 15–30 ml/min/1.73 m^2 were included to receive either bardoxolone methyl or placebo [82]. Administration of bardoxolone methyl to patients increased eGFR and urine ACR, and decreased BW. Patients given bardoxolone methyl had a mean increase in eGFR of 5.5 mL/min/1.73 m^2 from a mean baseline eGFR of 22.4 mL/min/1.73 m^2, whereas patients in the placebo

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group experienced a decline in mean eGFR of –0.9 mL/min/1.73 m2 from baseline after 48 weeks of treatment. Although patients treated with bardoxolone methyl sustained an increase in eGFR after the drug was withdrawn (~20% of the on-treatment increase in eGFR was sustained), eGFR declined in the bardoxolone group compared with the placebo group from end of treatment to 4 weeks post withdrawal, suggesting that bardoxolone methyl treatment is not renoprotective [83]. A significant number of patients in the bardoxolone methyl group reached a composite cardiovascular outcome event of nonfatal myocardial infarction, nonfatal stroke, hospitalization for heart failure, or death from cardiovascular causes [82]. Given the higher rate of cardiovascular events in the bardoxolone methyl group, the BEACON trial was terminated prematurely.

The effects of the bardoxolone methyl analogs, RTA 405 and dh404, in diabetic ZDF rats were reported in two experimental studies [84,85], after termination of the clinical trial. Treatment of ZDF rats with dh404 in both studies showed no major effects or worsening of DKD. By contrast, administration of RTA 405 in the study by *Zoja et al.* [84] caused significant weight reductions and increased proteinuria, glomerulosclerosis, and tubular damage, whereas no major effects was seen in the study by Chin *et al.* [85]. However, potential impurities were identified in the drug RTA 405 used in the study by Zoja *et al.* [84]. Although the clinical improvement in eGFR has not been replicated preclinically, the BEACON trial suggests that bardoxolone methyl does not have a renoprotective effect. In both experimental studies, the ZDF rat presented with mild proteinuria and mild to moderate glomerular changes, suggesting that the lack of transient beneficial effects of bardoxolone methyl was because the ZDF rats do not display a severe disease phenotype. For future experimental studies investigating whether bardoxolone methyl influences GFR, more advanced rodent models of DKD are recommend.

Emerging therapeutic approaches

Several randomized clinical trials are currently investigating new approaches for the treatment of DKD. Here, we highlight preclinical studies that might have contributed to the initiation of clinical drug development and testing (Table 2).

Fenofibrate, a peroxisome proliferator-activated receptor-α (PPAR-α) agonist, is associated with reduced proteinuria in experimental models [86,87]. Fenofibrate improves creatinine clearance and serum creatinine levels in STZ rats [87]. By contrast, fenofibrate-treated *db/db* mice showed decreased creatinine clearance and nonsignificant changes in serum creatinine levels [86]. The preclinical data showed contrasting results depending on whether fenofibrate was tested in a T1DM or a T2DM model of DKD. The effect of fenofibrate therapy in DKD is currently being evaluated in patients with T2DM in a Phase 3 clinical trial [88].

Pentoxifylline, a nonspecific phosphodiesterase inhibitor, reduces urinary protein excretion in patients with diabetes [89]. The antiproteinuric properties of pentoxifylline are related to a reduction in tumor necrosis factor (TNF)-α, a proinflammatory cytokine. Administration of pentoxifylline in STZ rats reduced albuminuria and several proinflammatory markers, including urinary TNF-α [90]. The effects of pentoxifylline in patients with T2DM and DKD is currently being evaluated in a Phase IV trial, the PENFOSIDINE study.

Colchicine, another drug that works by interfering with inflammatory pathways, is currently being tested in patients with T2DM and microalbuminuria, with the primary objective of slowing the progression of microvascular complications [91]. In a preclinical study, colchicine was shown to reduce albuminuria in STZ rats, but did not affect serum creatinine levels [92].

Clinical and preclinical data on additional therapeutic agents, including the AGE inhibitors DW1029 M and pyridorin, the ETA receptor antagonist atrasentan, the NADPH oxidase inhibitor GKT137831, and the vitamin D receptor agonists, as well as the antifibrotic pirfenidone, are presented in Table 3.

Concluding remarks

There is a disconnect in the study designs in preclinical experiments and clinical trials. To improve the validity of translational research, the rodent model used must represent the target patient population to be enrolled in the clinical trial in question. Evidently, the predictive value of an animal model is limited to the disease this model represents. In addition, similar end-point measurements need to be explored between preclinical studies and clinical trials. The selection of a preclinical model should also be based on the mechanism of action of the current drug. For example, both enalapril and liraglutide upregulate the gene encoding eNOS, suggesting that use of eNOS-deficient mouse models will affect treatment efficacy in such models.

Although numerous rodent models display pathophysiological characteristics of DKD, only a limited number of models are recurrently used in pharmacological studies. The best-characterized and most intensively used models are the STZ rat and the *db/db* mouse, although both models display varying degree of translatability to human disease. The KK-Ay mouse, KK-Akita mouse, STZ mouse, eNOS-deficient STZ mouse, and ZDF rat are also frequently used. Of these, only pharmacological studies in the KK-Akita mouse and KK-Ay mouse have shown translatability to clinical trials. Furthermore, the BTBR *ob/ob* mouse model appears in fewer published preclinical studies of clinically tested drugs, despite being characterized as an advanced model of DKD. Features of advanced DKD also appear in KK-Ay mice, eNOS-deficient STZ mice, and in accelerated DKD models in *db/db* mice. Notably, the addition of stressors to the *db/db* model accelerates the progression of DKD, hereby reflecting human DKD to a higher degree.

In conclusion, the selection of preclinical rodent models should be based on prior knowledge of target presence and regulation in the model and reflect clinical parameters of DKD in patients. In addition, it is recommended to use more than one DKD or CKD model for preclinical characterization of a compound to delineate effects on diabetes as well as nondiabetes-associated progression of kidney disease. Preclinical studies using advanced DKD models appear to have better translatability and should be favored for preclinical drug testing.

Conflict of interests

F.S.E., M.V.Ø., K.F., and L.N.F. are employees of Gubra ApS; N.V. and J.J. are owners of Gubra ApS.

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