Antisense Oligonucleotide for Chronic Kidney Disease

Subjects: Others

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Chronic kidney disease (CKD) is a global public health issue that places an increasing burden on the healthcare systems of both the developed and developing countries. CKD is a progressive and irreversible condition, affecting approximately 10% of the population worldwide. Patients that have progressed to end-stage renal disease (ESRD) require expensive renal replacement therapy, i.e., dialysis or kidney transplantation. Current CKD therapy largely relies on the use of angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs). However, these treatments by no means halt the progression of CKD to ESRD. Therefore, the development of new therapies is urgently needed. Antisense oligonucleotide (ASO) has recently attracted considerable interest as a drug development platform. Thus far, eight ASO-based drugs have been granted approval by the US Food and Drug Administration for the treatment of various diseases.

chronic kidney disease | antisense oligonucleotide | therapeutics

1. Introduction

1.1. Antisense Oligonucleotide

Antisense oligonucleotides (ASOs) are one of the most classical synthetic therapeutic oligonucleotides that are able to modify gene expression. ASOs are typically designed to regulate gene expression by specifically binding to the pre-mRNA or mRNA of the gene via Watson–Crick base pairing [1][2][3][4]. Since the first report on the ASO-mediated inhibition of gene expression in the late 1970s by pioneering researchers Zamecnik and Stephenson [5][6][7], ASO technology has become a well-established platform for ASO-mediated RNA-targeting therapy [8]. So far, the US Food and Drug Administration (FDA) has granted approval for eight ASO drugs for clinical usage (**Table 1**) [9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24][25]. These successful clinical translations inspire both the academia and pharmaceutical industry to develop ASO-based drugs for the treatment of various diseases, either by the downregulation of disease-causing gene expression or rescuing the expression of essential but defective genes.

Table 1. Antisense oligonucleotide (ASO)-based drugs approved by the FDA for clinical applications.

No.	ASO Drug	Approval Year	Indication	Mechanism	Ref.
1	Fomivirsen (Vitravene®)	1998	Cytomegalovirus (CMV) retinitis	Downregulate the gene encoding CMV immediate-early 2 protein	[9][10]

No.	ASO Drug	Approval Year	Indication	Mechanism	Ref.
2	Mipomersen (Kynamro®)	2013	Familial hypercholesterolemia (FH)	Downregulate the gene *APOB* encoding apolipoprotein B	[11] [12]
3	Eteplirsen (Exondys 51®)	2016	Duchenne muscular dystrophy (DMD)	Rescue the expression of dystrophin through exon-51 skipping of the mRNA of *DMD* gene	[13] [14] [15] [16]
4	Nusinersen (Spinraza®)	2016	Spinal muscular atrophy (SMA)	Increase the production of the survival motor neuron (SMN) protein by exon-7 inclusion of the mRNA of *SMN2* gene	[17] [18] [19] [20]
5	Inotersen (Tegsedi®)	2018	Hereditary transthyretin (TTR) amyloidosis	Downregulate the gene *TTR* encoding transthyretin	[21] [22]
6	Golodirsen (Vyondys 53®)	2019	DMD	Rescue the expression of dystrophin through exon-53 skipping of the mRNA of *DMD* gene	[23]
7	Viltolarsen (Viltepso®)	2020	DMD	Rescue the expression of dystrophin through exon-53 skipping of the mRNA of *DMD* gene	[24]
8	Casimersen (Amondys 45®)	2021	DMD	Rescue the expression of dystrophin through exon-45 skipping of the mRNA of *DMD* gene	[25]

2.2. Chronic Kidney Disease

Chronic kidney disease (CKD) is a leading public health issue worldwide [26]. Approximately 850 million people (~10% adult population) are affected by CKD [27]. CKD accounted for 1.3% of years of life lost (YLL) in 2012 [28] and will become the fifth most common cause of YLL worldwide by 2040 [29]. In the US, CKD affects ~13% of the population [30], with more than 100,000 new patients starting on dialysis every year [31]. CKD is defined as the persistently decreased function of the kidney for more than 90 days [32]. Kidney dysfunction is shown by a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2, or markers of kidney damage such as hematuria, albuminuria, and a variety of abnormalities detected by histology or imaging [32][33]. In CKD, the kidney undergoes a progressive and irreversible functional decline that can be classified into six stages (G1: normal, GFR ≥ 90 mL/min/1.73 m^2; G2: 60–89 mL/min/1.73 m^2; G3a: 45–59 mL/min/1.73 m^2; G3b: 30–44 mL/min/1.73 m^2; G4: 15–29 mL/min/1.73 m^2; and G5: end-stage renal disease/kidney failure, <15 mL/min/1.73 m^2). Patients who reach end-stage renal disease (ESRD) require kidney replacement therapy, i.e., dialysis or kidney transplantation, as their kidney is no longer able to maintain life in the long term [28]. However, less than half of patients needing kidney replacement therapy have access to treatment [34], as many governments and individuals are faced with the unaffordability of these costly therapies [35]. Moreover, kidney transplantation leads to a high risk of mortality as a result of unavoidable continuous immunosuppression (anti-rejection), which may cause infections and cancer development [36][37]. Therefore, it is imperative to delay or even prevent the progression of CKD from early stage to ESRD.

Although the causes of CKD vary geographically, diabetes accounts for 30–50% of CKD worldwide [28]. In developed countries, diabetes and hypertension are the leading causes of CKD [28]. Diabetic nephropathy is the main cause of ESRD in these countries and the burden of ESRD resulting from type 2 diabetes is projected to increase fourfold in decades to come [38][39], which is partly due to the increased prevalence of type 2 diabetes in young people [40]. In developing countries, CKD from glomerulonephritis and interstitial nephritis are more common as a result of the high prevalence of infections [41][42], such as streptococcal infections, acquired immunodeficiency syndrome (AIDS), schistosomiasis, leishmaniasis, hepatitis B, and hepatitis C. Notably, people affected by CKD are 5–10 times more likely to die prematurely due to the complications of CKD than ESRD [43]. This increased risk of death can be largely attributed to cardiovascular disease and cancer [44].

1.3. Cardiorenal Syndromes

Since the kidneys and heart have a bidirectional interorgan communication, dysfunction in one organ may cause dysfunction in the other organ, resulting in cardiorenal syndromes, a complex disorder of both the heart and kidneys [45]. Cardiorenal syndromes can be classified into five sub-types: type I [heart failure leading to acute kidney injury (AKI)], type II (chronic heart failure leading to CKD), type III (AKI leading to acute heart failure), type IV (CKD leading to heart failure), and type V (systemic condition such as diabetes mellitus leading to both renal and cardiac dysfunction) [45]. The common pathophysiological mechanisms in heart failure and CKD include dysfunction of the neurohormonal system (leading to the activation of the renin–angiotensin–aldosterone system), abnormal endothelial activation, reduced intestinal perfusion, and release of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 [46]. These mechanisms simultaneously and sequentially contribute to cardiorenal syndromes, ultimately resulting in fibrosis and dysfunction in both organs [46].

2. Antisense Oligonucleotides Targeting Chronic Kidney Disease

ASO-mediated gene knockdown by specifically reducing the mRNA production of genes enables the identification and/or verification of disease-relevant genes in CKD, leading to the expansion of knowledge regarding the molecular basis underlying the disease, thereby bringing new hope to identifying promising target genes for CKD therapy aimed at delaying or halting the progressive decline of kidney function. Furthermore, upon recognition of genes as potential therapeutic targets for CKD, the identified ASO-based gene inhibitors can directly serve as lead compounds, which speeds up the drug development process and bypasses the potential difficulties that development of small molecule-based inhibitors may face, such as the undruggability of target proteins and the toxicity of small molecules.

In fact, uniformly PS-modified ASOs and 2′-MOE-PS gapmer-like ASOs have been developed by different groups to target genes involved in the progression of CKD by downregulating the expression of those genes through the RNase H mechanism. In this section, we review the ASO-based research on genes that are relevant to CKD, including *THBS1* (also known as *TSP1*) that encodes thrombospondin-1 (TSP1), *CCN2* (also known as *CTGF*) encoding connective tissue growth factor (CTGF), *KRAS* encoding Kirsten rat sarcoma viral oncogene homolog

(KRAS), *MTOR* encoding mammalian target of rapamycin (mTOR), *AGT* encoding angiotensinogen (AGT), and *APOL1* that encodes apolipoprotein L1 (APOL1). These studies provide new insights into the molecular pathogenesis of CKD and explore the therapeutic potential of ASO-based gene inhibitors for the targeted therapy of CKD. In vitro screening of ASO candidates and subsequent in vivo investigations in these studies are shown in **Table 2** and **Table 3**, respectively.

Table 2. In vitro screening of ASOs and the identified best-performing candidates for subsequent in vitro and/or in vivo studies. Purple asterisks "*" in ASO sequences represent PS modification, red color represents 2′-MOE modification, blue color represents 2′-4′ constrained ethyl modification, black color represents deoxyribonucleotide. 2′-MOE: 2′-O-methoxyethyl, PS: Phosphorothioate internucleotide linkage, PO: Phosphodiester internucleotide linkage.

In Vitro Study (Initial Screen of ASO Candidates)			Best-Performing ASO Candidates	
Target Gene	**Chemistry**	**Cellular Target**	**ASO Sequences**	**Ref.**
TSP-1	Eleven 14–25 mer DNA^{PS} ASOs	Mesangial cell	**5′-T*T*C*T*C*C*G*T*T*G*T*G*A*T*T*G*A*A-3′** **5′-C*A*C*C*T*C*C*A*A*T*G*A*G*T*T-3′**	[47]
CTGF	20 mer 4-12-4 MOE^{PO}-DNA^{PS}-MOE^{PO} and MOE^{PS}-DNA^{PS}-MOE^{PS} ASOs	Rat mesangial cell line	**5′-CCACA*A*G*C*T*G*T*C*C*A*G*T*CTAA-3′** **5′-C*C*A*C*A*A*G*C*T*G*T*C*C*A*G*T*C*T*A*A-3′**	[48]
KRAS	20 mer 5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} ASOs	Rat renal fibroblast (NRK-49F)	**5′-A*T*T*C*A*C*A*T*G*A*C*T*A*T*A*C*A*C*C*T-3′** **5′-C*A*C*A*C*T*T*A*T*T*C*C*C*T*A*C*T*A*G*G-3′**	[49]
MTOR	~150 20 mer 5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} ASOs	Primary murine hepatocytes (for screening), type 1 Madin-Darby Canine Kidney cells (for other in vitro experiments)	**5′-T*C*C*A*C*T*T*T*T*C*A*C*A*G*C*A*C*T*G*C-3′**	[50]
AGT	~150 20 mer 5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} ASOs	Primary murine hepatocytes	**5′-T*C*T*T*C*C*A*C*C*C*T*G*T*C*A*C*A*G*C*C-3′**	[51]

In Vitro Study (Initial Screen of ASO Candidates)		Best-Performing ASO Candidates		
Target Gene	**Chemistry**	**Cellular Target**	**ASO Sequences**	**Ref.**
	Over 4000 16			

Target Gene	**In Vivo Studies of ASO Mediated Gene Silencing**		**Ref.**
TSP-1	**Type of CKD model**	**Animal model**	[47]
	Induced experimental mesangial proliferative glomerulonephritis (the anti-Thy1 model)	Sprague-Dawley rats (150–200 g)	
	Therapeutic regimen of ASO		
	ASOs were transferred into renal glomeruli via left renal artery perfusion. Five days after the administration, kidneys were isolated for analysis.		
	Renal function and/or renal damage markers		
	Inhibited glomerular extracellular matrix accumulation determined by significantly reduced collagen IV positive glomerular area (%): TSP-1 ASO-treated group (~16%), scrambled ASO-treated group (~31%), $p < 0.01$. Markedly reduced mesangial cell activation determined by significantly reduced smooth-muscle-actin positive glomerular area (%): TSP-1 ASO-treated group (~15%), scrambled ASO-treated group (~39%), $p < 0.01$.		
CTGF	**Type of CKD model**	**Animal model**	[48]
	Mice received streptozotocin (STZ) to develop an experimental model of type 1 diabetes induced diabetic nephropathy, and *db/db* mice with naturally developed diabetic nephropathy	C57BL/6 mice	
	Therapeutic regimen of ASO		
	Mice with type 1 diabetes: 20 mg/kg (twice a week) for 16 weeks. *db/db* mice: 5, 10, 20 mg/kg (twice a week) for 8 weeks		
	Renal function and/or renal damage markers		
	Mice with type 1 diabetes: Reduced kidney hypertrophy determined by reduced ratio (kidney weight/body weight): CTGF ASO-treated group (1.4%), vehicle-treated group (1.9%), $p < 0.02$. Attenuated mesangial matrix expansion (a.u.): CTGF ASO-treated group (~1.8), vehicle-treated group (~3.2), $p < 0.05$. Significantly reduced urinary albumin determined by reduced 24 h urinary albumin excretion (urinary albumin/urinary creatinine, ug/mg): CTGF ASO-treated group (~1.5), vehicle-treated group (~4.0), $p < 0.05$. *db/db* mice: Matrix expansion (%): 10 mg/kg CTGF ASO-treated group (~60%), vehicle-treated group (~100%), $p < 0.05$.		

Target Gene	In Vivo Studies of ASO Mediated Gene Silencing		Ref.
	Urinary albumin/urinary creatinine (ug/mg): 20 mg/kg CTGF ASO-treated group (~1.2), vehicle-treated group (~2.4), $p < 0.05$.		
	Type of CKD model	**Animal model**	
	Unilateral ureteric obstruction (UUO) model	Male Wistar rats	
	Therapeutic regimen of ASO		
KRAS	12.5 mg/kg for six days (administration was performed on alternate days)		[49]
	Renal function and/or renal damage markers		
	Significantly reduced fibrosis determined by reduced fibrosis score (%): KRAS ASO-1-treated group (17%), scrambled ASO-1 (~40%), $p < 0.001$; KRAS ASO-2-treated group (20.3%), scrambled ASO-2 (~36%), $p < 0.01$.		
	Type of CKD model	**Animal model**	
	An orthologous model of human autosomal dominant polycystic kidney disease (ADPKD) caused by a mutation in the *Pkd2* gene	C57BL/6 Pkd2WS25/− mice	
	Therapeutic regimen of ASO		
MTOR	Intraperitoneal injection at 100 mg/kg/week for the first 4 weeks and 50 mg/kg/week for the remaining 8 weeks		[50]
	Renal function and/or renal damage markers		
	Improved kidney function determined by reduced ratio (kidney weight/body weight): MTOR ASO-treated group (1.5%), scrambled ASO-treated group (2.4%), $p < 0.001$; and cyst volume density: MTOR ASO-treated group (15.1%), scrambled ASO-treated group (34.1%), $p < 0.01$.		
	Type of CKD model	**Animal model**	
	An orthologous model of human ADPKD caused by a mutation in the *Pkd2* gene	C57BL/6 Pkd2WS25/− mice	
	Therapeutic regimen of ASO		
AGT	Intraperitoneal injection at 100 mg/kg/week for the first 4 weeks and 50 mg/kg/week for the remaining 8 weeks		[51]
	Renal function and/or renal damage markers		
	Improved kidney function determined by reduced ratio (kidney weight/body weight): AGT ASO-treated group (1.5%), scrambled ASO-treated group (2.4%), $p < 0.01$; and cyst volume density: AGT ASO-treated group (22%), scrambled ASO-treated group (34.1%), $p < 0.05$.		

Target Gene	In Vivo Studies of ASO Mediated Gene Silencing		Ref.
	Type of CKD model	**Animal model**	
APOL1	Human *APOL1*-transgenic mice with induced proteinuria by IFN-γ challenge	Human *APOL1*-transgenic C57BL/6 mice	[52]
	Therapeutic regimen of ASO		
	Intraperitoneal injection at 50 mg/kg/week for four weeks		
	Renal function and/or renal damage markers		
	Prevention of IFN-γ induced proteinuria determined by urinary albumin-to-creatinine ratio (ACR) (ug Alb/mg Cre): APOL1 ASO-treated group (0), control ASO-treated group (~1000), $p < 0.001$.		[56][57]

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which achieved markedly reduced extracellular matrix accumulation [58]. However, TGF-β is not a suitable therapeutic target as it is a pleiotropic cytokine that exhibits other essential biofunctions in mammals, and TGF-β knockout mice survive for only a few weeks after birth [59][60][61].

Thrombospondin-1 (TSP1) is a major activator of TGF-β1 [62][63][64][65]. Nevertheless, unlike the TGF-β null mice, TSP1 knockout mice do not die prematurely and are healthy [64][65]. De novo *TSP1* expression in mesangial cells colocalizes with the upregulation of TGF-β1 in different experimental kidney disease models [66], including the anti-Thy1 model [55][67]. In order to investigate the role of TSP1 as a TGF-β activator in the development of renal fibrosis, Daniel et al. screened out two PS-modified DNA ASO sequences (an 18 mer ASO and a 15 mer ASO) from 11 candidates that were designed to inhibit TSP1 expression [47]. ASOs were selectively transferred to the glomeruli of Sprague-Dawley (SD) rats with anti-Thy1 antibody-induced mesangial proliferative glomerulonephritis through renal artery perfusion followed by electroporation. Six days after treatment, TSP1-specific ASOs achieved the efficient reduction of TSP1 expression by more than 60%, and the inhibition of active TGF-β secretion by 50%, while not affecting the total expression level of TGF-β [47]. The ASO treatment also led to an evident reduction in the glomerular number of nucleic positive for the phospho-Smad2/3 (a TGF-β-signaling molecule that is known as a marker of TGF-β activation), indicating a markedly decreased glomerular TGF-β activity, which was associated with a marked reduction in mesangial cell activation [47]. Furthermore, TSP1-targeting ASO therapy inhibited the accumulation of glomerular extracellular matrix so that extra-domain A of fibronectin was markedly reduced by nearly 96%, as well as the reduced accumulation of other extracellular matrix proteins such as collagen I and collagen IV [47]. This study reveals that TSP1 is a tightly regulated activator of TGF-β in the anti-Thy1 model, and is responsible for the accumulation of glomerular extracellular matrix by activating TGF-β. Moreover, the data of the study suggests that the ASO-based TSP1 inhibitor may be a feasible therapeutic for fibrotic renal disease.

2.2. Connective Tissue Growth Factor (CTGF)

Over 30% of ESRD is caused by diabetic nephropathy. Glomerulosclerosis, the pathological hallmark of diabetic nephropathy, is characterized by the extracellular matrix accumulation of mesangial cells and tubulointerstitial fibrosis [68]. Both in vitro and in vivo studies have established that TGF-β contributes to glomerulosclerosis and that

overexpression of TGF-β is associated with fibrosis and scarring in response to renal injury in diabetes [69][70][71][72][73]. However, as mentioned in the previous section, TGF-β is not a suitable target for drug development due to its multifunctionality. CTGF, a prosclerotic cytokine overexpressed during diabetes and acting downstream of TGF-β [74], directly contributes to the accumulation of extracellular matrix and tubulointerstitial fibrosis in diabetic nephropathy [74][75][76]. Okada et al. downregulated the expression of CTGF in tubular epithelium by the intravenous administration of a CTGF-targeting, PS-modified 18 mer DNA ASO in mice with subtotal nephrectomy (SNx) [77]. They found that decreased expression of CTGF caused by the ASO is associated with attenuated interstitial fibrosis resulting from the downregulated expression of genes involved in the expansion of the glomerular extracellular matrix, demonstrating that CTGF is a direct and significant contributor of TGF-β-dependent renal fibrogenesis [77]. This study suggested that the development of an ASO-based CTGF inhibitor may be a promising strategy for antifibrotic therapy in TGF-β-dependent CKD such as diabetic nephropathy.

Later, Guha et al. investigated the role of CTGF in the progression of diabetic nephropathy by administrating a CTGF-specific 20 mer ASO (either 4-12-4 MOE^{PO}-DNA^{PS}-MOE^{PO} or 4-12-4 MOE^{PS}-DNA^{PS}-MOE^{PS} gapmer) to nephropathic mice with streptozotocin-induced type 1 diabetes and db/db mice (mice with type 2 diabetes) with a dosage of 20 mg/kg (twice weekly) for 16 weeks and 8 weeks, respectively [48]. The CTGF-targeting ASO-inhibited hyperglycemia induced overexpression of CTGF in both diabetic mouse models. In the mice with type 1 diabetes, ASO treatment inhibited a variety of indices of renal disease, for instance, the development of renal hypertrophy was significantly attenuated in that the diabetes-induced increase of kidney weight was reduced by 32%, increases of serum creatinine and urinary albumin which are pathological features of diabetic nephropathy were reduced by 32% and 52%, respectively, and the diabetes-induced expansion of the mesangial matrix was attenuated by 43%, which was associated with the reduced synthesis of collagen 1, fibronectin, and TGF-β1 [48]. Furthermore, ASO treatment achieved the inhibition of profibrotic p38 mitogen-activated protein kinase (MAPK) and its downstream target transcription factor cAMP-response element binding protein (CREB) so that the activation of p38 MAPK and CREB was attenuated by 54% and 74%, respectively, indicating that the progression of diabetic nephropathy may be inhibited [48]. In addition, in the db/db mice, CTGF-targeting ASO reduced serum creatinine, urinary total protein, and urinary albumin by 37%, 41%, and 48%, respectively [48]. This study provides sound scientific evidence that the specific knockdown of CTGF by gapmer-like ASO holds significant promise as a potential therapy for diabetic nephropathy.

2.3. Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

Tubulointerstitial fibrosis, characterized by the excessive deposition of extracellular matrix resulting from an increased number of activated interstitial myofibroblasts, is a key determinant of progressive CKD [78]. RAS proteins, termed small guanosine triphosphatases (GTPases), play essential roles in the regulation of cell survival, proliferation, and differentiation by acting as signal transduction molecules in various extracellular pathways [79]. Bechtel et al. demonstrated that the activation of RAS is directly associated with renal fibrogenesis [80]. Therefore, RAS proteins may be potential therapeutic targets for the renal fibrosis of CKD [81][82][83][84][85][86][87][88]. Sharpe et al. demonstrated that KRAS is the predominant isoform of RAS expressed in human renal fibroblasts and that PS or 2′-MOE-PS-modified ASO-induced KRAS knockdown significantly suppresses the proliferation of fibroblasts [89]

[90]. Later, in order to investigate the profibrotic role of KRAS in CKD, the same group silenced the KRAS expression in rats with unilateral ureteric obstruction (unilateral ureteric obstruction is considered a model of renal fibrosis and CKD [91]) by the subcutaneous administration of KRAS-specific 20 mer gapmer-like ASOs (ISIS 104440 or ISIS 104419, 5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} design) on alternate days (for six days) at a dosage of 12.5 mg/kg [49]. Treatment of KRAS-specific ASOs significantly reduced the level of KRAS mRNA by 61% (ISIS 104440) and 97% (ISIS 104419) compared to their correspondent scrambled ASOs (negative control), which was associated with reduced renal fibrosis (fibrosis score was reduced to 17% by ISIS 104440 and 20.3% by ISIS 104419) and collagen deposition (collagen deposition score was reduced to 18.4% by ISIS 104440 and 17% by ISIS 104419) [49]. Furthermore, the upregulation of α-smooth muscle actin (α-SMA, a marker of myofibroblast activity) induced by obstructive nephropathy was inhibited by the KRAS-targeting ASOs so that the α-SMA expression was reduced from 53% (negative control group) to 3.9% and 20% by ISIS 104440 and ISIS 104419, respectively, indicating that an ASO-mediated KRAS knockdown could prevent the onset of fibrosis in rat models of unilateral ureteric obstruction [49]. This study suggests that ASO-induced KRAS inhibition could be a novel antifibrotic strategy for CKD as ASO treatment markedly inhibited renal fibrosis.

It is worth mentioning that Ross et al. recently evaluated another highly potent KRAS-specific ASO candidate (AZD4785 or ISIS 651987) for its anti-tumour effects in vitro and in vivo [92]. We recommend researchers compare the efficacy between AZD4785 and ISIS 104440/ISIS 104419 to identify the best-performing lead candidate for further anti-CKD therapeutic development.

2.4. Mammalian Target of Rapamycin (mTOR)

Autosomal dominant polycystic kidney disease (PKD), caused by a mutation in the PKD1 or PKD2 gene, with 50% of patients developing CKD, accounts for ~5–10% of ESRD in the US requiring dialysis or renal transplantation [93]. The mTOR signaling pathway plays a role in the regulation of cell growth and proliferation [94]. Specifically, mTOR complex 1 (mTORC1) controls protein synthesis and cell proliferation, and the hyperactivation of the mTORC1 signal is a feature of PKD [95][96][97][98]. mTOR complex 2 (mTORC2) modulates cell survival and arrangement of actin cytoskeleton by phosphorylating AGC kinases such as the pro-survival kinase $pAkt^{Ser473}$ [99][100][101]. Activation of mTORC2 is upregulated in PKD [96][97][98][99][100][101][102][103]. Sirolimus, an mTORC1 inhibitor, has been demonstrated to have a therapeutic effect on mice with PKD resulting from Pkd1 inactivation via the reduction of cyst growth and preservation of renal function [96]. However, sirolimus had no effect on renal function in Pkd2WS25/− mice (a model of human autosomal dominant PKD resulting from mutation in the Pkd2 gene) [104], and a clinical study showed that sirolimus treatment did not halt the growth of polycystic kidneys in humans [105]. One possible reason of the inefficacy is that sirolimus directly inhibits mTORC1 but not mTORC2, therefore, it is unable to inhibit mTORC2-dependent Akt-induced proliferation [106].

In order to determine the therapeutic effect of the combined inhibition of mTORC1 and mTORC2 on PKD, Ravichandran et al. developed an mTOR-specific 20 mer gapmer-like ASO (5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} design) through the screening of ~150 ASO candidates [50], as mTOR exists in both mTORC1 and mTORC2 [107]. ASOs were administered into Pkd2WS25/− mice via intraperitoneal injections at a sequential two-stage dosage of 100

mg/kg/week (first 4 weeks) and 50 mg/kg/week (the remaining 8 weeks) [50]. Treatment of mTOR ASO led to a significant reduction in the expression levels of mTOR, pS6 (a marker of mTORC1 signaling), and pAkt^{Ser473} [50], which was associated with the reduced ratio of two kidneys/total body weight from 2.4% (control ASO group) to 1.5% (mTOR ASO group), significantly decreased cyst volume density from 34.1% (control ASO group) to 15.1% (mTOR ASO group), and significantly reduced blood urea nitrogen from 43.4 mg/dL (control ASO group) to 29 mg/dL (mTOR ASO group) [50], indicating an ASO-induced melioration of PKD and normalization of renal function. Furthermore, mTOR ASO treatment significantly inhibited both the proliferation and apoptosis of tubular epithelial cells (proliferation and apoptosis of epithelial cells lining the renal tubular plays a key role in cyst growth [108]) so that the number of proliferating cell nuclear antigen positive cells was reduced from 1.9 per cyst (control ASO group) to 0.8 per cyst (mTOR ASO group), and the number of apoptotic cells was reduced from 3.6 per non-cystic tubule (control ASO group) to 1.2 per non-cystic tubule (mTOR ASO group) [50], suggesting that ASO therapy could inhibit cyst growth. This study demonstrated that the combined inhibition of both mTORC1 and mTORC2 holds therapeutic potential for autosomal dominant PKD [50], and an ASO-based mTOR inhibitor can be a promising approach for treating the disease owing to its capability of combined mTORC1/2 knockdown.

2.5. Angiotensinogen (AGT)

Enlargement of renal cysts in autosomal dominant PKD is associated with the activation of the renin–angiotensin system and the resultant production of proinflammatory and profibrotic angiotensin II [109][110][111], which contributes to cystogenesis by the induction of cellular proliferation, inflammation, and fibrosis [109][112]. The renin–angiotensin system also plays an important role in type IV cardiorenal syndrome and CKD [113]. Angiotensin II induces the differentiation of renal fibroblasts into myofibroblasts and stimulates the expression and activation of TGF-β [114][115]. Angiotensin II leads to renal damage by increasing the expression of proinflammatory cytokines and chemokines and renal leukocyte infiltration [116]. Despite the importance of the renin–angiotensin system in the pathogenesis of PKD, the single or combined use of renin–angiotensin system inhibitors, including ACEis, ARBs, and renin inhibitor, have limited efficacy and induce side effects [117][118]. Identification of other targets within the renin–angiotensin system may result in the development of more efficient therapeutics.

Angiotensinogen (AGT) is a substrate of the peptidase renin in the renin–angiotensin system, and AGT is cleaved by renin forming angiotensin I, which is then converted to angiotensin II by ACE [115]. In order to investigate the potential therapeutic effects of direct AGT inhibition on PKD, Ravichandran et al. developed an AGT-specific 20 mer gapmer-like ASO (5-10-5 MOE^{PS}-DNA^{PS}-MOE^{PS} design) through the screening of ~150 ASO candidates [51]. The administration of ASO was performed by intraperitoneal injections into Pkd2WS25/− mice at a sequential two-stage dosage of 100 mg/kg/week (first 4 weeks) and 50 mg/kg/week (the remaining 8 weeks) [51]. AGT-specific ASO treatment significantly reduced the AGT expression, which was associated with a reduced two kidney/total body weight ratio (AGT ASO group: 1.5%, control ASO group: 2.4%), decreased cyst volume density (AGT ASO group: 22%, control ASO group: 34.1%) and blood urea nitrogen (AGT ASO group: 34 mg/dL, control ASO group: 47 mg/dL), indicating that AGT-specific ASO treatment could lead to decreased PKD and normalization of renal function [51]. Furthermore, significant decreases in proinflammatory cytokines including C-X-C motif chemokine ligand 1 (CXCL1), interleukin 12 (IL-12), and the profibrotic TGF-β were observed in the AGT ASO treatment

groups (CXCL1: 0.6 pg/mg, IL-12: 8.8 pg/mg, TGF-β: 32 pg/mg) in contrast to the control ASO treatment groups (CXCL1: 3.4 pg/mg, IL-12: 37.3 pg/mg, TGF-β: 102 pg/mg), indicating that AGT-specific ASO treatment could lead to decreased proinflammatory and profibrotic molecules [51]. Although further investigation is required to elucidate the mechanism underlying the ASO-induced inhibition on cyst growth, chronic AGT inhibition by ASO may be a possible therapeutic strategy for autosomal dominant PKD in the future.

2.6. Apolipoprotein L1 (APOL1)

APOL1 is a newly evolved gene that is only present in a few primates such as humans, baboons, and gorillas [119][120]. The APOL1 protein functions as the trypanolytic factor in serum that lyses trypanosomes against African trypanosomiasis (sleeping sickness), a disease endemic to Africa [121][122][123]. One of the trypanosome species, Trypanosoma brucei rhodesiense, has evolved to resist the wild type APOL1 (G0), while the G1 and G2 mutants of APOL1 (commonly found in populations of African ancestry), discovered in 2010, overcome the resistance of Trypanosoma brucei rhodesiense [124]. However, mutant APOL1 also leads to toxic gain of function when overexpressed so that [125][126], compared to the wild type APOL1 (G0), the G1/G2 mutants are associated with an increased risk of CKD by 7- to 30-fold [124][127][128][129]. Furthermore, these mutants accelerate the GFR decline, and thus the progression of CKD [128][130]. In addition, G1/G2 mutants are strongly associated with various forms of nondiabetic nephropathy such as focal segmental glomerulosclerosis (FSGS) and interferon (IFN) therapy-related collapsing glomerulopathy [124][125][128]. As APOL1 is not essential for kidney development and function [119][131][132][133], reducing the expression level of APOL1 therapeutically will not result in any harmful effects other than increased susceptibility to African sleeping sickness in specific geographical regions [52]. Therefore, in an attempt to study APOL1 systemically and achieve proof of concept for APOL1 inhibition by antisense oligomer, Aghajan et al. established a transgenic mouse model for APOL1-related CKD (C57BL/6 mice with human APOL1 G1 mutant gene were challenged by IFN-γ leading to induced proteinuria in mice), developed a APOL1 specific, 2′-MOE or 2′-4′ constrained ethyl (cEt)-modified 16 mer gapmer ASO on a PS backbone (IONIS-APOL1$_{Rx}$) through the screening of over 4000 ASO candidates, and treated APOL1 G1 transgenic mice with IONIS-APOL1$_{Rx}$ at a weekly dose of 50 mg/kg for four weeks prior to IFN-γ challenge [52]. ASO treatment led to a significant decrease in the APOL1 mRNA levels in both the kidney (by ~50%–60%) and liver (by 95%), which completely prevented the occurrence of IFN-γ-triggered proteinuria, indicating that the CKD-relevant cell types (podocytes, endothelial cells, and mesangial cells that constitute the renal filtration barrier) are sensitive to ASO treatment [52]. It was also found that administration of IONIS-APOL1$_{Rx}$ at a weekly dosage as low as 6.25 mg/kg led to the significant inhibition of induced proteinuria, demonstrating the potency of ASO therapy and the renoprotective effect that it provided [52]. Although further study is definitely required to reveal the pathogenesis of the toxic gain of function resulting from mutant APOL1, IONIS-APOL1$_{Rx}$ holds promise to be developed as an efficient anti-CKD therapeutic option for patients with APOL1 nephropathies.

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