



CKJ REVIEW

Emerging targeted strategies for the treatment of autosomal dominant polycystic kidney disease

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a widespread genetic disease that leads to renal failure in the majority of patients. The very first pharmacological treatment, tolvaptan, received Food and Drug Administration approval in 2018 after previous approval in Europe and other countries. However, tolvaptan is moderately effective and may negatively impact a patient's quality of life due to potentially significant side effects. Additional and improved therapies are still urgently needed, and several clinical trials are underway, which are discussed in the companion paper Müller and Benzing (Management of autosomal-dominant polycystic kidney disease—state-of-the-art) *Clin Kidney J* 2018; 11: i2–i13. Here, we discuss new therapeutic avenues that are currently being investigated at the preclinical stage. We focus on mammalian target of rapamycin and dual kinase inhibitors, compounds that target inflammation and histone deacetylases, RNA-targeted therapeutic strategies, glucosylceramide synthase inhibitors, compounds that affect the metabolism of renal cysts and dietary restriction. We discuss tissue targeting to renal cysts of small molecules via the folate receptor, and of monoclonal antibodies via the polymeric immunoglobulin receptor. A general problem with potential pharmacological approaches is that the many molecular targets that have been implicated in ADPKD are all widely expressed and carry out important functions in many organs and tissues. Because ADPKD is a slowly progressing, chronic disease, it is likely that any therapy will have to continue over years and decades. Therefore, systemically distributed drugs are likely to lead to potentially prohibitive extra-renal side effects during extended treatment. Tissue targeting to renal cysts of such drugs is one potential way around this problem. The use of dietary, instead of pharmacological, interventions is another.

Keywords: ADPKD, dietary intervention, mTOR, pharmacological intervention, polycystic kidney disease

INTRODUCTION

There is no shortage of 'promising' molecular drug targets for the treatment of polycystic kidney disease (PKD). Work in many laboratories over the years has led to the identification of a vast number of signaling pathways, kinases, transcription factors,

metabolic pathways, etc. that are aberrantly up- or down-regulated in PKD. Many of these could be targeted with existing or novel drugs, which very often lead to amelioration of renal cyst growth and slowing down of functional deterioration of the kidneys. The caveat is that whereas these drugs are effective in

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animal models of PKD, mostly mice and rats, the translation of these findings into clinical success is a hurdle that has proved very difficult to overcome.

The main reason for this translation problem is that virtually all of the molecular drug targets that have been implicated in PKD are not unique to polycystic kidneys. In fact, most of them are ubiquitously expressed and serve important functions in many extra-renal tissues and organs. Therefore, drugs that affect PKD targets may be effective in inhibiting renal cyst growth but they also tend to cause adverse effects in both the kidneys and even more importantly extra-renal tissues that would be prohibitive in clinical practice.

A prime example is the mammalian target of rapamycin (mTOR) inhibitors. mTOR is a key signaling kinase that receives inputs from several upstream pathways and regulates fundamental cellular behaviors including cell growth, proliferation, survival and energy metabolism [1]. mTOR is aberrantly activated in cyst-lining cells in human autosomal dominant polycystic kidney disease (ADPKD) and most rodent models of PKD [2, 3]. This was an exciting finding because mTOR inhibitors, such as rapamycin, were already clinically approved as immunosuppressive drugs. Indeed, rapamycin proved to be highly effective in numerous PKD rodent models [3–7] in which it can be used at high doses during the relatively short treatment periods that are required, usually around 2 weeks. The high doses ensure that mTOR in the target organ, the polycystic kidneys, is indeed inhibited. The short treatment period ensures that long-term side effects become irrelevant. For example, immunosuppression as an unwanted effect of mTOR inhibition is generally not a problem for a mouse living for 2 weeks in a nearly pathogen-free animal facility. The problem is that ADPKD in humans is a very slowly progressing, chronic disease that leads to renal deterioration and eventually renal failure during the course of decades. To observe any beneficial effects in clinical trials, a study has to extend for at least a year and ideally longer. All the while, these patients live in environments that are nothing like a mouse cage. In hindsight, it may not have been surprising that clinical trials to test the efficacy of mTOR inhibitors in ADPKD failed to show beneficial effects [8–10]. The long-term tolerable doses of these drugs in humans are much lower than those that can be administered in the short term in rodents. It is likely that these low, tolerable doses have relatively little effect on mTOR in polycystic kidneys, but they still cause significant extra-renal adverse effects in patients. Simply increasing the dose is therefore not an option.

This example illustrates perhaps the most significant hurdle in finding a feasible pharmacological therapy for ADPKD. Due to the chronic, slowly progressive nature of the disease, any drug treatment for ADPKD would likely have to occur continuously over the course of years and decades. Treatment would also likely have to be initiated early in relatively nonsymptomatic patients to avoid permanent renal function loss as much as possible. This puts an extremely high burden on any drug to be used for ADPKD therapy to exhibit an extremely low long-term side effect profile.

Since many of the molecular targets implicated in ADPKD have already been pursued for other indications, primarily cancer, many drugs have already been developed or are even in clinical use for those other indications. The re-purposing of these compounds for ADPKD therapy is a promising avenue. To accomplish the lowest possible side effect profile, a pharmaceutical compound would ideally be highly specific to its intended molecular target. This is often difficult to achieve with small molecule drugs such as tyrosine kinase inhibitors. Biologics

such as monoclonal antibodies (mAbs) are a more specific approach, but there have been few attempts, and no success, so far in preclinical PKD studies. The targeting of compounds, both small molecules and antibodies, specifically to polycystic kidneys is a novel approach that has the potential to reduce extra-renal effects and to make the re-purposing of many compounds for long-term therapy in ADPKD potentially feasible. We will discuss tissue targeting further below. First, we will discuss select, molecular targets that have recently emerged in preclinical studies. The names of drugs or drug classes are shown in bold at first mention.

MOLECULAR TARGETS ON THE HORIZON

Catalytic mTOR inhibitors and dual kinase inhibitors

As mentioned above, **rapamycin** and its analogs (rapalogs) are highly effective in PKD rodent models. These drugs specifically target the activity of mTORC1 (see Figure 1), one of the two complexes in which the kinase mTOR acts. The other complex, mTORC2, has different functions but is also activated in PKD as deduced from the high levels of one of its downstream targets, phospho-Akt (Ser473), compared with wild-type kidneys [4, 11]. Since mTORC2 is not directly targeted by rapamycin, it has been investigated whether combined inhibition of both mTORC1 and mTORC2 may be beneficial. This was achieved *in vivo* utilizing a catalytic mTORC1/mTORC2 kinase inhibitor, **PP242**, in the Han:SPRD Cy/+ rat model of PKD [12]. This approach led to inhibition of the progression of renal cystic disease. However, in the absence of a head-to-head comparison with mTORC1-specific inhibition, it is difficult to conclude whether the added inhibition of mTORC2 was beneficial over inhibition of mTORC1 alone. A concern is that combined mTORC1 and -2 inhibitions may lead to increased extra-renal toxicity in the clinic compared with rapalogs, especially during long-term treatment.

In another study, the effect of dual inhibition of mTORC1/2 and phosphoinositide 3-kinase (PI3K) was tested utilizing **NVP-BEZ235** in the Han:SPRD Cy/+ rat model and a mouse *Pkd1* model [13]. The authors demonstrated that inhibition of mTORC1 with a rapalog activated both mTORC2 and ERK via two feedback loops. Dual inhibition of mTORC1/2 and PI3K with **NVP-BEZ235** was more effective than rapalog-mediated mTORC1 inhibition in terms of reducing cystic disease progression. Although this study highlights the theoretical utility of dual mTOR/PI3K inhibition in PKD, the main concern is the toxicity of this compound, which led to early termination of a Phase I trial [14].

Targeting inflammation

Numerous lines of evidence suggest an important role of inflammation in the progression of PKD. This is supported by the presence of pro-inflammatory markers in ADPKD urine and renal cyst fluid [15], accumulation of inflammatory cells and the role of renal macrophages in PKD progression [16, 17]. The inflammatory cytokine tumor necrosis factor (TNF- α) is present in ADPKD cyst fluid and can promote cyst growth with *ex vivo* and *in vivo* administration [18]. TNF- α was reported to disrupt the localization of polycystin-2 to the plasma membrane and primary cilia [18]. A TNF- α inhibitor, **etanercept**, is a Food and Drug Administration-approved biologic drug used for the treatment of autoimmune disorders. Etanercept acts as a decoy receptor for TNF- α and has been shown to inhibit renal cyst growth in *Pkd2*^{+/-} mice [18].

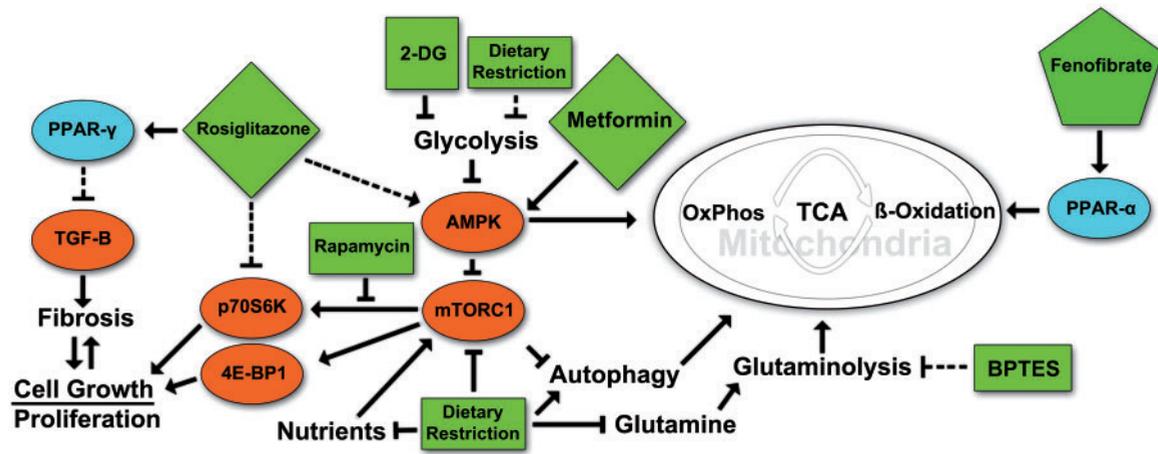


FIGURE 1: Targeted metabolic regulation in ADPKD. A highly simplified cartoon of some of the major pathways that relate to the pathogenesis of PKD and that are affected by some of the compounds discussed in this article. Rosiglitazone treatment activates PPAR- γ , causing heterodimeric binding to retinoid x receptor (RXR) followed by translocation to the nucleus, activating gene transcription of PPAR response element-regulated genes. This in turn leads to a decrease in TGF- β signaling and a subsequent reduction in fibrosis. Rosiglitazone also acts independently of PPAR- γ to inhibit p70S6K activation and ribosomal protein S6 phosphorylation. Treatment with 2DG leads to a reduction in glycolysis by inhibition of phosphoglucosomerase. Decreased glycolytic activity in turn may cause the activation of AMPK and subsequent inhibition of mTORC1, cell growth and proliferation with an increase in fatty acid oxidation. Metformin activates AMPK that directly represses mTORC1 signaling via phosphorylation. Treatment with BPTES inhibits glutaminase (GLS1) disrupting the breakdown of glutamine to glutamate preventing it from being used in the TCA cycle to produce α -ketoglutarate. Fenofibrate treatment activates PPAR- α to bind to PPAR response elements and increase transcription of genes involved in fatty acid utilization, oxidative phosphorylation and mitochondrial biogenesis. Rapamycin inhibits the ability of mTORC1 to activate the S6-Kinase branch of its downstream pathway but has less effect on the 4E-BP1 branch. In contrast, mTOR kinase inhibitors or mTOR ASOs would affect both downstream branches. Dietary restriction simulates the effects of targeted drug therapies by reducing nutrient intake, leading to reductions in key regulatory pathways. Pointed arrowheads indicate activating effects. Blunt arrowheads indicate inhibitory effects. Dashed arrows indicate indirect or multistep effects.

The natural polyphenolic compound **resveratrol** exerts anti-inflammatory, antioxidant and anti-proliferative effects through targeting of mTOR and nuclear factor (NF)- κ B [19, 20] and has been shown to slow disease progression through dampening inflammatory pathway activity in animal models of PKD [21]. Treatment of the Han:SPRD Cy/+ rat model with resveratrol led to a decrease in the pro-inflammatory cytokines MCP-1, CFB and TNF- α , and reduction in macrophage infiltration and some amelioration of renal cystic disease [21]. Although there is extensive *in vitro* and *in vivo* evidence that resveratrol could be used therapeutically in a wide array of diseases, a major challenge for clinical trials has been its poor bioavailability [22].

Histone deacetylase inhibitors

Histone deacetylases (HDACs) are a family of enzymes that modulate gene expression by removing acetyl groups from histones and regulate a diverse array of intracellular pathways by acting on nonhistone proteins [23]. A chemical modifier screen revealed the positive effect of HDAC inhibition in a *Pkd2*-deficient zebrafish model. In this study, a pan-HDAC inhibitor, **trichostatin A** and a Class I HDAC inhibitor, **valproic acid (VPA)**, were shown to affect both body curvature and laterality, two pathological changes associated with cystogenesis in zebrafish [24]. It was further demonstrated that VPA treatment decreased renal cyst progression and preserved kidney function in an orthologous mouse model (*Pkd1*^{flox/flox}; *Pkhd1*-Cre) [24].

The histone deacetylase, HDAC6, has heightened expression and activity in *Pkd1* mutant renal epithelial cells [25]. HDAC6 is predominantly localized to the cytoplasm and uniquely interacts with nonhistone proteins [26]. In ADPKD models, blocking HDAC6 with specific inhibitors, **ACY-1215** and **tubacin**, slows cyst growth *in vivo* and prevents cyst formation *in vitro* [27, 28]. Both ACY-1215 and tubacin are thought to inhibit proliferation

of cyst-lining epithelia by preventing deacetylation of α -tubulin, where deacetylation would typically promote cell cycle progression. In addition, both ACY-1215 and tubacin were found to downregulate cyclic adenosine monophosphate (cAMP); however, the exact mechanism underlying this finding requires further investigation [27, 28]. HDAC6 inhibition may also function by blocking EGF-mediated β -catenin nuclear localization, resulting in suppression of epithelial proliferation and an increase in EGF receptor (EGFR) degradation [29].

With the promising results of HDAC inhibition in animal models of ADPKD, there has been a focus on the role of histone acetylation, which by modifying chromatin structure can recruit DNA-binding factors such as bromodomain and extra-terminal proteins (BET) [30, 31]. It has been shown that an inhibitor of the Brd4 BET protein, **JQ1**, was effective in suppressing cyst growth and kidney size, and maintaining kidney function in two *Pkd1*-mutant mouse models [32].

RNA-targeted therapeutic strategies

The two primary pharmacological approaches to target RNA that have emerged over the past decades are antisense oligonucleotides (ASOs) and RNA-mediated interference, including micro-RNA (miRNA) [33], which we will discuss in the context of PKD.

Antisense oligonucleotides. ASOs are short oligonucleotides with complementary sequence to a specific mRNA designed to reduce the expression of a target mRNA and its protein product. Antisense strategies against certain targets have been exploited in a number of diseases [34], leading to the approval of four ASO-based therapies as of 2017 [35].

As has already been noted above, the mTOR pathway is highly activated in PKD. To determine whether downregulation of mTOR is an effective treatment strategy, an ASO targeting

mTOR expression has been investigated in the *Pkd2*^{WS25/-} mouse model [36]. The mTOR ASO effectively reduced mTOR levels and reduced the activity of mTORC1 and mTORC2, as assessed by surrogate markers. This resulted in a significant improvement of various aspects of the renal cystic phenotype [36].

The renin-angiotensin system has been shown to be upregulated in PKD [37, 38], and clinical trials utilizing **angiotensin-converting enzyme (ACE) inhibitors** have been conducted in ADPKD patients [39, 40]. Although ACE inhibitors did not affect the decline in renal function in these clinical studies [41], the feasibility of targeting angiotensinogen (AGT) levels directly with ASOs has been investigated in preclinical studies [42–44]. In all three studies, the **AGT ASO** was shown to accumulate in the kidney and reduce AGT mRNA and protein in kidney, serum and urine. In terms of therapeutic efficacy, AGT ASOs, but not scrambled ASOs, significantly ameliorate several aspects of the renal cystic disease phenotype in both the PKD2WS25 model [42] and a global, tamoxifen-inducible knockout model of PKD (*Pkd1*^{flox/flox}; CAGG-CreER) [43, 44].

Taken together, these studies suggest that targeting AGT with ASOs may be superior to ACE inhibitors. These studies also suggest that the utilized second-generation ASOs accumulate in kidneys and/or cysts, but this is unlikely to be a specific effect. Nevertheless, such ASOs could be considered to target the expression of other proteins implicated in PKD. For example, **AZD9150**, an ASO that targets STAT3, has shown promising initial activity in lymphoma patients in a Phase 1 clinical trial [45]. Given that STAT3 is activated in ADPKD and multiple preclinical models of PKD [46–49], the potential efficacy of ASOs targeting STAT3 may be of interest.

Targeting miRNAs. miRNAs are a class of endogenous small (~22 nt) noncoding RNAs that regulate the expression of target mRNAs and their protein product [50]. In the context of PKD, several miRNAs have been shown to be expressed and modulated in the disease state [51–53]. Kidney-specific, collecting duct knockout of key miRNA pathway genes has been shown to result in epithelial-to-mesenchymal transition and fibrosis [54], suggesting that these miRNAs may also play a role in fibrosis in PKD. A number of studies have demonstrated that the miR-17-92 cluster [55, 56], which encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1), is upregulated in several models of PKD [57–59], and *Pkd1* has been shown to be a direct target of miR-20 [60]. Overexpression or knockdown of the miR-17-92 cluster influences the development of PKD [58]. Utilizing the slow-progressing *Pkd1*^{RC/RC} mouse model, a kidney-targeted **anti-miR-17 oligonucleotide** proved to be effective in reducing kidney growth, decreasing proliferation and fibrosis and extending animal survival [57].

Outside of the miR-17-92 cluster, several other miRNAs have been associated with PKD. These include miR-21 [61], miR-20b-5p and miR-106a-5p [62], miR-199a-5p [63] and miR-501-5p [64] and suggest that targeting miRNAs pharmacologically may represent a new paradigm in PKD treatment [50].

Glucosylceramide synthase inhibitors

Glycosphingolipids (GSLs) play fundamental roles in a variety of cellular processes [65, 66], and their dysregulation leads to a number of diseases, including Gaucher, Fabry [67, 68] and renal diseases [69]. A potential role for GSLs in PKD has been suggested based on upregulation of certain GSLs in preclinical models and ADPKD samples [70–73]. The glucosylceramide synthase (GCS) inhibitor **Genz-123346** was tested in multiple animal

models of PKD and showed remarkable efficacy in terms of reducing kidney size, cystic index and improving renal function [71]. Genz-123346 treatment led to inhibition of glucosylceramide (GlcCer), lactosylceramide (LacCer) and GM3 production, and was shown to inhibit key cellular pathways known to be activated in PKD, including Akt-mTOR and various cell cycle proteins. Genetic ablation experiments revealed that deletion of GM3 synthase or sphingokinase 1 either ameliorated or exacerbated the renal cystic phenotype in *jck* mice, respectively, suggesting that specific GSLs, or combinations of multiple GSLs, are important modifiers of renal cyst growth [74]. The GCS inhibitor, **venglustat**, has recently entered a Phase 3 clinical trial in which its efficacy will be tested in ADPKD patients with rapidly progressing disease (see ClinicalTrials.gov Identifier: NCT03523728). In future work, it will be important to better understand the biological mechanisms underlying the upregulation of GSLs in PKD and the consequences this may have on signaling pathways [75], membrane properties and trafficking [76–78].

Targeting metabolism

A series of recent findings [79–82] have suggested that cysts in PKD exhibit an altered energy metabolism characterized by a high rate of glycolysis and a low rate of mitochondrial oxidative phosphorylation similar to the Warburg effect in many cancer cells. A concrete mechanistic explanation for the Warburg effect in cancer is still lacking and, not surprisingly, it is lacking for PKD as well. Similar to many cancer cells, PKD cells exhibit an increased need for glycolysis [79], a defect in fatty acid oxidation [83] and increased mitochondrial damage [80, 84]. Persistent mTORC1 activity in PKD (see above) could play a role in mitochondrial damage because it would antagonize autophagy [85], which would thwart the removal and replacement of defective mitochondria [86]. Even though the metabolic changes in PKD have been described as ‘aerobic’ glycolysis, renal cystic tissue has been found to be hypoxic [87, 88]. Therefore, these metabolic changes may be a survival adaptation of cyst-lining cells to the hypoxic environment. Just as in cancer, increased glycolysis with reduced oxidative phosphorylation is also expected to lead to increased generation of glycolytic intermediates that form the building blocks for anabolic pathways needed to sustain cell growth and proliferation.

AMP-activated protein kinase (AMPK), a metabolic sensor that negatively regulates mTORC1 and affects many other pathways, has been reported to be less active in some PKD models [79, 81, 89], but not in all [90], and may play a role in mediating the metabolic changes in PKD [91]. **Metformin**, a widely used diabetes medication and a known AMPK activator, was shown to inhibit disease progression in PKD mouse [92] and zebrafish models [90] and is currently being tested in a Phase II clinical trial in ADPKD patients [93]. Numerous other compounds exist—including widely used ones—that function at least partly as **AMPK activators**, and it could be contemplated to investigate their efficacy in PKD [91].

Due to the presumed glucose dependency of cystic cells, targeting glucose utilization has been attempted in PKD models with promising results. **2-deoxy glucose (2DG)** is a potent inhibitor of glycolysis [94] by inhibiting conversion of glucose-6-phosphate by phosphoglucosomerase in the second step of glycolysis [94] with resultant inhibition of hexokinase [95]. Mouse embryonic fibroblasts (MEFs) lacking functional PC1 display increased levels of ATP, ERK and mTORC1 signaling [79]. Increased levels of ATP and lactate have also been observed in

human ADPKD cell cultures and are reduced following treatment with 2DG [89]. Upon glucose withdrawal, *Pkd1*^{-/-}-MEFs were unable to activate autophagy and displayed increased apoptosis [79]. Treatment of a *Pkd1* mouse [79, 96] and the Han:SPRD Cy/+ rat models [89] of PKD with 2DG led to reduced cystic burden and kidney volume [89, 96]. Timing of the knock-out of *Pkd1* in mice at either postnatal day 12 or 25 using a tamoxifen-inducible transgene leads to differences in the rate of disease progression, and in both rapidly and slowly progressing PKD, 2DG treatment was effective in retarding disease progression [96]. 2DG is reasonably well tolerated based on clinical trials in the cancer setting [97]. Whether long-term therapy in ADPKD is feasible remains an open question.

Besides increased reliance on glycolysis, cystic cells appear to have altered metabolic pathways that may be due to mitochondrial defects. Cells from the *cpk* mouse model have an altered tricarboxylic acid cycle (TCA) cycle and overproduce citrate and the oncometabolite 2-hydroxyglutarate, most likely due to increased utilization of glutamine that requires the activity of glutaminase for entry into the TCA cycle [98]. The tumor suppressor *Lkb1* activates AMPK and has been shown to have decreased activity in PKD [79]. Concomitant ablation of *Lkb1* and the mTORC1 regulator *TSC1* leads to PKD and glutamine dependence [99]. Utilizing a primary human ADPKD cell line, inhibition of glutaminolysis with Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) and **CB-839** prevented forskolin-induced cystogenesis [100]. It has been suggested that these cells are glutamine addicted and that **glutaminase inhibitors** may be effective in PKD [98, 100].

Fatty acid beta oxidation, which also occurs in mitochondria, has been found to be impaired in cystic cells [83, 101]. The mitochondria in these cystic cells may no longer function primarily as energy centers but instead primarily produce metabolic intermediates to supply anabolic pathways. Structural and functional abnormalities of mitochondria as well as reduced mitochondrial numbers have been found in human ADPKD and animal models [80]. These mitochondrial defects may involve dysregulation of the peroxisome proliferator-activated receptor (PPAR) family of transcription factors that control a suite of genes involved in fatty acid utilization, inflammation, lipid uptake and lipogenesis [102]. Cells lacking *Pkd1* also show altered levels of the PPARs with either decreased PPAR- α [101] or increased PPAR- γ expression [103].

Of interest is the PPAR- α activator **fenofibrate** and the PPAR- γ activators **rosiglitazone** and **pioglitazone**. PPAR- α levels have been reported to be reduced in human ADPKD cells and aggressive models of mouse PKD [57]. Reduced fatty acid oxidation is observed in the *Pkd1*^{RC/RC} mouse model of PKD along with decreased levels of PPAR- α mRNA [101]. Use of fenofibrate restored fatty acid oxidation and decreased the cystic phenotype following 10 days of administration [101]. Fenofibrate-treated mice also showed increased expression of pyruvate dehydrogenase kinase 4—a negative regulator of glycolysis—and an increase in the mitochondrial biogenic protein PPARC1A and fatty acid β -oxidation [101]. Additionally, a reduction in cell proliferation and inflammation was observed following fenofibrate treatment [101]. PPAR- γ activators have been used in the treatment of diabetes to promote glucose uptake via insulin sensitization [104]. Use of the PPAR- γ agonists rosiglitazone and pioglitazone have each shown efficacy in treating animal models of PKD [105, 106]. Rosiglitazone and pioglitazone treatment reduced levels of the profibrogenic cytokine TGF- β [107–109], in turn decreasing fibrogenesis [108]. Rosiglitazone and pioglitazone also affect the activity of several overactive pathways in PKD

including p70S6K [109, 110], phospho-S6 [109] and phospho-ERK [108]. The reduction in p70S6k by rosiglitazone is not directly mediated through mTOR as concomitant treatment with rapamycin further reduced levels of p70S6K in rodents [110]. The non-PPAR-mediated effects of rosiglitazone have been observed previously in nonsmall-cell lung carcinoma and shown to activate AMPK [111]. This supports an additional mechanism by which rosiglitazone may provide therapeutic potential. Additionally, treatment with rosiglitazone was also able to inhibit proliferation in ADPKD cyst-lining cells by increasing levels of the cell cycle regulators p21 and p27, decreasing levels of cyclin D1 and Cdk4, causing G1-arrest [103]. This was coupled with an increase in apoptosis by a reduction in Bcl-2 and increased levels of Bax [103]. Taken together these studies provide strong evidence for the use of PPAR agonists in the treatment of PKD, with a clinical trial currently testing the efficacy of pioglitazone (see ClinicalTrials.gov Identifier: NCT02697617). However, it is important to keep in mind that the safety of rosiglitazone has been controversial due to conflicting reports of cardiovascular risks that led to suspension of this drug from European markets [112].

Dietary restriction

As detailed above, mTORC1 is typically activated in PKD cysts and is a driver of cyst growth. Given that mTORC1 is not only regulated by growth factor signaling but also by nutrient supply and the energy status of a cell, it could be that these latter factors contribute to mTORC1 activation in PKD. Two groups tested this independently by subjecting three slowly progressive mouse models (*Pkd1*^{RC/RC}, *Pkd2*^{WS25/-} and *Pkd1*^{fllox/fllox}; Nestin-Cre) to food restriction, without malnutrition, and found this treatment to be surprisingly effective [113, 114]. Mild to moderate reduction of food intake—even a reduction as low as 10% below that of controls—resulted in very significant inhibition of renal cyst growth, proliferation, fibrosis and markers of inflammation. In these studies, food intake was reduced overall meaning that all macro- and micronutrients were proportionally reduced. Food reduction by 40% not only inhibited the rate of cyst growth but also actually led to a decrease of existing cystic burden and therefore reversed the disease. Such a reversal has previously only been seen from mTORC1 inhibition with a high dose of rapamycin [4].

Food restriction led to inhibition of not only the S6 branch downstream of mTORC1 [113, 114], but also even more substantial inhibition of the 4E-BP1 branch [114] (see Figure 1). Although previous work mainly evaluated the S6 branch, this new finding suggests that 4E-BP1 may be a more important driver of renal cyst growth. Rapamycin effectively inhibits the S6 branch, but is a less effective inhibitor of the 4E-BP1 branch [115]. This consideration may possibly help to explain why high-dose rapamycin is effective in rodent models of PKD but low-dose rapamycin failed in clinical trials (see above).

A mechanistic explanation for the surprisingly high efficacy of food restriction in PKD mouse models still remains outstanding. However, the translational potential of these findings is very high. It seems possible that ADPKD may prove to be treatable by dietary interventions in many patients. If so, this would provide a safe and inexpensive therapy but may encounter resistance and potential conflicts of interest [116, 117] because it would also undermine significant investments by academic investigators, patient advocacy organizations and the biotech and pharmaceutical industry to develop new drugs for ADPKD therapy.

Tissue targeting approaches

Many of the compounds discussed above would likely be too toxic to be used for long-term therapy in ADPKD patients. A possible way to circumvent unwanted extra-renal effects is to target these compounds specifically, or at least preferentially, to polycystic kidneys. Work in our laboratory has led to two successful strategies, one for the targeting of small molecules to PKD kidneys and the other for the targeting of antibodies to cyst lumens in PKD kidneys (Figure 2). Because small molecule drugs

and biologics (such as mAbs) differ dramatically in their pharmacological properties, very different approaches are needed to direct them to a specific target tissue.

Targeting of small molecules to renal cysts via the folate receptor. In this approach, drug payloads are chemically conjugated to the B vitamin, folic acid. The resulting **folate-conjugated drugs** bind to the high-affinity folate receptor (FR) and are internalized into cells by endocytosis (Figure 2B). Tissue

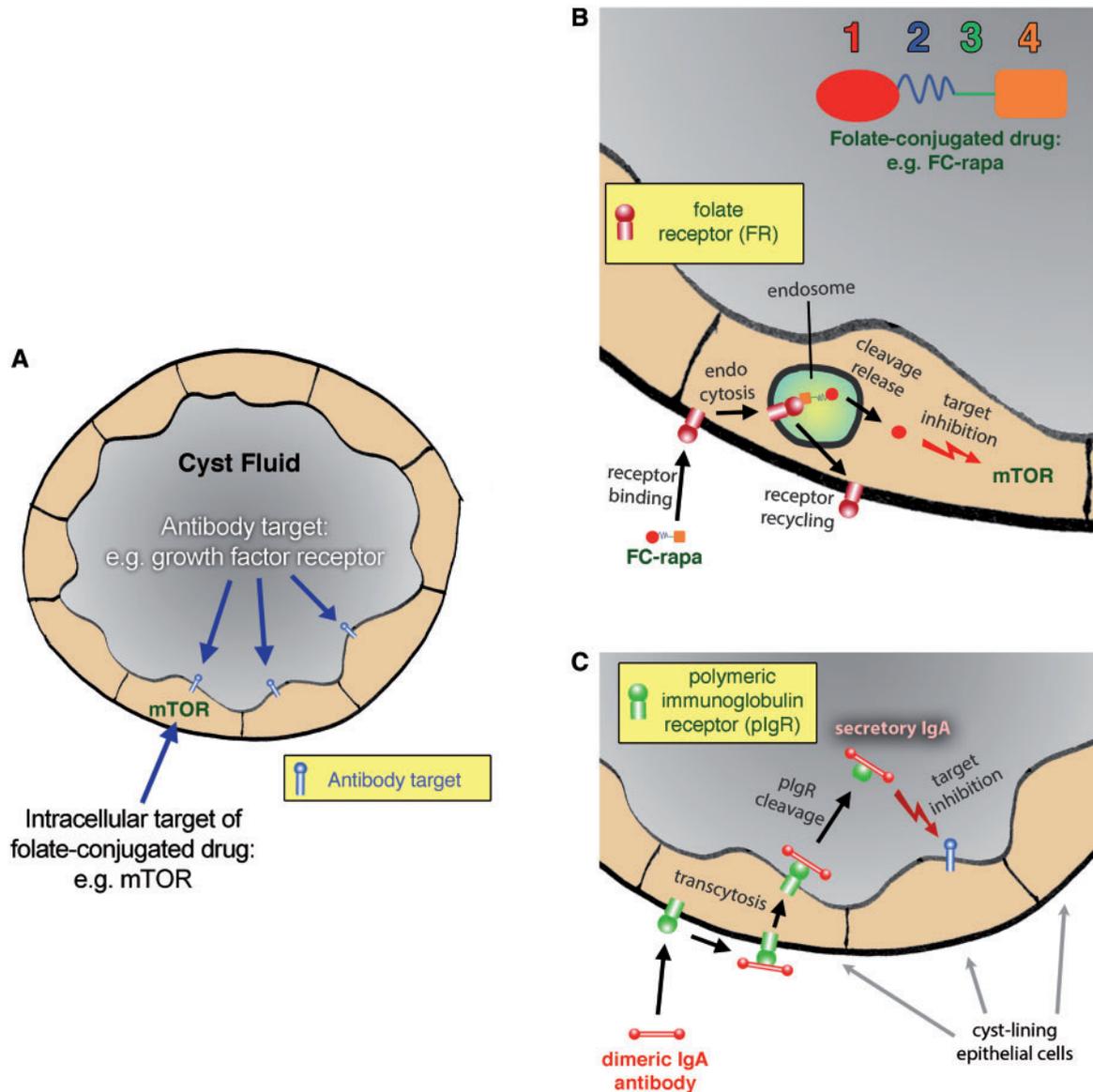


FIGURE 2: Drug targeting to renal cyst lumens in PKD. (A) Cartoon of a cross-section of a renal cyst in PKD that is lined by a single-layer epithelium forming an enclosed space. The kinase mTOR is depicted as an example of an intracellular pharmacological target that can be reached via folate-conjugated small molecule drugs as shown in (B). The cyst lumen contains growth factors and cytokines that activate receptors on the apical plasma membranes of the cyst-lining cells. These growth factors and their receptors are the intended targets of antagonistic mAbs in dIgA format as shown in (C). (B) Magnified cartoon. A depiction of a folate-conjugated small molecule drug consisting of the ligand folate (1), a hydrophilic spacer (2), a cleavable bond (3) and the payload (4), which could be rapamycin in the case of FC-rapa. FC-rapa binds to the FR on the plasma membrane of cyst-lining cells and is internalized via receptor-mediated endocytosis, followed by cleavage and release of the drug in the endosome. The free, activated drug is subsequently released into the cytoplasm where it can inhibit its intended target mTOR. The FR is subsequently recycled back to the plasma membrane for additional rounds of drug uptake. Whereas cysts in PKD express the FR, most other cells instead utilize the reduced folate carrier (RFC) for folate uptake. Because the RFC cannot transport folate-conjugated drugs, these cells will be unaffected. (C) An antagonistic antibody in dIgA format (red) binds to the pIgR (green) on the basolateral surface of cyst-lining cells and is transcytosed to the apical surface. After arrival at the apical surface, the pIgR is cleaved which leads to the release of the complex between dIgA and the ectodomain of the pIgR (secretory IgA) into the cyst fluid. The dIgA antibody can then inhibit its intended target such as a growth factor or growth factor receptor (blue). Because renal cysts have enclosed spaces, the dIgA antibody will accumulate in the cyst fluid. In contrast, pIgR-mediated transcytosis will lead to excretion and loss of the dIgA antibody in other epithelial tissues such as the intestinal epithelium.

specificity of these drugs is due to the fact that most cells in the body do not express the FR, but instead import folate via the reduced folate carrier, which does not allow the import of folate-conjugated drugs [118, 119]. The FR α isoform has a restricted pattern of expression in normal tissues [120] but is expressed at high levels in renal proximal tubule epithelial cells [120, 121]. In addition, FR α is highly expressed in several epithelial cancers, which is why folate-conjugated drugs preferentially target to cancer cells and have been developed for tumor therapy [122, 123].

FR α expression was also found to be high in renal cysts in mouse models of PKD and human ADPKD samples [11, 124], which suggested that PKD could be amenable to FR-targeting strategies. A folate-targeted form of the mTORC1 inhibitor (see above) rapamycin (FC-*rapa* or EC0371) was developed that included a hydrophilic spacer and a self-immolative linker designed to be cleaved intracellularly to reconstitute the active drug [11]. In the nonorthologous, early-onset *bpk* model of PKD, FC-*rapa* administered by intraperitoneal injection proved highly effective in inhibiting mTOR activity in the kidneys—but not in other organs—and led to strong attenuation of cyst growth and proliferation and preserved renal function [11]. In a follow-up study, FC-*rapa* was tested at lower doses in an orthologous *Pkd1^{fllox/-}*: Nestin-Cre mouse model and compared head-to-head to unconjugated rapamycin. Both, FC-*rapa* and unconjugated rapamycin were similarly effective in inhibiting PKD disease progression. However, FC-*rapa* exhibited much reduced extrarenal effects, including effects on the immune system and reduced systemic toxicity as assessed by body weight gain over time [124]. In the same study, it could also be directly demonstrated that renal cysts are accessible to folate-conjugated compounds as assessed using a novel fluorescent folate-conjugated reporter [124]. This reporter was efficiently taken up by the collecting duct-derived cysts that are prevalent in the *bpk* mouse model [124], and FC-*rapa* was effective in inhibiting the growth of these cysts [11]. These results indicate that the FR is expressed on collecting duct-derived cysts and not restricted to cysts that originate from proximal tubules.

Although the preclinical data to date suggest that targeting drugs to PKD kidneys via FR is a feasible approach [125], it has yet to be tested in a clinical trial in ADPKD patients. In addition to targeting FR with folate-conjugated small molecules, other FR-targeting approaches have been investigated that may also have application in PKD. The most advanced is **mirvetuximab soravtansine**, an FR-targeted antibody–drug conjugate, which consists of an anti-FR antibody linked to maytansinoid DM4, a potent tubulin-disrupting agent. Preclinical and clinical studies suggest that the overall efficacy of mirvetuximab soravtansine is linked to the relative expression of FR [126–128], and its relative efficacy is currently being tested in patients with platinum-resistant ovarian cancer [129] including a pivotal Phase 3 clinical trial in this patient population [130].

Targeting of mAbs to renal cysts via the polymeric immunoglobulin receptor. As opposed to small molecule drugs, a major advantage of mAbs is their typically higher target specificity that greatly reduces or eliminates off-target adverse effects [131, 132]. The disadvantage due to lack of oral bioavailability is partially overcome by much longer half-lives of mAbs compared with small molecules, which means that dosing is required much less frequently.

Many potential targets for PKD therapy are cytokines, growth factors and their receptors. For many of these targets, very effective **antagonistic mAbs** have already been

developed—and are even in clinical use, primarily for cancer therapy. A prime example is the EGFR, which is over-expressed in PKD. Small molecule tyrosine kinase inhibitors against the EGFR effectively inhibit renal cyst growth in PKD mouse models [133]. However, the known toxicity of small molecule EGFR inhibitors would seem to make them poor candidates as long-term therapeutics for ADPKD. Antagonistic EGFR mAbs would seem much more promising at first glance. However, to our knowledge, no publication reporting efficacy of any mAb in rodent models of PKD has yet appeared. Our own efforts to use existing mAbs against several targets in PKD mouse models have led to failure. It seems likely that other labs have found similar results, but such failed studies are rarely published.

A likely explanation for these failures is that many of the growth factors/receptors implicated in PKD are localized to the luminal compartment of renal cysts. For example, the EGFR has been found to be activated and apically localized in cysts [134], and EGFR ligands have been found in cyst fluid [135]. Since cysts are enclosed, epithelial-lined spaces [136], any growth factors secreted into cyst fluid should be able to stimulate their cognate receptors that are present on apical membranes of cyst-lining cells. This would lead to continuous auto- and paracrine activation of cyst cells and may lead to an inescapable, permanent state of activation once cysts are formed.

The biotech industry exclusively uses IgG isotypes to develop mAb therapeutics. However, IgG antibodies are not capable of crossing the epithelial barrier of renal cysts and should therefore never gain access to this compartment. Considering this problem, it is not surprising that mAbs in IgG format should be ineffective in PKD if the intended target resides in cyst lumens.

To overcome this problem, we have utilized antibodies of a different isotype, IgA, specifically **dimeric IgA (dIgA)**. The purpose of dIgA in nature is to cross epithelial barriers so that it can be excreted into external secretions as a first line of defense against pathogens. This is accomplished by transcytosis via the polymeric immunoglobulin receptor (pIgR) that binds dIgA at the basolateral side of epithelial cells and releases it into the apical space in complex with the ecto-domain of the pIgR [137, 138]. This final ‘secretory IgA’ (sIgA) consists of the dIgA molecule tightly bound to the pIgR ecto-domain, which also provides the antibody increased stability and protection from proteases.

We found that the pIgR is highly expressed on cyst-lining cells in human ADPKD and mouse models, and that its expression is driven by the aberrant activation of the STAT6 pathway [139] that is one of the driving forces of renal cyst growth [140, 141]. Importantly, we found that dIgA administered by intraperitoneal injection in PKD mouse models is indeed targeted to polycystic kidneys and accumulates in renal cyst fluid [139]. In contrast, injected IgG does not measurably reach renal cyst lumens [139]. Because pIgR is a sacrificial transporter, it can only transcytose dIgA unidirectionally into cyst lumens but not back out. Because the majority of renal cysts have lost their connection to the tubular system, once dIgA has reached cyst lumens, it will be trapped there. Therefore, administered dIgA can accumulate in renal cyst lumens (Figure 2C). In contrast, due to the relatively short serum half-life of dIgA, the remainder of injected dIgA would be rapidly cleared systemically by secretion, primarily via the intestinal epithelium and via the bile [142, 143]. The net effect is that parenterally administered dIgA is specifically targeted to renal cyst lumens and would be expected to have minimal systemic effects.

Using this approach, we estimated that concentrations of dIgA in the microgram per milliliter range can be achieved in

renal cyst lumens in mice, which far exceeds the 50% effective concentrations of any IgG therapeutic mAb currently in use. These findings suggest that it is feasible to utilize antagonistic mAbs against any number of growth factors/receptors implicated in PKD, provided that the mAbs are in dIgA format. This approach would allow the re-purposing of numerous existing mAbs, after re-formatting to dIgA—including those mAbs that are already in clinical use such as mAbs against the EGFR. Since the pIgR can also transcytose pentameric IgM antibodies, this isotype could potentially also be utilized. However, the large size of pentameric IgM is likely to create additional challenges with regard to manufacturing and pharmacokinetics.

CONCLUSIONS

In conclusion, numerous pharmacological agents targeting a multitude of pathways and molecules have shown promise in preclinical studies. Although renal and extra-renal side effects are a concern for the long duration of therapy needed in ADPKD, novel approaches for targeting of drugs to renal cysts may overcome this problem. New findings suggest that ADPKD therapy may even be possible without drugs but instead using dietary intervention.

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CONFLICT OF INTEREST STATEMENT

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