The Cellular Pathways and Potential Therapeutics of Polycystic Kidney Disease

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Abstract

Polycystic Kidney Disease (PKD) refers to a group of disorders, driven by the formation of cysts in renal tubular cells and is currently one of the leading causes of end-stage renal disease. The range of symptoms observed in PKD are due to mutations in cilia-localising genes, resulting in changes in cellular signalling. As such, compounds that are currently in preclinical and clinical trials target some of these signalling pathways that are dysregulated in PKD. In this review, we highlight these pathways including cAMP, EGF and AMPK signalling and drugs that target them and may show promise in lessening the disease burden of PKD patients. At present, tolvaptan is the only approved therapy for ADPKD, however, it carries several adverse side effects whilst comparatively, no pharmacological drug is approved for ARPKD treatment. Aside from this, drugs that have been the subject of multiple clinical trials such as metformin, which targets AMPK signalling and somatostatins, which target cAMP signalling have shown great promise in reducing cyst formation and cellular proliferation. This review also discusses other potential and novel targets that can be used for future interventions, such as β-catenin and TAZ, where research has shown that a reduction of the over-expression of these signalling components results in amelioration of disease phenotype. Thus, it becomes apparent that well-designed preclinical investigations and future clinical trials into these pathways and other potential signalling targets are crucial in bettering disease prognosis for PKD patients and could lead to personalised therapy approaches.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ADPKD</td>
<td>Autosomal Dominant Polycystic Kidney Disease</td>
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<tr>
<td>AKI</td>
<td>Acute Kidney Injury</td>
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<tr>
<td>Akt</td>
<td>RAC-alpha serine/threonine-protein kinase</td>
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<td>AMPK</td>
<td>AMP-activated protein kinase</td>
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<td>AQP2</td>
<td>Aquaporin-2</td>
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<td>ARPKD</td>
<td>Autosomal Recessive Polycystic Kidney Disease</td>
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<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<td>cAMP</td>
<td>cyclic Adenosine Monophosphate</td>
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<tr>
<td>CDK4</td>
<td>Cyclin-dependent kinase 4</td>
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<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
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<td>CKD</td>
<td>Chronic Kidney Disease</td>
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<tr>
<td>c-Met</td>
<td>Tyrosine-protein kinase Met</td>
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<td>c-Src</td>
<td>Proto-oncogene tyrosine-protein kinase</td>
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<tr>
<td>DZIP1L</td>
<td>DAZ Interacting Zinc Finger Protein 1 Like</td>
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<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
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<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
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<tr>
<td>ErbB1/2</td>
<td>Receptor Tyrosine-Protein Kinase ErbB 1/2</td>
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<tr>
<td>ERK1/2</td>
<td>Extracellular signal-regulated protein kinase 1/2</td>
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<tr>
<td>ESRD</td>
<td>End Stage Renal Disease</td>
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<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<td>GLI2</td>
<td>GLI Family Zinc Finger 2</td>
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<tr>
<td>Glis2</td>
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<tr>
<td>GSK</td>
<td>Glycogen Synthase Kinase</td>
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<tr>
<td>HDAC</td>
<td>Histone deacetylases</td>
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<td>HGF</td>
<td>Hepatocyte Growth Factor</td>
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<td>Hh</td>
<td>Hedgehog</td>
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<td>JAK</td>
<td>Janus Kinase</td>
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<tr>
<td>jck</td>
<td>juvenile cystic kidney</td>
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<td>LPK</td>
<td>Lewis Polycystic Kidney</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-Activated Protein Kinase</td>
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<tr>
<td>MATE1-K1/2</td>
<td>multidrug and toxin extrusion 1/2</td>
</tr>
<tr>
<td>mTOR</td>
<td>mammalian Target Of Rapamycin</td>
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NF-κB  Nuclear Factor kappa B
PC1/2  Polycystin 1/2
Pck  Polycystic kidney
PCP  Planar Cell Polarity
PDGF  Platelet-Derived Growth Factor
PKD  Polycystic Kidney Disease
PKD1/2  Polycystic Kidney Disease 1/2
PKHD1  Polycystic Kidney And Hepatic Disease 1
PPAR  Peroxisome Proliferator-Activated Receptor
QTC  corrected QT
SIRT1  Sirtuin-1
SKI-606  Bosutinib
SRC  Proto-oncogene tyrosine-protein kinase
STAT3  Signal transducer and activator of transcription 3
TAZ  Transcriptional coactivator with PDZ-binding motif
TGF-β  Transforming Growth Factor Beta
TSV  Tesevatinib
V2R  Vasopressin 2 Receptor
VEGFR2  Vascular Endothelial Growth Factor Receptor 2
YAP  Yes-Associated Protein

**Introduction**

Polycystic Kidney Disease (PKD) is a subset of Chronic Kidney Disease (CKD) marked by the gradual loss of renal function, due to the bilateral formation of cysts within the kidney’s tubular epithelia. Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common form of PKD and the most prevalent inherited progressive kidney disease with an estimated incidence of 1:400 – 1:1,000[1-2]. Autosomal Recessive Polycystic Kidney Disease (ARPKD) is rarer than ADPKD, with an incidence of 1:20,000 live births[3]. Despite the age of ARPKD onset being considerably earlier than ADPKD, with ARPKD usually presenting during the neonatal period, both diseases feature a similar presentation including kidney enlargement and multiple cyst development[1]. The development of kidney cysts differs between ADPKD and ARPKD. In ADPKD, renal cysts develop in and branch off all segments of the nephron, whereas ARPKD arises due to the development of
cysts within collecting duct cells\[1\]. Both diseases feature extra-renal presentations of different manifestation. ADPKD patients may display liver and pancreatic cysts and intracranial aneurysms\[4\], while ARPKD individuals may present with hepatic fibrosis, biliary duct hyperplasia and portal fibrosis because of ductal plate malformations\[4\]. Additionally, 30–50\% of early ARPKD cases may suffer from respiratory distress, due to pulmonary hypoplasia and excessive kidney enlargement possibly resulting in the compression of the lungs\[4\]; it has yet to be confirmed that the pulmonary hypoplasia is not a direct consequence of the loss of fibrocystin function. As such, ARPKD is a rare paediatric disease of variable manifestation, with outcomes that vary from death in utero to kidney/liver transplantation early on in life to milder manifestations in adulthood with predominantly liver presentations. Successful therapeutic interventions can thus become challenging, since PKD pathogenesis and disease severity can vary significantly between patients, although molecular interaction studies suggest possible overlapping mechanisms, despite the differences in PKD presentation and progression \[5-9,20,24\]. Accordingly, recent investigations are looking to understand the possible mechanisms behind this and to identify ways to address them.

At the molecular level, Polycystic Kidney Disease 1 (PKD1) and Polycystic Kidney Disease 2 (PKD2) are mutated in ADPKD, whereas Polycystic Kidney and Hepatic Disease 1 (PKHD1) and DAZ Interacting Zinc Finger Protein 1 Like (DZIP1L) mutations are associated with ARPKD\[10-13\]. DZIP1L mutations are involved in a rarer subset of patients featuring a moderate ARPKD presentation\[13\]. PKD1, PKD2 and PKHD1 encode the cilia localising proteins Polycystin-1 (PC-1), Polycystin-2 (PC-2) and Fibrocystin (FPC) respectively\[14-21\]. DZIP1L encodes a protein that localises to the ciliary diffusion barrier and loss of DZIP1L results in the inability of proteins associated with PKD1 and PKD2 to localise to cilia\[13\]. The exact roles of PC-1, 2 and FPC within ciliary processes are under investigation. Both PC-1 and PC-2, as well as FPC and PC-2 have been reported to form complexes that may be involved in controlling the levels of cellular calcium via cilia\[16,22-24\]. However, recent data has criticised this model, showing that cilia do not evoke calcium signalling in a mechanosensory manner\[25\]. Additionally, although the three aforementioned proteins are all reported to localise to cilia, it is unclear whether the PKD phenotype
is driven by cilia dysfunction alone, given that all three proteins also localise to other cellular compartments. PC-1 localises to lateral membranes in cell–cell contact areas, such as desmosomes and cellular junctions. PC-2 localises to mitotic spindles, the Golgi and the Endoplasmic Reticulum, where it may play a role in intracellular calcium signalling. FPC localises to basal bodies and mitotic spindles. All three proteins are present in exosome-like vesicles. Increased proliferation, increased extracellular matrix deposition and abnormal fluid secretion are detected in PKD. Many animal models have been employed to study PKD and these have been excellently reviewed; they constitute powerful tools in understanding disease pathogenesis and have contributed to early drug investigations in preclinical trials. Nonetheless, no single PKD animal model fully recapitulates the human manifestation. Thus, a number of rodent models have been used in ADPKD and ARPKD research; these are summarised in Table 1. As becomes evident in our review which focuses on kidney treatments for PKD, the effect on signalling pathways and the outcome of treatment in preclinical trials may vary between the various PKD animal models. Hence, the data extrapolated from PKD animal models requires careful consideration, as it sometimes is model-dependent and may involve restricted timepoints of intervention and challenges in long-term study.

Dysregulation of various cellular pathways have been implicated in PKD (Figure 1); so far, cyclic AMP (cAMP) and Epidermal Growth Factor Receptor (EGFR) have been extensively studied. cAMP activation drives proliferation via the ERK/MAPK pathway in response to decreased intracellular calcium. cAMP was hyperactivated in multiple PKD animal models and in cellular models of ADPKD and ARPKD renal epithelia. Calcium reduction is not the only mechanism by which cAMP becomes activated in PKD. Studies in rodent PKD models suggest that Vasopressin 2 Receptor (V2R) plays a role in cAMP activation. Aquaporin-2 (AQP-2), another channel regulated by cAMP was also increased in response to increased cAMP and V2R levels in rodent PKD models, although AQP2 was not expressed in all cysts of a Pkd1 mouse model. Additionally, cAMP could drive fluid secretion into renal cysts via the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in human ADPKD cells, by regulating the insertion of CFTR into the apical cellular membrane. However, not all human ADPKD cysts were
positive for CFTR and even in CFTR-expressing cysts, it was not expressed on all cystic cells\cite{43}. Furthermore, although cAMP was hyperactivated in both ADPKD and ARPKD, CFTR was not required for the development of cysts in ARPKD\cite{46}.

Similar to cAMP, EGFR has been associated with ADPKD, ARPKD and in cyst development in multiple animal models of PKD\cite{54-64}. EGFR was mislocalised from the basolateral cellular membrane to the apical membrane in human cellular models of both ADPKD and ARPKD and in multiple rodent models of PKD\cite{54-57,63-65}. Alongside the increased EGFR expression, increased growth factor and EGF precursor expression was detected in cystic fluid, further driving the activation of EGFR signalling in PKD\cite{58,59,66,67}. The impact of EGFR in driving cystogenesis has been observed in rodent studies that decreased EGFR activity\cite{61,63,64}. However, neither increased EGFR expression nor EGFR mis-localisation was observed in the Polycystic Kidney (PCK) rat and treatment with EGFR inhibitors aggravated the cystic phenotype\cite{68}, indicating that EGFR signalling may not be required for cystogenesis in all cases of PKD. Multiple downstream EGFR cellular processes are also reported to be activated in PKD, such as MAPK, mTOR, SRC and STAT3 signalling\cite{39-44,62,69-76}.

cAMP and EGF are not the only pathways associated with PKD. In ADPKD and ARPKD animal models, PCK rat livers and in ADPKD patient samples, aberrant disease\cite{77-80} signalling via TGF-β has been associated with hepatic and renal disease\cite{77-80}. Hedgehog signalling was linked to renal and hepatic cystogenesis in rodent ADPKD models, the PCK rat and ADPKD derived cell lines\cite{81-83}. Activation of canonical WNT and WNT/Ca²⁺ signalling has been found in ADPKD models, with PC-1 suggested to potentially act as a WNT receptor\cite{84,85}. Additionally, activated canonical WNT signalling may drive the epithelial-to-mesenchymal transition in the PCK rat cystic epithelial\cite{86}. Planar cell polarity (PCP) genes and proteins displayed increased expression in ARPKD kidney tissues and may drive cyst development in ARPKD\cite{87}; mice lacking the PCP gene Fat4 also develop cystic kidneys\cite{88}. The role of PCP and PCP signalling in PKD remains unclear and requires further study of the individual signalling components. Hippo signalling is activated in ADPKD cells, tissues and in ADPKD rodent models\cite{89-92}.

**cAMP, EGF and AMPK signalling clinical trials for PKD**
Within the last decade, tolvaptan was approved in Europe and the US for the treatment of ADPKD [4]. Tolvaptan is a V2R antagonist and acts by binding to V2R [93] (Table 2). Subsequently, vasopressin activity is reduced and leads to decreased cAMP levels, reducing cAMP-mediated activation of MAPK and decreasing CFTR activity [93]. Tolvaptan was effective in reducing cyst burden in ADPKD and ARPKD rodent models [94,95] (Table 2). Results from short-term and long-term clinical trials of ADPKD patients have demonstrated a rapid response to treatment [96-98]. In the TEMPO study [98], ADPKD patients demonstrated reduced cyst growth and a decrease in Glomerular Filtration Rate (GFR) decline throughout the three-year period [97,98]. However, tolvaptan was associated with adverse events, such as thirst and polyuria [97,98], thus careful precaution may be needed before its prescription for ADPKD [99]. Tolvaptan clinical trials in ARPKD have recently started (NCT04782258; NCT04786574).

Other V2R antagonists, showing beneficial effects in rodent ADPKD models have also been trialled, such as lixivaptan and mozavaptan [53,94,100,101]. Similar to tolvaptan, lixivaptan has completed one phase 2 ADPKD clinical trial with another phase 3 clinical trial starting soon (Table 2). However, the impact of vasopressin antagonists on the development of the fibropolycystic liver disease in PKD is likely to not be beneficial due to the lack of expression of V2R in the liver [45,68,94,95]. Additionally, a study looking at the impact of dose titration on V2R antagonists in a Pkd1 mouse model found that delayed mozavaptan administration did not impede ADPKD progression in the later stages of disease [101]. This may be due to either loss of functioning nephrons, loss of V2R when the nephrons disconnect from the tubular system or V2R de-differentiation. Decreased V2R mRNA was detected at later stages and only 20% of cysts were expressing AQP-2 in a Pkd1 deletion mouse model [53], implying that even if V2R antagonists worked, they may be able to only partially prevent cyst development in PKD.

Metformin has been highlighted as a potential effective therapy for ADPKD treatment via AMPK activation [102,103]. AMPK is inhibits both the mTOR and CFTR pathways [102,104-106]. In a retrospective study, patients treated with metformin displayed a reduced rate of GFR decline together with stabilised GFR [102]. In addition, individuals experienced little or no adverse side effects, a finding replicated in a similar phase 2 study [102,103]. Nevertheless, several limitations were reported in
both studies, such as the lack of a control group\textsuperscript{[103]}, small population size\textsuperscript{[103]}, the inability to conclude whether metformin was the driving factor in GFR stability\textsuperscript{[102]} and limitations in the procedures testing total kidney volume\textsuperscript{[102]}. Additionally, a report published in 2019\textsuperscript{[107]} did not find any beneficial impact for metformin at clinically relevant serum levels on another ADPKD mouse model. This \textit{Pkd1} conditional knockout mouse line responded better to salsalate treatment than to metformin\textsuperscript{[107]}, although a similar daily metformin dosage was used as in previous studies\textsuperscript{[108]}. It is possible that the different mouse ADPKD models could contribute to this discrepancy and it thus remains crucial that the most relevant, closely mimicking PKD models are used in preclinical studies. At present, metformin is undergoing investigation in a phase 3 clinical trial (Table 2).

Somatostatins inhibit intracellular cAMP production\textsuperscript{[109]}, however, they are promptly eliminated \textit{in vivo}, requiring analogues with a longer half-life\textsuperscript{[109]}. Octreotide, lanreotide, and pasireotide are manufactured analogues used for a variety of conditions, but none so far have been exclusively registered for the treatment of PKD\textsuperscript{[109]}. Investigations in \textit{Pck} rats have revealed that simultaneous administration of octreotide and pasireotide was beneficial in PKD treatment. However, octreotide treatment alone was less effective than pasireotide alone or both together at producing the same effects, such as reducing renal tissue cAMP levels\textsuperscript{[109-111]}. Similarly, another study\textsuperscript{[112]} demonstrated that the combined therapy of octreotide and sorafenib, a tyrosine protein kinase and RAF kinase inhibitor, was effective at reducing cellular proliferation and cyst size in \textit{Pkd2} mutated mice\textsuperscript{[109,112]}. Contradictory to this, an earlier study\textsuperscript{[110]} highlighted octreotide as more effective in a single ADPKD patient over a period of 6 months and thus, further research is required to establish the effectiveness of octreotide either alone or combined with an additional inhibitor\textsuperscript{[109,113]}.

In addition, combination treatment of tolvaptan with pasireotide was effective in suppressing renal cyst development in a hypomorphic \textit{Pkd1} mouse model\textsuperscript{[95,109]}. Comparatively, one clinical trial\textsuperscript{[114]} revealed that sole treatment with lanreotide was able to attenuate kidney and liver cyst growth with lasting beneficial effects on total kidney volume, a finding further emphasised in another study\textsuperscript{[115]} which also revealed significantly reduced symptoms in ADPKD patients\textsuperscript{[109,114,115]}. Importantly, no
significant difference was observed in the rate of GFR loss upon lanreotide treatment and thus, it might be assumed that combined treatment of lanreotide with either another somatostatin analogue or tolvaptan may reveal a better therapeutic outcome.

Tesevatinib (TSV) is a multi-tyrosine kinase inhibitor that blocks EGFR and cAMP signalling, whilst reducing phosphorylation of c-Src, Receptor Tyrosine-Protein Kinase ErbB-2 (ErbB2) and Vascular Endothelial Growth Factor Receptor 2 (VEGFR2)\textsuperscript{[116]}, leading to decreased cellular proliferation due to a consequent reduction of MAPK activity and reduced angiogenesis\textsuperscript{[116]}. TSV treatment in the BALB/c-(Bicc1/Bicc1) (Bpk) mouse and the PCK rat ARPKD models significantly reduced the phenotypic signs of ARPKD. In both models, TSV treatment resulted in a reduction of both kidney and liver body: weight ratios and improved renal and liver morphology, including reduced cyst size, in a dose dependent manner \textsuperscript{[116]}. TSV has been the subject of multiple clinical trials, with one phase 2 clinical trial for treatment of ADPKD still underway (Table 2). Preliminary data from the currently ongoing trial highlights TSV as a safe ARPKD therapy with limited adverse effects, such as diarrhoea and nausea in patients treated with 100 mg/d\textsuperscript{[116]}. On the other hand, previous \textit{in vitro} studies have demonstrated mild serum creatinine level increases upon TSV treatment, an observation possibly explained through a consequent Multidrug And Toxin Extrusion 1/2 (MATE1/2-K) transporter inhibition\textsuperscript{[117]}. 

Bosutinib (SKI-606) is a Src tyrosine kinase inhibitor that has recently been explored as a potential therapeutic PKD intervention\textsuperscript{[118]}. Initial research associated increased Src activity with ARPKD progression in the Bpk mouse and PCK rat \textsuperscript{[119]}. Both Bpk and PCK animal models were treated with SKI-606 and demonstrated a significant reduction in renal Src activity with a consequent reduction in cystic kidney size, renal collecting tubule cysts, improved kidney weight: body weight ratio and improved liver morphology \textsuperscript{[119]}. Importantly, Src activity levels correlated with ErbB1 in the Bpk mice and ErbB2 and ERK1/2 in the PCK rat, emphasising the roles these signalling pathways play in the pathogenesis of PKD\textsuperscript{[119]}. A later study highlights the association between increased Src activity and cell proliferation, motility and apoptosis and demonstrates increased Src activity in cyst-lining epithelia in human and mouse ADPKD\textsuperscript{[120]}. Investigations of mouse and human ADPKD cells showed
that treatment with SKI-606 inhibited epithelial cell proliferation and decreased cell matrix adhesion, with *in vivo* studies demonstrating the positive effect Src inhibition has on cyst development\[^{120}\]. In a recent study\[^{121}\] bosutinib positively impacted cell viability in both wildtype and *Pkd1*-null cells, whilst also slowing down cyst progression\[^{121}\]. Bosutinib has been the subject of a recent clinical trial that investigated its efficacy and safety in ADPKD (Table 2)\[^{118}\]. The annual kidney growth rate was 66% slower upon bosutinib treatment (200 mg/d) compared to placebo, with no significant difference in the annual rate of GFR decline\[^{118}\]. Although bosutinib was previously associated with liver and gastrointestinal side effects, upon investigation, no new toxicities were identified\[^{118}\].

Sirolimus also referred to as Rapamycin is a promising therapeutic drug with potential in treating ADPKD by indirectly suppressing mTORC1\[^{74}\]. Sirolimus treatment ameliorated PKD in conditionally inactivated *Pkd1*\[^{69}\] and *Pkd2* mutant mice\[^{72}\] and reduced cyst enlargement in *Lewis Polycystic Kidney* (LPK) rats and juvenile cystic kidney (*jck*) mice\[^{122}\]. In a hypomorphic *Pkd1* mouse model (*Pkd1*\[^{RC/RC}\]), sirolimus was compared to Torin2, an ATP competitive mTOR inhibitor\[^{74,123}\]; both inhibitors were equally effective in reducing cyst formation and in ameliorating kidney function in the *Pkd1*\[^{RC/RC}\] kidneys\[^{74}\]. Nevertheless, a recent study\[^{124}\] investigating sirolimus treatment over an 18-month period showed no effect in polycystic kidney growth in adult ADPKD patients\[^{124}\], manifesting the need for further research to determine its effectiveness. Everolimus (Certican) is an approved immunosuppressant for organ transplant rejection with additional applications in renal cancer treatments\[^{125}\]. In ADPKD patients, the progression of renal impairment was not decelerated when treated with everolimus for a period of 2 years, although it did delay total kidney volume expansion\[^{126}\]. The combined rapamycin/everolimus treatment on cultured *PCK* cholangiocytes, arrested cellular proliferative activity by inducing apoptosis\[^{127}\], stipulating that when used alongside another drug, everolimus demonstrates greater effects.

As briefly discussed above, salsalate was effective in attenuating renal cystic disease in an adult-onset *Pkd1* mouse and weakened metabolic reprogramming and inflammation in *Pkd1* cystic kidneys\[^{107}\]. Salsalate inhibits the expression of TGF-β downstream, by preventing TGF-β-induced phosphorylation and transcriptional
activity of Smad2/3 and it has been shown to reverse fibrogenic responses\cite{128}. Equivalently, transcriptional activity of Yes-Associated Protein (YAP) is suppressed when AMPK is activated, as AMPK phosphorylates YAP, preventing nuclear translocation\cite{129,130}. To date, no clinical trials have used combination treatment with salsalate and tolvaptan to treat PKD, but it has been speculated that there may be additive therapeutic effects by downregulating cAMP levels\cite{107}.

**Potential and novel targets of PKD**

Aside from the above signalling cascades, further investigations into cellular signalling have revealed that additional pathways are dysregulated in PKD and may be of promise in ameliorating disease symptoms upon modulation. Upregulation of β-catenin in a transgenic mouse model displayed severe PKD-related phenotypes including renal cysts\cite{131}. In \textit{Vil}^{\text{Cre}}\textit{Pkd2}^{\text{f/f}} mice\cite{84}, depleted PC2 and a consequent elevation of β-catenin contributed to the ADPKD phenotype. Wnt inhibitors XAV939 and LGK974 ameliorated the cystic phenotype in \textit{Vil}^{\text{Cre}}\textit{Pkd2}^{\text{f/f}} mice and via canonical Wnt signalling pathway suppression, resulted in downregulation of β-catenin expression\cite{100}. Interestingly, through a combination of experimental studies, inhibition of β-catenin as well as ERK1/2, S6 and mTOR by Cardamonin was discovered as a potential therapeutic target for ADPKD\cite{132}. Treatment of Endo-IWR-1, which also inhibits β-catenin via AXIN stabilisation, reduced \textit{in vitro} cyst growth in siRNA-mediated \textit{Pkd1}-silenced cells\cite{91}. Furthermore, upregulation of Glycogen Synthase Kinase (GSK3β) expression was observed in the cystic kidneys of \textit{Cys}^{\text{fok}}\textit{mice}, \textit{PKD1}^{\text{flo}}.\textit{PKHD1}^{\text{cre}} and human ADPKD kidneys. Pharmacological inhibition and collecting duct specific gene deletion of GSK3 in both ARPKD and ADPKD mouse models reduced cyst area and number, as well as the proliferation rate of cyst lining epithelial cells and overall kidney size\cite{133}. The above observations open room for a targeted approach that investigates whether inhibitors of canonical Wnt signalling are effective as therapeutic interventions for PKD, presenting novel targets for symptom amelioration.

It should be noted that many signalling pathways converge and as a result, dysregulation in one affects the other. In human ADPKD renal cystic tissue, an upregulation of GLI Family Zinc Finger 2 (GLI2) was observed resulting in
inappropriate activation of Hh signalling\textsuperscript{[134]}. As a result, upon inhibition of Hh signalling, the abnormally elevated levels of serum liver enzymes observed in \textit{PCK} rats were downregulated, and this, in turn, attenuated the development of kidney cysts and bile duct dilation\textsuperscript{[83]}. This evidence highlights that inhibition of GLI2, potentially by employing Glis Family Zinc Finger 2 (Glis2), a parologue of GLI2 and a repressor of the Hh pathway\textsuperscript{[134]}, may be a novel therapeutic target for PKD. In addition, the loss of Transcriptional co-activator with PDZ-binding motif (TAZ), a Hippo signalling effector, in \textit{Pkd1}-deficient mice reduced cyst formation through a PKD1-TAZ-Wnt-β-catenin-c-MYC signalling axis\textsuperscript{[91]}. \textit{In vivo} and human ADPKD investigations found an increase in TAZ, c-MYC and β-catenin expression in cyst lining epithelia in the kidneys. An increase in \textit{Yap/Taz} target gene expression was observed in \textit{Pkd1}-deleted kidneys \textsuperscript{[93]}. When Taz was inhibited, c-MYC and β-catenin expression was reduced, in turn ameliorating the PKD phenotype\textsuperscript{[91]}.

Peroxisome proliferator-activator receptor-γ (PPAR-γ), with a known role in insulin sensitisation and adipogenesis, is also expressed in the kidney and liver\textsuperscript{[135-137]}. Since PPAR-γ agonists such as thiazolidinediones (TZDs) have demonstrated roles in processes that are altered in ADPKD, such as regulating cell proliferation via ERK signalling and inhibiting CFTR protein synthesis, research has used these drugs to establish a protective role in the kidneys\textsuperscript{[138,139]}. The suppression of TGF-β via treatment with pioglitazone, a type of TZD, ameliorates renal fibrosis and inflammation\textsuperscript{[140]}. Maternal administration of pioglitazone and troglitazone ameliorated the cystic phenotype in \textit{Pkd1}-/- mouse embryos with inhibited PKD disease progression being observed in several other \textit{PCK} rat models upon treatment with TZDs\textsuperscript{[2,12,141-144]}. Pioglitazone-treated rats presented reduced weight and cystic areas in both the kidney and liver through cell proliferation and fibrosis inhibition and improved renal function; prolonged survival of Han:SPRD rats was demonstrated upon rosiglitazone treatment, another PPAR-γ agonist\textsuperscript{[2,145]}. Pioglitazone treatment also resulted in altered expression of renal genes involved in several metabolic processes including cell proliferation and fatty acid metabolism\textsuperscript{[146]}. Contradictory to this, pioglitazone had little effect in slowing down PKD progression in another study, however, combined treatment of pioglitazone with tolvaptan and tolvaptan alone displayed improved renal survival and a reduced rate of PKD progression, albeit statistically insignificantly\textsuperscript{[11]}.
cardiac enlargement and oedema have been reported in some cases upon PPAR-γ-agonist drug treatment and thus critical assessment is required\cite{12,145}. Recent advancements have found pioglitazone become the subject of a clinical trial to determine its efficacy in ADPKD treatment\cite{11}.

In ARPKD, renal fibrosis has been noted to be a result of elevated TGF-β signalling\cite{147}. It has been established that interstitial fibrosis can be reduced through the suppression of the TGF-β/Smad3 pathway\cite{77} and cardamonin was shown to ameliorate fibrosis and decelerate progression of PKD by downregulating TGF-β/Smad signalling\cite{132}. In Smad3 knockout/cpk double-mutant mice, the absence of Smad3 also inhibited abnormal cellular proliferation in cystic epithelia by suppressing the JNK/CDK4-dependent pSmad3L cascade\cite{77}. Therefore, for therapeutic purposes, targeting TGF-β-mediated SMAD signalling could prevent the development of fibrosis, hence bettering the ARPKD phenotype\cite{148}.

**Concluding remarks**

It becomes obvious that targeting one pathway that plays a role in PKD could possibly have an effect on other relevant pathways, making the study of these interactions and any resulting side effects important areas of research. A number of clinical trials that are currently underway show promise, in particular for ADPKD treatment and many pre-clinical studies are in place to address potential therapeutic effects in both ADPKD and ARPKD animal models. Nevertheless, contradictory findings are observed in preclinical trials for the same drug and well-designed studies that allow for the preservation of kidney function into old age and are well tolerated remain challenging. It is thus crucial that both preclinical and clinical PKD trials are well-designed, have got accurately defined endpoints and clear, measurable outcomes. In addition, as PKD therapies would be of benefit even if they only slow down cyst progression, it is onerous to measure the success of clinical trials, when only looking for incremental changes over a long period of treatment. Curcumin, a natural compound that decreases STAT3 phosphorylation, is planned for a Phase 4 trial for PKD\cite{149}. Triptolide, which prevents the phosphorylation of JAK2/STAT3\cite{1506}, has also shown promise in ADPKD treatment through a small study where patients were treated with triptolide-containing formulation and displayed stabilised renal function and decreased proteinuria\cite{151}.
Importantly, limitations of the study lie with the small sample size and short study duration, however, a more recent study\[^{152}\] highlighted that short-term treatment of triptolide in a *Pkd1* animal model also decelerated disease progression\[^{153,154}\]. Another drug of interest is resveratrol, which decreased the levels of pro-inflammatory proteins NF-κBp65, p105, and p50 in human ADPKD renal epithelial cells\[^{149,150}\] and reduced cyst number in Madin–Darby canine kidney epithelial cells\[^{120,151}\], suggesting that resveratrol has the potential to impede cyst development and reduce inflammation in future clinical trials.

It should also be noted that although there are some similarities in the mechanisms of ADPKD and ARPKD, there are also a few differences in manifestation, age of onset and severity and molecular mechanisms, which in addition to the great variability in symptoms observed from one individual to the other, make therapeutic interventions challenging and the contradictory outcomes of preclinical and clinical trials hard to interpret. The current rodent models could potentially be good predictors of liver treatments for PKD, but it still remains onerous to consistently improve kidney function. Although in both diseases, therapies only have to slow down disease progression, in ARPKD, interventions are usually needed at a much earlier age and these have to be well-tolerated for a lifetime and must allow for the preservation of kidney function into old age. Hence little or no side effects have to be observed, making the application of certain drugs such as kinase inhibitors too strident in the long run. Furthermore, there are no great ARPKD animal models currently available, accentuating the need for better-designed models that carry the human mutations and fully represent the ARPKD phenotype. It would be valuable if distinct therapeutic goals depending on the stage of diagnosis and the rate of disease progression are studied in PKD animal models, allowing for targeted interventions, e.g. neonatal survival, improved kidney function, ameliorated liver activity. We are still in the tunnel on finding effective PKD therapies that work for everyone, but there is light at the end of the tunnel, thanks to genomic and personalised medicine and collective scientific and medical efforts to put the spotlight on PKD.
<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Characteristics of model</th>
<th>Disease model</th>
<th>Drug Tested</th>
<th>Associate d Pathway</th>
<th>Experimental design</th>
<th>Effects and measurable outcome</th>
<th>References</th>
</tr>
</thead>
</table>


| PKD rat                                                                 | Kidney cysts in the thick ascending loops of Henle, distal tubules, and cortical collecting ducts. Liver cysts, mild bile duct dilation by day 1. Focal interstitial fibrosis and inflammation by day 70. Gender dysmorphism, males express more severe disease progression. | 1. ADPKD 2. ARPKD 3. ARPK D 4. PKD 5. ADPKD 6. ARPKD 1. OPC312 60 2. V2 agonist 1-deamino-8-d-arginine vasopressin (DDAVP) 3. octreotide (Oct) 4. EKI-785/EKB-569 5. N/A 6. Cyclopamine | 1. cAMP 2. cAMP 3. cAMP 4. EGFR 5. TGF-β 6. Hedgehog | 1. 24 hr urine outputs in metabolic caggages recorded weekly. Tailing blood pressures (BP) recorded. N=22 2. 24 hr urine outputs in metabolic caggages and tail-cuff BP recorded weekly for 3 wks. Blood from cardiac puncture for determination of serum electrolytes and blood urea nitrogen levels. N=4 per genotype/gender a) Treatment with Oct/vehicle 4-16 weeks, n=60 b) Treatment with vehicle. Oct, pasireotide or Oct/pasireotide 12 wks, n=24 4. Treatment occurred between 3810wks of age. Blood from cardiac puncture for determination of serum electrolytes and blood urea nitrogen levels. EKI-785 n=14, EKB-569 n=27 (intraperitoneal administration). EKB-569 n=83 (enteral administration). 5. At 2, 4 and 6 months of age expression of the RAS components (angiotensin converting enzyme (ACE) were examined by quantitative real time PCR, n=27 6. 10 mg/kg cyclopamine intraperitoneally daily between 4-8 wks of age. Rats were weighed, and blood obtained at 48s, n=5 7. Urine obtained by bladder puncture. Blood obtained by cardiac puncture for determination of serum creatinine and BUN levels, n=11 7. Urine concentration defect, which led to renal failure | 1. Renal cAMP expression reduction was significant 2. Increased renal cAMP levels; recovered cystic phenotype of PCK AVP - rats 3. Reduction of kidney weight, cystic volume, cellular proliferation. Reduced cAMP levels in pasireotide or Oct/pasireotide 4. Administration had no effect or worsened PKD. No effect on the development of fibrocystic liver disease. Renal cAMP & vasopressin V2 receptor expression upregulated in EKI-785-treated rats 5. ACE gene expression was increased, 8 and 17-fold in 4- and 6- month old liver, respectively. Progressive fibrosis and increased hepatic collagen recorded 6. Significantly attenuated kidney cyst formation and bile duct dilation. Reduced abnormally elevated serum liver enzymes | 1. 44 2. 48 3. 110 4. 68 5. 80 6. 83 156 |
| pcy mouse                                                                 | Caused by a missense mutation in NPHP3. Linked to adolescent Nephronphthisis | 7. ARPKD | 7. OPC31260 | 7. cAMP | 7. Urine obtained by bladder puncture. Blood obtained by cardiac puncture for determination of serum creatinine and BUN levels, n=11 | 7. Urine concentration defect, which led to renal failure | 7. 45 |
| jck mouse                                                                   | Nek8, encoding protein is responsible for cystic kidneys. Protein mapped to cilia. Renal cysts develop in the cortical collecting ducts, distal tubules, and loop of Henle. Gender dysmorphism. | 8. ADPKD | 8. Dihydrotestosterone (DHT) | 8. cAMP | 8. 50 mg/kg of DHT administered subcutaneously from 26 to 64 days. Serum urea nitrogen levels recorded n=15 | 8. Gender differences in cystogenesis and survival. Enlargement of kidneys. | 8. 157 47 |
| Model                  | Hypomorphic model, homoygous viable. Global reduction of functional PC1 in every cell. Viable for ~1 year with slow progressive PKD. Tubule dilations, cyst formation, extensive fibrosis & loss of renal function. | Treatment early or late
| OPCK10££ mouse          | 10. ADPKD
14. ARPKD
13. N/A
14. Thiadiazoli dinones (TDZD) TDZD-8
13. TGF-β
14. GSFKβ
13. The cpk mouse was originally derived from a spontaneous Cysflox mutation in the C57BL/6J strain. Genotyping of the offspring completed via PCR from tail or toe biopsies. n= 5 mice/group.
14. Cysflox mice were genotyped on P2; treated with TDZD-8 Smg/kg from P3 until P14 by single, daily, intra-peritoneal injections. n=6/group
14. Upregulation of GSFKβ expression recorded in the cystic kidneys. Reduced cyst area and smaller kidney size |
| OPK10££ mouse          | 17. ADPKD
17. Not applicable
17. TGFβ
17. n=5 or more per genotype
17. TGFβ upregulated, cyst formation, renal fibrosis |
| OPK10££ mouse          | 18. ADPKD
18. Not applicable
18. Hedgehog
18. n=3 or more per genotype
18. Small Molecule Hh Inhibitors reduced renal cystogenic potential |
| Am- Ksp-Cad-CreERT2; Pkd1flox−/flox-11 | 19. ADPKD
19. V2RA OPC-31260
19. cAMP
19. Administered high (0.1%) and low (0.05%) dose for 3 and 6 weeks; n=10 or more per group
19. Cyst ratio and kidney weight reduced after 3 weeks; no further significant reduction after 6 weeks, even on high dose. |
| Am- Ksp-Cad-CreERT2; Pkd1flox−/flox-11 | 20. ADPKD
20. Mozavapta n (V2RA OPC-31260)
20. cAMP
20. Administered to mice in fixed foes or in titrated dose. Treatment early or late P21 or P42, n=123
20. ADPKD progression was not suppressed |
| Ksp-Pkd1del condition al knock-out | 21. ADPKD
21. Salsalate &Metformin
21. mTOR
21. Administered with 150 mg/kg of tamoxifen by oral gavage at days P18 and P19 . n=20 (male/group)
21. Renal failure at ~ 4 months of age. Combination of drugs did not differ from salsalate independently or metformin |
| AmCre/Pk 4££             | 22. ADPKD
22. β-NF
22. Hippo
22. Administered β-NF daily from P8 to P111, n=3
22. Extensive enlargement of kidneys after 3 weeks, cysts in most nephron segments. Died within 4 weeks after injections. |
| PKD1flox−/flox KHD1££     | 23. ADPKD
23. Thiadiazoli dinones (TDZ)
23. GSFKβ
23. Administered TDZ-8 via injection from P10 until P21, n=6 mice/group
| Alm− mouse            | 24. ARPKD
24. Not applicable
24. Wnt
24. n=8 per genotype
24. Kidney, liver and lung abnormalities. Prominent lung hypoplasia, embryonic lethality
Table 1. A table summarising various rodent models that have been employed in PKD research. It presents the research approaches and preclinical studies conducted on the rodent models and displays the pathways targeted, experimental study design and measurable outcomes.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Target pathway</th>
<th>Clinical trial phase</th>
<th>Clinical trial number</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolvaptan</td>
<td>cAMP signalling</td>
<td>Phase 3 completed Phase 3 in progress Phase 2 completed Phase 3 Just set-up Phase 3 Just set-up</td>
<td>NCT00428948 NCT02964273 NCT01336972 NCT04782258 NCT04786574</td>
<td>93-98,155</td>
</tr>
<tr>
<td>Lixivaptan</td>
<td>cAMP signalling</td>
<td>Phase 3 in progress Phase 2 Completed</td>
<td>NCT04064346 NCT03487913</td>
<td>100</td>
</tr>
<tr>
<td>Octreotide</td>
<td>cAMP signalling</td>
<td>Phase 3 completed Phase 2 in progress (combined therapy with tolvaptan)</td>
<td>NCT01377246 NCT03541447</td>
<td>109-113</td>
</tr>
<tr>
<td>Lanreotide</td>
<td>cAMP signalling</td>
<td>Phase 3 completed</td>
<td>NCT01616927</td>
<td>109</td>
</tr>
<tr>
<td>Pasireotide</td>
<td>cAMP signalling</td>
<td>Phase 2 completed</td>
<td>NCT01670110</td>
<td>109-111</td>
</tr>
<tr>
<td>Tesevatinib</td>
<td>EGFR, cAMP and MAPK signalling</td>
<td>Phase 2 completed Phase 2 in progress</td>
<td>NCT01559363 NCT03203642</td>
<td>116</td>
</tr>
<tr>
<td>Metformin</td>
<td>AMPK signalling</td>
<td>Phase 3 in progress Phase 2 Completed</td>
<td>ISRCTN93749377 NCT03764605 NCT02656017</td>
<td>102-104</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>EGFR and MAPK signalling</td>
<td>Phase 2 completed</td>
<td>NCT01233869</td>
<td>114,119,120,145</td>
</tr>
<tr>
<td>Curcumin</td>
<td>STAT3 signalling</td>
<td>Phase 4 in progress</td>
<td>NCT02494141</td>
<td>149</td>
</tr>
<tr>
<td>Triptolide</td>
<td>JAK2/STAT3 signalling</td>
<td>Phase 3 completed</td>
<td>NCT02115659</td>
<td>151,152</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>mTOR signalling</td>
<td>Phase 3 completed</td>
<td>NCT02055079</td>
<td>69,72,74,122-124</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR signalling</td>
<td>Phase 4 completed</td>
<td>NCT00414440</td>
<td>126,127</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>ERK, mTOR and TGF-β signalling</td>
<td>Phase 2 completed</td>
<td>NCT02697617</td>
<td>130,138</td>
</tr>
</tbody>
</table>
Table 2. A table featuring the potential PKD therapeutic treatments currently under investigation, their target pathways and the current stage of clinical trials. Clinical trial information has been extracted from clinicaltrials.gov.

Perspectives

- PKD is a chronic kidney disease that affects millions of people globally and is a major cause of renal failure worldwide. Current treatments for ADPKD are limited with the majority of treatments focused at treating symptoms in order to lower disease burden. Currently, only one pharmacological treatment (Tolvaptan) has been approved within the UK and Europe for ADPKD treatment; no ARPKD pharmacological cures exist.

- Several pathways e.g., cAMP, EGFR, mTOR etc are of promise in clinical trials for PKD. Of the 13 drugs mentioned in this review, 2 are currently in phase 4, with Everolimus having completed the final stage, ready for FDA approval. The remaining majority are at phase 3, targeting a range of signalling pathways from JAK2/STAT3 to AMPK. In the foreseeable future, Everolimus could be used in routine clinical practices for PKD, as an alternative to Tolvaptan, with hopes of more options being available to treat PKD soon.

- Gene therapy interventions using small intereference (si)-RNAs that target signalling pathways like Wnt may be of great promise in the treatment of PKD. This approach is yet to be explored and could be of great merit.

Author contributions

T.R, K.M., S.A.M. and P.G. all together devised the idea for the mini-review, wrote the manuscript and prepared the figure and tables.

Declaration of Interests

No conflicts of interest to declare.

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Figure Legends

Figure 1. A schematic representation of the signalling pathways dysregulated in Polycystic Kidney Disease (PKD) and potential therapeutics. PC1 (orange rectangle), PC2 (orange rectangle) and FPC (orange oval) localise to cilia\cite{13-21}. FPC and DZIP1L also localise to the basal body\cite{13,18,21}. PC1 localises to cell junctions\cite{20,26-28}, PC2 at the Endoplasmic Reticulum\cite{29,31} and both FPC and PC2 localise to Golgi\cite{31,34}. V2R and cAMP (green rounded rectangle) are upregulated in both ADPKD and ARPKD and drive the activation of CFTR (green rectangle) in ADPKD\cite{50,51}, as well as the activation of AQP2, SRC and MAPK in PKD\cite{53}. EGFR and associated pathways MTOR, MAPK, SRC and STAT3 are upregulated in PKD\cite{39-44,62,69-76}. STAT3 activator JAK is also reported to play a role in ADPKD\cite{150}. Activation of canonical WNT signalling has been reported in ADPKD\cite{84}. WNT/Ca2+ signalling was activated in ADPKD\cite{85} and WNT/Planar Cell Polarity (green oval) genes and proteins were increased in ARPKD\cite{87,88}. Hippo is decreased in ADPKD (red rectangle) resulting in activation of YAP/TAZ\cite{89-92}. Increased Hedgehog and TGF-β signalling were detected in models of PKD\cite{77-80,81-83}. Activation of AMPK in ADPKD (white rectangle) and PPAR-γ in PKD (white rounded rectangle) could be potential methods of TGF-β intervention\cite{102-104,138-144}. Anumber of compounds (top rounded grey rectangles) have been tested for PKD treatment. AMPK Agonists consist of metformin and salsalate, MTOR inhibitors include everolimus and sirolimus and WNT Inhibitors comprise Endo-IWR-1 and XAV839. Autosomal Dominant Polycystic Kidney Disease (ADPKD); AMP-Activated Protein Kinase (AMPK); Aquaporin-2 (AQP2); Autosomal Recessive Polycystic Kidney Disease (ARPKD) Calcium (Ca2+); Bosutinib (SKI-606); Cyclic AMP (cAMP); Cystic Fibrosis Transmembrane Conductance Regulator (CFTR); DAZ Interacting Zinc Finger Protein 1 Like (DZIP1L); Epidermal Growth Factor Receptor (EGFR); Fibrocystin (FPC); Hedgehog (Hh); Janus Kinase 2 (JAK2); Mitogen-Activated Protein Kinase (MAPK); Mammalian Target of Rapamycin (MTOR); Planar Cell Polarity (PCP); Polycystin-1 (PC-1); Polycystin-2 (PC-2); Peroxisome Proliferator-Activated Receptor-Gamma (PPARY-γ)Proto-Oncogene Tyrosine Protein Kinase (SRC);
Signal Transducer and Activator of Transcription 3 (STAT3); Tesevatinib (TSV); Thiazolidinediones (TZD); Transforming Growth Factor-Beta Receptor (TGF-βR); Vasopressin Receptor 2 (V2R); Wingless-Related Integration Site (WNT); Yes-Associated Protein/ Transcriptional coactivator with PDZ-binding motif (YAP/TAZ).

References


(43) Tamio Yamaguchi, Darren P. Wallace, Brenda S. Magenheimer, Scott J. Hempson, Jared J. Grantham, James P. Calvet. Calcium Restriction Allows cAMP


(71) WAHL PR, SERRA AL, LE HIR M, MOLLE KD, HALL MN, WÜTHRICH RP. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). Nephrology, dialysis, transplantation 2006;21(3):598-604.


(155) Boertien WE, Meijs E, de Jong PE, Bakker SJL, Czerwiec FS, Struck J, et al. Short-term renal hemodynamic effects of tolvaptan in subjects with autosomal


