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Experimental Therapies and Ongoing Clinical Trials to Slow Down Progression of ADPKD

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Abstract

The improvement of imaging techniques over the years has contributed to the understanding of the natural history of autosomal dominant polycystic kidney disease, and facilitated the observation of its structural progression. Advances in molecular biology and genetics have made possible a greater understanding of the genetics, molecular, and cellular pathophysiologic mechanisms responsible for its development and have laid the foundation for the development of potential new therapies.

Therapies targeting genetic mechanisms in ADPKD have inherent limitations. As a result, most experimental therapies at the present time are aimed at delaying the growth of the cysts and associated interstitial inflammation and fibrosis by targeting tubular epithelial cell proliferation and fluid secretion by the cystic epithelium.

Several interventions affecting many of the signaling pathways disrupted in ADPKD have been effective in animal models and some are currently being tested in clinical trials.

Keywords

Autosomal Dominant Polycystic Kidney Disease; AMP-activated protein kinase (AMPK) activators; Cystic fibrosis transmembrane conductance regulator (CFTR) inhibitors; mammalian target of rapamycin (mTOR) inhibitors; Somatostatin analogues; Src inhibitors; Peroxisome Proliferator Activated Receptors (PPAR γ) agonists; Vasopressin V2 receptor antagonists

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a multisystem disease, characterized by multiple bilateral renal cysts and various extra renal manifestations such as cysts in other organs, cardiovascular and muscle skeletal abnormalities. It is the most frequent hereditary renal disease, with a prevalence estimated between 1:400 to 1:1000 individual worldwide, and a similar occurrence rate between races. Two different disease-causing genes; *PKD1* and *PKD2*, hundreds of different pathogenic mutations, as well as

CONFLICT OF INTEREST

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modifier genes and environmental factors, account for the large phenotypic variability among ADPKD patients. The improvement of imaging techniques over the years has contributed to the understanding of its natural history, and facilitated the observation of its structural progression. Advances in molecular biology and genetics have made possible a greater understanding of the genetics, molecular, and cellular pathophysiologic mechanisms responsible for its development and have laid the foundation for the development of potential new therapies.

NATURAL HISTORY OF ADPKD

The majority of the renal complications in ADPKD arise mainly as a consequence of the cyst expansion along with associated inflammation and fibrosis. Cyst growth requires excessive cell proliferation and apoptosis, fluid secretion, and remodeling of the surrounding tissue. One of the major challenges of therapies for ADPKD is due to the natural history of the disease. During most of its course, the renal function remains normal and by the time the renal function starts to decline, most of the kidneys have been replaced by cysts. Because of this, therapies that are implemented at later stages are less likely to be successful since the patient may already have reached a point of no return in chronic kidney disease (CKD). On the other hand, when therapies are started at early stages, it is difficult to identify a response based solely on glomerular filtration rate (GFR). Consequently, the notion of kidney volume (KV) as a marker of disease progression gained attention.

The Consortium for Radiologic Imaging Study of PKD (CRISP) was created to develop imaging techniques and analyses, to follow disease progression and evaluate treatments in ADPKD. CRISP has provided invaluable information on how cysts develop and grow, as well as baseline predictors of disease severity and renal function decline [1].

CRISP I recruited 241 participants (between the ages of 15 and 45; with a creatinine clearance >70 ml/min) in 2001, and followed them with baseline and three yearly (YR1 to YR3) visits including measurements of total kidney volume (TKV) by magnetic resonance (MR), and GFR by iothalamate clearance. Two thirds of the patients had an increased risk for renal insufficiency defined by presence of hypertension. In most subjects, kidney and cyst volumes increased exponentially from year to year. At baseline TKV was 1060 ± 642 mL and mean increase over three years was 204 mL (5.27%) per year. Rates of kidney and cyst enlargement were strongly correlated and varied widely from subject to subject. Baseline and subsequent rate of increase in TKV were associated with declining GFR. The correlation between TKV and GFR slopes was significant ($r = -0.186$, $P = 0.005$). However, when patients were stratified into 3 groups according to their baseline TKV (<750 mL, $750 - 1500$, and >1500 mL), only patients with TKV higher than 1500 mL had a significant decrease in GFR slopes (-4.3 ± 8.07 mL per minute per year, $P < .001$). Extrapolation of TKV in individual CRISP subjects back to an age of 18 was consistent with volumes observed by direct measurement in the subset of patients who were 18 years old during the study. The good fit of extrapolated and measured values indicates that TKV growth rate is a defining trait for individual patients [2].

A subset of CRISP I participants (N=131), had renal blood flow (RBF) measurements by MR. RBF decreased from year to year, while overall GFR remained stable. Analysis of baseline predictors of disease progression showed that TKV, RBF, and urinary sodium excretion (UNaE), independently predicted TKV increase. The association between UNaE and structural progression suggests that sodium intake may affect cyst growth. RBF, in addition to TKV, was an independent predictor of GFR decline [3].

After completion of CRISP I, participants were invited to participate in CRISP II (NCT01039987), and 201 reenrolled. Further analysis of multiple baseline covariates on the rate of renal enlargement over 6.3 years of follow-up (Y6), confirmed that TKV and UNaE are independent predictors of renal growth. This time, RBF was excluded from the analysis, and lower HDL-cholesterol was found to be an independent predictor of renal growth as well. However, in the final regression model to predict GFR decline, only two baseline covariates, in addition to lnTKV, had an independent significant effect: body surface area (BSA) and 24-hour urine osmolality; and UNaE and serum HDL-cholesterol were not independently significant [4].

The latest analysis from CRISP II participants (194 patients have completed 7.9 ± 0.6 years of follow-up or Y8) aimed to determine if height-adjusted TKV (htTKV) could predict the onset of renal insufficiency (CKD 3 or $\text{GFR} < 60 \text{ mL/min per } 1.73 \text{ m}^2$). TKV was adjusted to ascertain a more accurate correlation with the severity of the disease. Of all the variables tested (baseline height, weight, BSA, or body mass index), height was the best reference for TKV (htTKV), with a male/female ratio of 1.037. By multivariate analysis, it was determined that for each 100-cc increment of htTKV at baseline, the odds of reaching CKD 3 within 7.9 years increased 1.48-fold. These findings may imply that a single determination of htTKV in an adult patient could be used to determine the likelihood of developing significant renal insufficiency; further validation studies initiated in children are needed. Longer follow-up periods (Y6-Y8) identified significant changes in GFR from baseline and a significant htTKV increase within 1 year with a continuous increase to $> 55\%$ from baseline to Y8 of follow-up. By receiver operator characteristic (ROC) analysis, it was concluded that a htTKV value of 600 cc/m could predict the development of renal insufficiency within 8 years, with good sensitivity and specificity in adults with ADPKD [5].

HIGH PHENOTYPIC VARIABILITY

ADPKD presents with large phenotypic variability, and while many ADPKD subjects would benefit from a therapy capable to slow down disease progression, others with mild disease would not require treatment even if one becomes available. The heterogeneity at the genic and allelic level, but also genetic and environmental modifying factors, are responsible for this phenotypic variability. Mosaicism can also modulate disease presentation and result in marked intrafamilial variability. It is important to consider this variability in ADPKD when designing clinical trials.

Genetic Heterogeneity

Individuals with mutations in *PKD1* tend to have a more severe clinical presentation, higher number of cysts, earlier diagnosis, higher incidence of hypertension and hematuria, and end-

stage renal disease (ESRD) occurring on average 20 years earlier (54.3 vs. 74.0 years in PKD2). Consistent with larger renal volumes being associated with more severe disease, patients with PKD1 present with larger kidneys due to the development of a higher number of cysts at an early age, not to faster cyst growth [6]. Because of its lower severity, the prevalence of PKD2 associated disease might have been underestimated. Population based studies in Olmsted County and Newfoundland, have shown relative frequencies of PKD2 (36 and 29%, respectively) higher than those in clinical studies (10–15%) [7, 8].

Allelic Factors

The *PKD1* and *PKD2* genes present high level of allelic heterogeneity; 929 and 167 different pathogenic mutations have been identified for *PKD1* and *PKD2*, respectively. <http://pkdb.mayo.edu/> No clear correlation between mutation type and disease severity has been identified in *PKD1* or *PKD2*. Patients with mutations in the 5' region of *PKD1* may have more severe disease (18.9% vs. 39.7% with adequate renal function at 60 years) and are more likely to have intracranial aneurysms and aneurysm ruptures than patients with 3' mutations. However, mutation position has not been correlated with disease severity in *PKD2*. Recently, hypomorphic or incompletely penetrant *PKD1* or *PKD2* alleles have been described [9]. A hypomorphic allele alone may result in mild cystic disease; two such alleles cause typical to severe disease, and in combination with an inactivating allele may be associated with early onset disease [9].

Modifier Genes

Large intra-familial variability of ADPKD highlights the importance of genetic modifying factors. In a small proportion of patients either substantial renal enlargement *in utero* or infancy may occur. Its high recurrence risk in affected families suggests a familial modifying background. The best documented example is the contiguous deletion of the adjacent *PKD1* and *TSC2* (Tuberous Sclerosis Complex), characterized by childhood PKD with additional clinical signs of TSC. Likewise, bilinear inheritance of a *PKD1* and *PKD2* mutant allele, as well as *PKD1* or *PKD2* hypomorphic alleles can variably enhance single gene phenotypes. Many additional modifier genes may also contribute to differences in phenotype. However, the majority of studies of candidate loci have shown disappointing results [10–16]. Although genome-wide association studies are feasible (GWAS), large populations of well characterized patients are required.

Gender Effects

Age-adjusted male/female sex ratios greater than unity (1.2–1.3) for yearly incidence rates of ADPKD-caused ESRD in Japan, Europe and United States may imply a more progressive disease in men compared to women. Consistent with this, a survival analysis of 1,391 parent/offspring pairs showed a significant male gender effect (HR, 1.424; 95% CI, 1.180 to 1.719) on age at ESRD (58 and 57 years for female and 54 and 54 years for male parents and offspring) [17]. Other studies have reported an effect of gender in PKD2, but not in PKD1[18].

Environmental Factors

Despite their likely importance, the influence of environmental factors on the progression of ADPKD remain to be elucidated. Smoking increases the risk of progression of renal failure in ADPKD patients [19]. Similarly, high sodium intake may accelerate the structural progression of the disease [3]. Studies in animal models suggest that vasopressin (AVP) contributes to cyst and kidney enlargement in ADPKD [20] and to progression of the renal insufficiency in CKD [21]. A recent study has shown an association between plasma copeptin concentrations (marker of endogenous AVP levels) and disease severity [22].

ADPKD GENES AND THEIR PROTEIN PRODUCTS

ADPKD is genetically heterogeneous with two genes identified, *PKD1* (chromosome 16p13.3; 85% cases) and *PKD2* (4q21; 15% cases) [23–25]. Inheritance of two *PKD1* or two *PKD2* alleles with inactivating mutations is embryonically lethal. Individuals with trans-heterozygous mutations involving both *PKD1* and *PKD2* are viable to adulthood but have more severe renal disease [26].

The *PKD1* and *PKD2* proteins, polycystin-1 (PC1~460 kDa) [24] and polycystin-2 [27] (PC2~110 kDa), are both membrane bound glycoproteins, and constitute a subfamily (TRPP) of transient receptor potential (TRP) channels [28]. Both PC1 and PC2 are located in the plasma membranes of the primary cilia. PC1 is also found in plasma membranes at focal adhesion, desmosomes, and adherens junction sites, whereas PC2 can be found also in the endoplasmic reticulum (ER).

PC1 is comprised of 4302 amino acids, with a large extracellular N-terminal (3074 aa) region, eleven-trans-membrane domains (1032 aa), and a short intracellular C-terminal region (197 aa). The extracellular region is composed of a variety of domains, including 16 immunoglobulin-like domains named PKD repeats (related to protein-protein and protein-carbohydrate interactions), a receptor for egg-jelly (REJ) domain, and a G-protein-coupled receptor proteolytic site (GPS). The intracellular C-terminal region contains a coiled-coil domain that mediates interaction with PC2, and a G-protein-binding domain. The final six-transmembrane region of PC1 share sequence homology with the PC2 transmembrane domains, although PC1 is a distant TRP homolog.

Overall, PC1 structural features resemble an adhesion molecule, mediating cell-cell and cell-extracellular matrix interactions. PC2 is a non-selective cation channel with great permeability to calcium and is comprised of 968 aa with 6 transmembrane domains; both C and N termini are cytoplasmic [29]. PC1 and PC2 interact through their respective C-terminal region. Additionally, PC2 interacts with ryanodine 2 (RyR2) and inositol 1, 4, 5-triphosphate receptor (IP3R), and TRP channels TRPC1, TRPC4, and TRPV4. These receptors control the release of calcium from the intracellular deposits.

In primary cilia, the polycystin complex senses and transmits the mechanic stimulation in a calcium inflow which results in an additional release of calcium by the ER. The reduction in one of both polycystins (PC1 or PC2) beyond a critical level results in a phenotypic change which is characterized by the inability to maintain planar cellular polarity, an increase in the

proliferation and apoptosis, the expression of a secretory phenotype, and remodeling of the extracellular matrix. The cellular mechanisms implied in these phenotypic changes include the intracellular deregulation of calcium, adenosine 3',5'-cyclic monophosphate (cAMP) accumulation and activation of protein kinase A (PKA), activation of mitogen activated protein (MAPK) and mammalian target of rapamycin (mTOR) kinases, canonical Wnt signaling and other intracellular signaling mechanisms (Fig. 1).

THERAPIES TARGETING GENETIC MECHANISMS

Understanding the molecular genetic mechanisms responsible for the cystic disease in ADPKD has treatment implications.

Somatic mutations to one or multiple genes are thought to be important in ADPKD. Therefore, treatments directed at reducing the rate of these mutations could be considered. Their effectiveness would depend on whether they occur mostly during the development, maturation and growth of the kidney or at later stages in association with environmental exposures and cellular senescence. Data from CRISP suggests that most of the renal cysts develop in utero where they have the fastest rate of growth, followed by a much slower rate of development and growth through the patient's life [6]. This makes this therapeutic approach very difficult, since the administration of drugs or other procedures in pregnant women or newborns to delay cyst formation would require a great degree of safety.

If ADPKD was a recessive disease at the cellular level, gene replacement therapy would be theoretically possible. The feasibility of gene replacement therapy has been demonstrated by the rescues of the Oak Ridge National laboratory polycystic kidney (orpk) mouse or the *Pkd1*^{del34/del34} knock-out mouse by the expression of wild-type orpk gene or *PKD1* genes as transgenes [30, 31]. The large size of the PKD genes and the requirement for highly selective, efficient, and durable gene transfers to somatic cells, safety issues, and the observation that the overexpression of *PKD1* also results in a cystic phenotype cast doubt on the feasibility of gene replacement as a successful treatment for ADPKD.

Other forms of gene therapy (antisense mediated exon skipping and stop-codon read-through) have not been explored in ADPKD. Exon skipping is achieved by antisense oligonucleotides that target specific exons to hide them from the splicing machinery during the splicing process [32, 33]. Exon skipping restores the open reading frame and allows the production of a partially functioning protein. This approach is mutation specific and in ADPKD is hindered by the fact that most PKD mutations are unique and evenly distributed along the length of large genes. Another approach in some patients with nonsense mutations due to single-nucleotide substitutions could be the use of compounds that force the cell to ignore premature stop-codons, allowing the production of full-length proteins [34].

Increasing evidence from clinical observations and animal models suggests that cysts can develop even if PC1 or PC2 is not completely lost. *Pkd1* animal models with hypomorphic alleles have shown that a reduced level of *Pkd1* is sufficient to initiate cystogenesis and vascular defects [35–37]. The temperature sensitive nature of one of these hypomorphic mutations (R3277C) suggest that interventions to promote more efficient folding of PC1 may increase the level of functional protein [37]. This indicates that next to the currently

available therapeutics, chaperone enhancing or proteasome inhibiting strategies could be effective in some patients with missense mutations to *PKD1* or *PKD2*. A dosage model of ADPKD suggests that approaches to increase the level of the normal *PKD1* (*PKD2*) allele in typical ADPKD patients, even by a modest amount, could have a dramatic clinical benefit.

Stem cell therapy possesses additional barriers in ADPKD; the cells introduced would need a proliferative advantage over the existing mutant cells, and autologous stem cells could not be used as they would carry the same mutation as the existing renal tissue [38].

Because of these limitations most experimental therapies at the present time are aimed at delaying the growth of the cysts and associated interstitial inflammation and fibrosis by targeting tubular epithelial cell proliferation and fluid secretion by the cystic epithelium. Table 1 provides a summary of selected therapies that have been tried in various animal models of PKD.

THERAPIES TARGETING CELL PROLIFERATION

Therapies Targeting Intracellular Calcium Homeostasis

In tubular epithelial cells the polycystin complexes in primary cilia sense and transduce mechanic stimulation by urine flow into a calcium inflow which in turn results in additional calcium release from the ER where PC2 interacts with IP3R and RyR2 [39–41]. In addition, PC2 affects intracellular calcium homeostasis through interactions with other TRP channels such as TRPC1 and TRPV4. ADPKD cyst cells lack flow-sensitive calcium signaling and exhibit reduced endoplasmic reticulum calcium stores, store-depletion-operated calcium entry and, under certain conditions, intracellular calcium concentrations [42].

Triptolide, an active diterpene in a traditional Chinese medicine, induces PC2 mediated calcium release from the ER, arrests growth of *Pkd1*^{-/-} cells, and reduces cyst burden in constitutive, kidney-specific conditional and inducible *Pkd1* knockout mice [43–45]. A clinical trial of triptolide for ADPKD is currently active in China (NCT00801268).

TRPV4 channel activators increase intracellular calcium, inhibit B-Raf, Erk1/2, and cell proliferation in PCK cholangiocytes, blunt the growth of PCK cysts in 3-D-culture, and attenuate the development of renal cysts and fibrosis in PCK rats [46].

Calcimimetics are allosteric activators of the calcium sensing receptor. By coupling to Gq proteins, calcium sensing receptors activate phospholipase C-protein kinase C signaling and mobilize calcium from intracellular stores. By coupling to Gi proteins, they also inhibit adenylyl cyclase-cAMP signaling. The calcimimetic R-568 inhibits cyst growth and fibrosis in Han:SPRD Cy/+ rats (when administered between 34 and 38 weeks of age [47] and in pcy mice [48], but had no effect on cyst growth in PCK rats and *Pkd2*^{-/WS25} mice [49]. These inconsistent effects may be due to the hypocalcemia associated with the administration of R-568 offsetting the calcimimetic effect on intracellular calcium and cAMP [50]. Indeed, inhibition of cyst growth was associated with a reduction in renal cAMP, whereas cAMP levels remained unchanged when no effect on cyst growth was detected.

Therapies Targeting cAMP Signaling: Vasopressin V2 Receptor Antagonists

The central role of cAMP/PKA in the pathogenesis of PKD and the ability to hormonally modulate cAMP in a cell-specific manner provide opportunities to treat PKD with relative safety. The AVP V2 receptor (V2R) is an attractive target to achieve this goal. Cysts in ADPKD and ARPKD derive predominantly from AVP sensitive tubular segments expressing V2R, i.e. collecting duct and distal nephron. AVP acting on V2R is the main adenylyl cyclase (AC) agonist in collecting ducts. Mammals live under constant tonic action of AVP to avoid dehydration. Circulating AVP levels and renal V2R expression are increased in rodent PKD, possibly due to AVP resistance manifested by a urinary concentrating defect. Almost exclusive renal expression of V2R makes unexpected side effects from V2R antagonism less likely.

Preclinical and clinical studies support further investigation of V2R antagonism as a potential PKD therapy. Serum copeptin (a surrogate for AVP) levels associated with disease severity in a cross-sectional analysis of 102 ADPKD (CKD 1–4) subjects [22], and with renal enlargement and GFR decline in a three-year longitudinal study of 241 ADPKD (CKD 1–2) subjects [51]. Mozavaptan and/or tolvaptan (V2R antagonists) attenuated PKD progression in rodent models of nephronophthisis, ARPKD, *PKD2* and *PKD1* (when treatment started shortly after induction of *PKD1* knockout) [52–56]. Suppression of AVP by forced water intake (3.5-fold increase in urine output) attenuated PKD progression in an ARPKD model (PCK rat) [57]. Cyst development was markedly inhibited in PCK rats lacking circulating AVP (generated by crossing PCK and Brattleboro rats), while administration of V2R agonist 1-deamino-8-d-arginine-vasopressin (dDAVP) fully rescued the cystic phenotype [58]. Satavaptan (V2R antagonist) blocked tubular expression of sFRP4, a secreted frizzled related protein that affects Wnt signaling, is overexpressed in polycystic kidneys, and promotes cystogenesis of zebrafish pronephros [59]. Tolvaptan inhibited AVP-induced chloride secretion and cyst growth of human ADPKD cells in collagen matrices [60], and its administration to ADPKD subjects (CKD 1–3) for one week reduced KV by 3.1%, likely due to its anti-secretory action and more noticeably in patients with preserved function [61].

Open-label studies in North America and Japan have ascertained long-term safety and tolerability of tolvaptan and generated pilot efficacy data [62]. KV was measured at baseline and months 2 (North America), 6 (Japan), 12, 24 and 36. Estimated GFR was measured thrice yearly. Fifty-one of 63 subjects (81%) completed 3 years of therapy. All experienced adverse events, mild to moderate in severity and consistent with its mechanism of action, accounting for 6 of 12 withdrawals. ADPKD subjects were randomly matched (1:2) to historical controls by gender, hypertension, age and baseline KV or eGFR. Baseline KV (controls 1422, tolvaptan 1635 mL) and eGFR (both 62 mL/min/1.73m²) were similar. KV increased 5.8 versus 1.7 %/year (p<0.001) and annualized eGFR declined –2.1 versus –0.71 mL/min/ 1.73m²/year (p=0.01) in 51 patients completing three years of treatment. Results from sensitivity analyses including withdrawn subjects were similar. Mixed model repeated measures sensitivity analyses were significant at each year for KV and non-significant for eGFR. Increasing KV correlated with decreasing eGFR.

Limitations of these studies include small number of patients and utilization of historical controls. Therefore, a phase 3, double-blind, placebo-controlled trial was implemented to determine long-term safety and efficacy of tolvaptan; 1445 ADPKD subjects (age 50 years, eCrCl 60 mL/min, and KV 750 mL) were randomized to tolvaptan or placebo (2:1) with stratification by baseline hypertension, eGFR, and KV [63]. Three split-dose regimens (45/15, 60/30, 90/30 mg) were tested. Treatment began with the lowest dose, titrated to the next higher dose until intolerability or highest dose was reached, and maintained on the highest tolerable dose for 36 months. Primary efficacy endpoint is rate of KV change. Composite secondary efficacy endpoint is time to multiple clinical progression events (blood pressure, pain, albuminuria, renal function). Results are expected in late 2012.

Therapies Targeting cAMP Signaling: Somatostatin Analogues

Somatostatin is a peptide hormone that acts on five Gi protein-coupled receptors (GiPCR) (SSTR1-5), present on cholangiocytes and renal tubular epithelial cells, inhibiting the activity of AC6 and the generation of cAMP. Since somatostatin has a very short half-life, approximately 3 minutes, more stable synthetic peptides (octreotide, lanreotide and pasireotide) have been developed for clinical use. Octreotide and lanreotide bind mainly to SSTR2 and SSTR3, whereas pasireotide has high affinity SSTR1-3 and SSTR5. In preclinical studies, octreotide halted the expansion of hepatic cysts from PCK rats *in vitro* and *in vivo* [64]. Similar effects were observed in the kidneys.

Three small randomized, placebo controlled studies of octreotide or lanreotide (NCT00309283, NCT00426153, NCT00565097) have been completed [65–68]. A cross-over study compared the effect of a 6 months treatment regime of octreotide (40 mg i.m. once every 28 days) with no treatment in 14 ADPKD patients (mean baseline GFR 57.1 mL/min, range 24.4 – 95.3 mL/min). GFR, measured as iothexol clearance, did not change significantly during both treatment periods. TKV increased significantly by 71 ± 107 mL ($p < 0.05$) and 162 ± 114 mL ($p < 0.01$) in octreotide versus not treated patients, respectively. Consequently, there was a 60% reduction in TKV increase in the octreotide group ($p < 0.05$). In a secondary *post hoc* analysis, significant changes in liver volumes between the two treatment periods were found. Liver volumes significantly decreased by 71 ± 57 mL during octreotide versus 14 ± 85 mL increase while no treatment. In addition, changes in liver volume significantly correlated with the changes in TKV ($r = 0.67$) during octreotide but not during placebo treatment [68].

A 6-month study compared treatment with Lanreotide (120 mg once every 28 days subcutaneously) to placebo in 54 patients with polycystic liver disease (PLD) (of which 32 had ADPKD) [66]. Total liver volume decreased significantly by 2.9% in Lanreotide, as compared to a 1.6% increase in placebo treated patients in the overall study population ($p < 0.01$). A *post hoc* stratification for ADPKD/PLD revealed similar changes in liver volume between them, and in both diseases, changes between lanreotide and placebo group were significant (both $p < 0.01$). In ADPKD subjects, TKV decreased by 1.5% in the Lanreotide group and increased by 3.4% in the placebo group ($p < 0.02$). Lanreotide also improved general health perception. Forty-one patients (25 ADPKD) re-enrolled in a 6 month open label extension to complete a total 12 months of lanreotide treatment. After 12

months of lanreotide treatment, median decrease in liver volume was 4% ($p<0.05$) in the overall study population. When results were analyzed by treatment periods, it was observed that 6 months of lanreotide treatment induced a 4% ($p<0.05$) reduction in liver volume, which was maintained with the additional treatment for 6 months. Interestingly, a 4% rebound growth in liver volume was observed in 22 patients that had a CT scan 6 months after stopping 12 months of lanreotide treatment. In 25 ADPKD patients that completed 12 months of lanreotide treatment, the change in TKV was not significant ($p=0.33$) [69].

Another study compared treatment with octreotide (40 mg i.m every 28 days) to placebo (2:1) for 12 months in 42 patients with PLD (of which 34 had ADPKD). (67) Baseline GFR was 71 (range 20–124) ml/min/1.73m². Total liver volume decreased 4.95% in the octreotide group compared with an increase of 0.92% in the placebo group. This difference in change in total liver volume was significant ($p=0.048$). Among ADPKD patients, TKV remained practically unchanged in octreotide treated patients (+0.25%) but increased significantly in non-treated patients (+8.61%). Consequently, kidney growth rate was significantly reduced in the octreotide group when compared to non-treated patients ($p=0.045$). GFR decreased 5.1% and 7.2% in the octreotide and the non- treated patients, respectively (NS). Forty-one patients re-enrolled in an open label extension and continued octreotide treatment or crossed over to octreotide for an additional 12 months. In the patients that received initially octreotide, the change in liver volume was maintained, but no significant change was observed during the following 12 months (–0.77% year 1 vs. year 2 $p=0.57$). Patients that were randomized to placebo and crossed to octreotide experienced a significant 7.66% reduction in liver volume by the end of the octreotide treatment period (year 1 to year 2 $p=0.01$) compared to the initial 0.92% increase during placebo (Baseline to year 1). The effect observed in TKV differed to the one in liver volume by the end of the second treatment period. While octreotide inhibited renal enlargement in the first treatment period, a significant 6.49% increase was observed by the end of the second treatment period (year 1 vs. year 2 $p<0.01$). The patients initially randomized to placebo showed no significant change in TKV during treatment with octreotide (+0.41% from year 1 to year 2 $p=0.90$), compared to the significant increase of 8.61% observed by the end of the first period (Baseline to year 1 $p=0.046$) [70].

Currently, two phase 3, randomized clinical trials: ALADIN 2 (NCT01377246, double-blind, octreotide/placebo) and DIPAK1 (NCT01616927, open label, lanreotide/control) are recruiting patients.

Other Therapies Targeting cAMP

Other Gs protein-coupled receptors (GsPCR) in addition to vasopressin V2R may affect the generation of cAMP and cystogenesis in renal tubules. Catecholamines, adenosine and prostaglandin E₂ (PGE₂) stimulate the generation of cAMP in cultured ADPKD cells. PGE₂ enhances cAMP production and growth of Madin-Darby canine kidney (MDCK) cysts in collagen gels [71]. The effects of PGE₂ on cAMP accumulation and cystogenesis are mediated by two GsPCR; E-prostanoid (EP) receptor 2 (EP₂) and EP₄. The effectiveness of EP₂ or EP₄ receptor blockers on the development of PKD *in vivo* has not been investigated [71].

The hydrolytic capacity of cAMP phosphodiesterase (PDE) exceeds the maximum rate of synthesis by AC. A small-molecule, non-selective PDE activator, was shown to reduce cAMP and inhibit the growth of MDCK cysts [72]. At present the efficacy of these compounds is limited by their lack of selectivity and potential toxicity *in vivo*.

Therapies Targeting c-Src and Ras-Raf-Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase (MAPK/ERK) Signaling

Under normal conditions, activation of PKA inhibits MAPK signaling and cell proliferation. However, in PKD or in conditions where intracellular calcium is reduced, PKA activates MAPK kinase (MEK) in a Src, Ras and B-raf dependent manner. MEK in turn phosphorylates and activates MAPK, also known as extracellular signal-regulated kinase (ERK). Src and MAPK also mediate downstream signaling from growth factors (GF) and their receptor tyrosine kinases which are upregulated in ADPKD. Therefore, cAMP and receptor tyrosine kinase signaling converge in the activation of c-Src, a nonreceptor tyrosine kinase.

The Src inhibitor SKI-606 retarded cyst growth in PCK rats and bpk and *Pkd1* heterozygous mice [73, 74]. PLX5568, a raf kinase inhibitor, attenuated cyst enlargement *in vitro* and in Han:SPRD cy/+ rats, but failed to prevent renal enlargement, promoted hepatic and renal fibrosis and did not improve renal function [75]. Sorafenib, a raf kinase inhibitor that also has activity against receptor tyrosine kinases, including vascular endothelial growth factor receptor (VEGFR) and Platelet-derived growth factor receptor (PDGFR), inhibited cAMP-dependent activation of B-Raf and MEK/ERK signaling, caused a concentration-dependent inhibition of cell proliferation induced by cAMP, epidermal growth factor (EGF), or combination of both, and blocked *in vitro* cyst growth of human ADPKD cells in a three-dimensional collagen gel [76]. However, the administration of sorafenib to mice with conditionally knocked-out *Pkd2* caused an unexpected increase in liver cyst area, cell proliferation and expression of pERK, possibly due to the ability of Raf-inhibitors to transactivate Raf-1 when a PKA-activated Ras promotes Raf-1/B-Raf heterodimerization [77].

The MEK inhibitor PD184352 inhibited cyst growth in the slowly progressive pcy mouse model [78], but another MEK inhibitor, UO126, failed to affect cystic progression in an acute perinatal *Pkd1* conditional knockout mouse [79].

A phase II, multicenter, randomized, double blind, placebo control clinical trial with bosutinib (a Src/Abl inhibitor) is currently ongoing (NCT01233869).

Therapies Targeting mTOR Signaling

From humans to fish, the *PKD1* and *TSC2* genes lie immediately adjacent to each other on chromosome 16 in a tail-to-tail orientation. Contiguous germline deletions affecting both genes lead to much earlier onset and more severe PKD than mutations in the individual genes, suggesting that PC1 and tuberlin function in a common pathway. mTOR is activated in cystic tissues. PC1 physically interacts and retains tuberlin at the plasma membrane thus preventing it phosphorylation by AKT (protein kinase B) and its ability to inhibit the mTOR

pathway [80]. Other studies have shown that ERK activation, downstream from PKA activation, leads to phosphorylation of tuberlin preventing its association with hamartin and the inhibition of Rheb and mTOR by the tuberlin-hamartin complex [81, 82].

Preclinical trials of mTOR inhibitors in rodent models of PKD were mostly encouraging, but differences between rat and mouse studies raise questions on whether doses of mTOR inhibitors necessary for effectiveness in PKD are feasible in the clinical setting. Tolerated doses and blood levels are much lower in rats than in mice. At doses and blood levels achievable in humans, sirolimus and everolimus were effective in a rat model of PKD affecting the proximal tubules [83–87], but not in an orthologous model affecting the distal nephron and collecting duct [88]. At doses and blood levels higher than those achievable in humans, they were consistently effective in multiple orthologous and non-orthologous mouse models [89–91].

The results of clinical trials have been mostly discouraging, possibly because blood levels capable to inhibit mTOR activity in peripheral blood mononuclear cells are not sufficient to inhibit mTOR activity in the kidney [92]. A randomized, open label, placebo controlled, eighteen month trial (SUISSE) of sirolimus in 100 ADPKD patients with a mean estimated creatinine clearance of 92 mL/min and KV of 907 (sirolimus) and 1003 (placebo) mL, had no effect on KV or GFR [93]. Doses of sirolimus were low due to toxicity and patient retention was excellent (96% in the sirolimus group). A six-month cross-over trial (SIRENA) of sirolimus in 21 patients with a mean GFR of 77 mL/min/1.73 m² and KV of 1874 mL, showed less increase in KV on sirolimus compared to placebo, but no effect on GFR [94]. Sirolimus blood levels were higher than in the SUISSE study, but patient retention (71%) was lower. Finally, a randomized, double blinded, placebo controlled, two year trial of everolimus in 433 patients with a mean eGFR of 55 mL/min/1.73 m² and KV of 2028 (everolimus) and 1911 (placebo) mL, showed inhibition of kidney growth but more decline in eGFR [95]. Limitations of the trial included advanced stage of CKD (6.3% in CKD stage IV) and low patient retention (67% in the everolimus group).

All together, these results cast doubt on the role of sirolimus and everolimus in the management of ADPKD, but do not negate the importance of mTOR activation in its pathogenesis, nor the possibility that other strategies targeting this pathway could be successful. One possible approach to overcome mTOR inhibitor systemic toxicity, while achieving a sufficient level to inhibit mTOR activity in the kidney, could be to target the drug specifically to the kidney. Previous studies have shown that folate receptor is overexpressed in the apical membranes of proximal tubule cells compared with most normal cells, raising the possibility for folate-conjugated drugs as candidates for kidney-specific therapeutics. Recent data from bpk mouse model treated with FC-rapa (synthetic folate-conjugated rapamycin) at 0.3 µmol/kg per day has shown to be effective in reducing renal cyst growth and preserving kidney function, without additional systemic effects [96].

Prospective, randomized clinical trials of sirolimus and everolimus are in progress (NCT00414440, NCT00286156, NCT00934791, NCT01009957, NCT01223755).

Therapies Targeting AMP-Activated Protein Kinase (AMPK) Signaling

AMPK is an intracellular energy sensor that decreases energy-consuming processes when cellular AMP levels are high and ATP levels are low. AMPK activation inhibits cell growth through inhibition of mTOR and stimulation of the p53/p21 axis. AMPK activation also decreases epithelial fluid secretion by directly inhibiting cystic fibrosis transmembrane conductance regulator (CFTR). Metformin, an AMPK activator, has been shown to inhibit the growth of MDCK cysts in collagen gels and renal cyst growth in metanephric organ cultures and in kidney specific conditional and in inducible *Pkd1* knockout mice [97].

Therapies Targeting Wnt/ β -catenin Signaling

It has been proposed that the polycystin proteins modulate Wnt signaling. The Wnt ligands are a family of secreted polypeptides that bind to cell surface receptors composed of a transmembrane protein called frizzled and a low-density lipoprotein receptor-related protein. Binding of a Wnt polypeptide to frizzled leads to activation of the protein disheveled, which then interacts with a “destruction” complex containing axin, adenomatous polyposis coli, and glycogen synthase kinase-3 β (GSK-3 β), preventing it from targeting β -catenin for proteasomal degradation and allowing β -catenin to enter the nucleus, where it binds to and activates the TCF transcription factor. Many of the genes whose expression is controlled by TCF stimulate cell division, including c-myc, c-jun, and cyclin D1 (canonical Wnt signaling). Activation of the frizzled receptor can also elicit several β -catenin-independent effects intimately involved in the establishment of planar cell polarity (non-canonical Wnt signaling). Disruption of Wnt signaling has been thought to play an important role in the pathogenesis of ADPKD [98]. Nevertheless, recent studies have suggested that neither disruption of canonical or non-canonical pathways is essential for cystogenesis and the role of Wnt signaling in the pathogenesis of PKD remains uncertain. At present, drugs that target Wnt signaling directly have not been used in PKD, but many therapeutic strategies (e.g. inhibition of PKA signaling) and drugs (e.g. curcumin, cyclooxygenase-2 inhibitors, peroxisome proliferator activated receptors (PPAR)- γ agonists, etc) [99–101] used in preclinical trials for PKD, also affect Wnt signaling.

Peroxisome Proliferator Activated Receptors (PPAR)- γ

PPARs are a family of transcription factors that belong to the nuclear receptor superfamily. PPARs form heterodimers with the 9-cis retinoic acid receptor, RXR α . Activation of PPAR:RXR α by PPAR ligands and/or RXR α ligands, allows the heterodimers to bind peroxisomal proliferator response elements in target genes and modulate gene transcription. PPAR γ is activated by its natural ligands, e.g. prostaglandin J2 and fatty acid derivatives, by conjugated linoleic acid, and by synthetic ligands such as the thiazolidinediones (pioglitazone, troglitazone, and rosiglitazone). Activation of PPAR γ causes cell cycle withdrawal by a variety of mechanisms that include repressed transcription and enhanced proteasome-dependent degradation of cyclin D1 [102–107].

Administration of conjugated linoleic acid reduced macrophage infiltration, interstitial inflammation, and interstitial fibrosis in male Han:SPRD rats [108]. Pioglitazone administered to pregnant mice inhibits renal cyst growth, cardiac abnormalities, and subcutaneous edema of *Pkd1*–/– embryos [109]. Rosiglitazone attenuated PKD progression

and prolonged survival in Han:SPRD rats [110]. Pioglitazone inhibited renal and hepatic cyst growth and fibrosis in the PCK rat [111, 112]. This effect was associated with downregulation of ERK, mTOR and transforming growth factor-beta (TGF- β) signaling and with reduced expression and apical localization of CFTR. Contrary to these favorable results, pioglitazone had no significant effect on cyst growth in principal cell specific, conditional *Pkd1* knockout mice [113].

Histone Deacetylases (HDACs)

Acetylation of histones by histone acetyl transferases increases accessibility of transcription factors to gene promoter regions whereas deacetylation by HDACs has the opposite effect. HDACs also deacetylate specific transcription factors to decrease their DNA binding activity. HDAC1 deacetylates p53 repressing *PKD1* gene transcription [114]. Polycystin signaling increases intracellular calcium and activates protein kinase C which directly or indirectly phosphorylates HDAC5, disrupts its association to myocyte enhancer factor 2C (MEF2C), and releases this transcription factor to affect transcription [115]. HDACs also regulate cellular functions through transcription-independent mechanisms [116]. HDAC6 deacetylates α -tubulin and regulates the stability of microtubules and cilia disassembly during the cell cycle. HDAC6 regulates the deacetylation of β -catenin which is important for canonical Wnt signaling. Valproic acid, an inhibitor of class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8) and trichostatin A, an inhibitor of class II HDACs (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10), suppress cyst formation and retard renal failure in embryonic *Pkd1* and *Pkd2* knockout and in kidney specific, conditional *Pkd1* knockout mice [115, 117, 118].

Therapies Targeting Cell Cycle Regulation

Increased cell proliferation is driven by cyclin-dependent kinases (CDKs). PC1 has been shown to activate Janus kinase-2 (JAK2)/Signal transducer and activator of transcription-1 (STAT1), up-regulate p21waf (a cell cycle inhibitor), inhibit Cdk2 and induce cell cycle arrest in G0/G1 in a PC2-dependent manner [119]. PC2 has been shown to bind Id2 (a helix-loop-helix protein), and prevent its translocation to the nucleus and suppression of p21waf, thus preventing Cdk2 activation and cell cycle progression [120]. In PKD, p21waf levels have been shown to be reduced, while Cdk2 and Cdc25 (cell division cycle) are up-regulated thus leading to increased cell proliferation.

Roscovitine (Seliciclib, CYC202), a CDK inhibitor, inhibits cystogenesis and improves renal function in two models of PKD (*jck* and *cpk* mice), acting through transcriptional regulation, blockade of the G1-S phase in the cell cycle, and inhibition of apoptosis [121]. Like PC1, roscovitine increases the levels of p21, which is downregulated in PKD [122]. In PLD the expression of miR15a is decreased, while that of its target, the cell-cycle regulator Cdc25A, is up-regulated. Cdc25A is a phosphatase that plays an essential role in cell cycle progression by activating CDKs. Menadione lowers the levels of Cdc25A and other cell cycle proteins and inhibits renal and hepatic cyst growth in both PCK rats and *Pkd2*^{ws25/-} mice [123].

Therapies Targeting Cytokine Signaling

Recent studies have provided strong support for the activation of innate immune mechanisms and paracrine and autocrine effects of cytokines in the pathogenesis of PKD. Preclinical studies have shown the feasibility of targeting tumor necrosis factor- α (TNF- α) signaling and cytokines acting on receptors activating STAT3 or STAT6 pathways.

TNF- α is one of many cytokines found in cyst fluids. TNF- α , TNF- α receptor (TNFR-I) and TNF- α converting enzyme are overexpressed in cystic tissues. Administration of TNF- α promotes cyst formation in *Pkd2*^{+/-} mice, while etanercept (TNF- α inhibitor) has an inhibitory effect [124]. The aggravation of PKD by TNF- α may be due to its enhancement of the expression of FIP2 (TNF- α -induced protein), a protein that physically interacts with PC2 and prevents its transport to the plasma membrane and primary cilium. Alternatively, TNF- α also activates IKK β (inhibitor of κ B kinase- β), which physically interacts and phosphorylates hamartin, suppressing TSC1-TSC2 function and activating mTOR [125]. An inhibitor of TNF- α -converting enzyme was shown to ameliorate the polycystic disease in the *bpk* mouse, a recessive model of PKD [126]. Nevertheless, transforming growth factor- α (TGF- α) does not appear to be critical for the development of PKD, since *bpk* mice with two TGF- α null alleles are not protected [127].

Many cytokines bind to receptors that use a JAK/STAT pathway to transduce their signals. Upon ligand binding, receptor-associated JAKs become activated leading to the phosphorylation of specific receptor tyrosine residues, and recruitment, phosphorylation and release of specific STATs. Activated STAT form dimers that translocate to the nucleus and bind to specific enhancer elements.

STAT3 transduce signals for the Interleukin-6 (IL-6) family in addition to other cytokines and several growth factors. IL-6 is present in renal and hepatic cyst fluids. The anti-parasitic drug pyrimethamine and the small molecule S3I-201, inhibit STAT3 activation and cyst formation and growth in inducible *Pkd1* knockout mice [128]. Curcumin, a natural product derived from the plant *curcuma longa*, inhibits forskolin-induced MDCK cell proliferation, *in vitro* MDCK cystogenesis, and cyst growth in metanephric organ cultures [100] and in inducible, kidney specific *Pkd1* knockout mice [99], possibly by blocking STAT3 signaling among other mechanisms.

STAT6 mediates IL-4 and IL-13 signaling. IL13 is present in cyst fluid and the IL4/13 receptor is overexpressed in cystic tissues. Genetic inactivation of STAT6 in a PKD mouse model leads to significant inhibition of proliferation and cyst growth and preservation of renal function. Teriflunomide, the active metabolite of leflunomide, a drug approved for treatment of arthritis, inhibits STAT6 in renal epithelial cells. Treatment of *bpk* mice with this drug leads to amelioration of the renal cystic disease similar to genetic STAT6 inactivation.

Therapies Targeting Growth Factor Signaling

A number of growth factors are found at high concentrations in cyst fluids and their receptors are overexpressed in cystic tissues.

Evidence from a number of laboratories provided strong support for an important role of the EGF/TGF- α /EGF receptor (EGFR or ErbB1) axis, in promoting tubular epithelial cell proliferation and cyst formation [129]. Pharmacologic inhibition of EGFR tyrosine kinase activity inhibits the development of cystic disease in bpk and orpk mice, two autosomal-recessive models of rapidly progressive cystic disease, and in Han:SPRD rats, an autosomal dominant model of slowly progressive renal cystic disease [130]. They had no effect, however in the PCK rat, an orthologous model of ARPKD [131], probably because ErbB2 but not EGFR (Erb-B1) is overexpressed in this model [73].

The expression of vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFR) are up-regulated in the renal and hepatic cystic epithelium. VEGFR-1 and VEGFR-2 ribozyme treatment inhibited cystogenesis and improved renal function in Han:SPRD rats. (132) The VEGF receptor inhibitor SU-5416 reduced cystic development of the liver, but did not affect renal cysts in *Pkd2*^{WS25/-} mice [133]. The same inhibitor in a different study blunted hepatic cystogenesis in conditional *Pkd2* but not *Pkd1* knockout mice [134]. In contrast with these results, administration of an anti-VEGF-A antibody worsened the renal cystic disease in Han:SPRD rats [135] and treatment of young wild-type mice with a VEGFR-2 antibody induced renal cyst formation [136].

Hepatocyte growth factor (HGF) and c-Met (HGF receptor) are overexpressed by cyst-lining cells in kidneys from individuals with PKD or acquired cystic disease [137]. In *Pkd1*-null cells, Casitas B-lineage lymphoma (c-Cbl), an E3-ubiquitin ligase for c-Met, is sequestered in the Golgi apparatus with $\alpha 3\beta 1$ integrin, resulting in the inability to properly ubiquitinate and degrade c-Met after stimulation by HGF. Treatment of mouse *Pkd1*-null cystic kidneys in organ culture with a c-Met pharmacological inhibitor blocked cystogenesis.

Insulin-like growth factor-1 (IGF-1) is found in cystic fluids and the IGF-1 receptor is overexpressed in cystic tissues from ADPKD patients. In the Han:SPRD rat, the kidney IGF-1 content increased in parallel with the severity of cystic disease. Dietary lowering of kidney IGF-1 concentrations (soy bean diet) resulted in a parallel reduction in cystic disease severity [138]. Kidney IGF-1 mRNA is increased by four-fold in the recessive pcy mouse model characterized by rapidly progressive cystic disease [139]. Mice transgenic for human growth hormone display pronounced cystic tubular dilatation. PC1 deficiency is associated with increased sensitivity to the proliferative effect of IGF-1 in conditionally immortalized tubular epithelial cells generated from ADPKD patients.

Therapies Targeting Structural Glycosphingolipids

Sphingolipids and glycosphingolipids regulate many cellular processes and cell signaling pathways. Ceramide is a precursor for structural glycosphingolipids (glucosylceramide $\rightarrow$ lactosylceramide $\rightarrow$ ganglioside GM3) and bioactive signaling sphingolipids ($\rightarrow$ sphingosine $\rightarrow$ sphingosine-1-phosphate). Glucosylceramide and lactosylceramide are elevated by approximately 2–3 folds in ADPKD patients and may stimulate proliferation *via* the activation of MAPK. The glucosylceramide synthase inhibitor Genz-123346, was found to suppress mTOR signaling, induce G1/S cell cycle arrest, and inhibit cystogenesis in mouse models orthologous to human autosomal dominant PKD (*Pkd1* conditional knockout mice) and nephronophthisis (jck and pcy mice). More recently, jck mice were crossed with

mice carrying a targeted mutation in the GM3 synthase gene, to determine the role of GM3 in PKD progression. GM3-deficient *jck* mice displayed milder PKD. To determine the role of sphingosine-1-phosphate, *jck* mice were crossed with sphingosine kinase 1 mutants. Genetic loss of sphingosine kinase 1 was associated with increased levels of glucosylceramide and GM3, and exacerbated cystogenesis. These structural glycosphingolipids are components of lipid rafts and may affect cell surface receptor interactions and cystogenesis. Together, these data suggest that glucosylceramide synthase and GM3 synthase may be therapeutic targets for PKD.

Additional Therapeutic Targets and Experimental Therapies

Purinergic Signaling Pathway—Blockade of the P2X7 receptor reduces cyst formation *via* ERK-dependent pathways in *pkd2*-morphant zebrafish, a zebrafish model of polycystic kidney disease, suggesting that P2X7 antagonists may have therapeutic potential in ADPKD.

The 20-hydroxyeicosatetraenoic acid (20-HETE), an endogenous cytochrome P450 metabolite of arachidonic acid, has known mitogenic properties, and is markedly increased in microsomes from *bpk* compared to wild-type mice [140]. The daily administration of a 20-HETE synthesis inhibitor (HET-0016), has been reported to reduce kidney size by half and doubled survival. Transfection of principal cells isolated from wild-type mice with *Cyp4a12* induced a four- to five-fold increase in cell proliferation, which was completely abolished when 20-HETE synthesis was inhibited. These observations suggest that 20-HETE contributes to the proliferation of epithelial cells in the formation of renal cysts and provide another potential target for intervention.

Increased apoptosis accompanies increased cell proliferation in PKD. Caspases, the major mediators of apoptosis, are increased in PKD. The development of the cystic disease and renal insufficiency in Han:SPRD rats was inhibited with a caspase inhibitor (IDN-8050) that reduced epithelial cell apoptosis and proliferation [141]. Double mutants with PKD (*cpk* mice) and a knockout of caspase 3, had less severe cystic disease and lived longer than *cpk* mice with intact caspase 3 [142].

THERAPIES TARGETING FLUID SECRETION

In the cystic cells, chloride enters across basolateral NaK2Cl cotransporters, driven by the sodium gradient generated by basolateral Na-K-ATPase, and exits across apical cAMP-stimulated CFTR, leading to cyst enlargement. Basolateral recycling of potassium may occur *via* KCa3.1 channels which play a critical role in transcellular chloride secretion and net fluid transport into the kidney cysts of patients with ADPKD [143, 144]. Active accumulation of chloride within the cyst lumen drives sodium and water secretion down transepithelial potential and osmotic gradients. This model for cyst fluid secretion requires that the paracellular pathway is sealed by tight junctions impermeable to chloride which remains intact even in late-stage cysts [145].

Work using 3D cultures of MDCK cells, metanephric organ cultures, and conditional *Pkd1* knockout mice has shown that CFTR inhibitors slowed cyst growth [146, 147]. Consistent with this, reports in families with both ADPKD and cystic fibrosis confirmed that

individuals with both diseases had less severe cystic disease than those with only ADPKD [148]. CFTR inhibitors that achieve high concentrations in the kidney and urine may find a place in the treatment of ADPKD because their accumulation in the lungs is minimal and CFTR inhibition has to exceed 90% to affect lung function, thus making the development of cystic fibrosis-like lung disease unlikely. There are no clinical trials yet for the use of CFTR inhibitors in ADPKD.

A KCa3.1 inhibitor, TRAM-34 (an analogue of desimidazolyl clotrimazole), inhibited forskolin stimulated transepithelial chloride secretion in filter-grown polarized monolayers of MDCK, NHK (normal human kidney), and ADPKD cells, as well as MDCK and ADPKD cell cyst formation and enlargement in collagen gels [143, 144]. Although the efficacy of KCa3.1 inhibitors in ADPKD still needs to be demonstrated in animal models of the disease, it is encouraging that pharmacological inhibition or knockdown of KCa3.1 suppresses tubulointerstitial damage and protects functional renal parenchyma in an animal model of unilateral ureteral obstruction [149]. Senicapoc (ICA-17043), a KCa3.1 inhibitor, has been used successfully in a phase 2 trial and has shown little or no toxicity in a phase 3 trial for sickle cell disease [150].

A number of therapies that effectively suppress cell proliferation in PKD also inhibit Chloride driven fluid secretion.

PKA can phosphorylate and activate CFTR Cl⁻ channels, increasing fluid secretion.

AMPK Activators

AMPK activators not only inhibit cell growth through inhibition of mTOR, but also decrease epithelial fluid secretion by directly inhibiting CFTR.

PPAR γ Agonists

PPAR γ agonists inhibit the expression and apical localization of CFTR, AVP-stimulated Cl⁻ secretion *via* CFTR in the MDCK cells, and renal and hepatic cyst growth in the PCK rat [111, 151].

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Fig. 1. Diagram depicting hypothetical pathways in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

In tubular epithelial cells the polycystin complexes in primary cilia sense and transduce mechanic stimulation by urine flow into a calcium inflow which in turn results in additional calcium release from the ER. In ADPKD, the reduction in one of both polycystins beyond a critical level results in dysregulation of intracellular calcium and accumulation of cAMP. cAMP stimulates MAPK/ERK signaling and cell proliferation in a Src- and Ras-dependent manner. Phosphorylation of tuberin by ERK (or inadequate targeting to the plasma membrane because of defective interaction with PC1) may lead to the dissociation of tuberin and hamartin and activation of Rheb (Ras homolog enriched in the brain) and mTOR. Upregulation of Wnt signaling stimulates mTOR and β -catenin signaling. ERK and mTOR activation promotes G1/S transition and cell proliferation. STAT3 transduces signals for IL-6 (found in cyst fluids) in addition to other cytokines and several growth factors. STAT6 mediates IL-4 and IL-13 signaling and the IL4/13 receptor is overexpressed in cystic tissues. Activation of tyrosine kinase receptors by ligands present in cystic fluid also contributes to the stimulation of MAPK/ERK signaling and cell proliferation. Increased cAMP levels also contribute to cystogenesis by stimulating chloride and fluid secretion. PC1, Polycystin 1; PC2, Polycystin 2; ER, Endoplasmic reticulum; cAMP, adenosine 3',5'-cyclic monophosphate; PKA, protein kinase A; MAPK/ERK, mitogen-activated protein kinase/extracellularly regulated kinase; mTOR, mammalian target of rapamycin; STAT3/6, Signal

transducer and activator of transcription 3/6; N, Nucleus; TNF- α , Tumor necrosis factor-alpha; AMPK, AMP-activated protein kinase; CFTR, cystic fibrosis transmembrane conductance regulator; IL-6, Interleukin-6; IL-4, Interleukin-4; IL-13, Interleukin-13.

Table 1
Effectiveness of Selected Therapeutic Interventions in Animal Models of Polycystic Kidney Disease

	Rats		Mice									
	Han:SPRD	PCK	orp	bpk	cpk	jck	pcy	<i>Pkd1^{-/-}</i>	<i>Pkd1^{+/c}</i>	<i>Pkd1^{hsp}</i>	<i>Pkd2^{c/c}</i>	<i>Pkd2^{-WS25}</i>
Protein restriction	yes	----	----	----	----	----	yes	----	----	----	----	----
Soy-based protein	yes	----	----	----	----	----	yes	----	----	----	----	----
Flax seed	yes	----	----	----	----	----	----	----	----	----	----	----
Bicarbonate/citrate	yes	no	----	----	----	----	no	----	----	----	no	----
Pacitaxel	no	----	no	----	yes	----	no	----	----	----	----	----
Methylprednisolone	yes	----	----	----	----	----	yes	----	----	----	----	----
Triptolide	----	----	----	----	----	----	yes	yes	yes	----	----	----
TRPV4 activator	----	yes	----	----	----	----	----	----	----	----	----	----
Calcimimetics	yes	no	----	----	----	----	yes	----	----	----	no	----
V2R antagonist	----	yes	----	----	yes	----	yes	----	yes	----	yes	----
SST analogs	----	yes	----	----	----	----	----	----	----	----	yes	----
c-Src inhibitor	----	yes	----	yes	----	----	----	----	----	----	----	----
Raf inhibitor	no	----	----	----	----	----	----	----	----	no	----	----
MEK inhibitor	----	----	----	----	----	----	yes	----	no	----	----	----
Rapalogues	yes	----	yes	yes	----	----	yes	----	yes	yes	yes	----
Metformin	----	----	----	----	----	----	----	----	yes	----	----	----
PRAR γ agonist	yes	yes	----	----	----	----	----	yes	no	----	----	----
HDAC inhibitors	----	----	----	----	----	----	----	yes	yes	----	----	----
CDK inhibitors	----	----	----	----	yes	----	----	----	----	----	----	----
Cdc25A inhibition	----	yes	----	----	----	----	----	----	----	----	yes	----
c-myc antisense	----	----	----	----	yes	----	----	----	----	----	----	----
TNF α inhibition	----	----	----	yes	----	----	----	----	----	----	yes	----
STAT3 inhibitors	----	----	----	----	----	----	----	----	yes	----	----	----
STAT6 inhibitors	----	----	----	yes	----	----	----	----	----	----	----	----
EGFR TK inhibitor	yes	no	yes	yes	----	----	----	----	----	----	----	----
ErbB2 TK inhibitor	----	yes	----	yes	----	----	----	----	----	----	----	----

	Rats		Mice									
	Han:SPRD	PCK	orpK	bpK	cpK	jek	pcy	<i>Pkd1</i> ^{-/-}	<i>Pkd1</i> ^{+/c}	<i>Pkd1</i> ^{hyp}	<i>Pkd2</i> ^{c/c}	<i>Pkd2</i> ^{-/WS25}
VEGFR inhibitor	yes								no		yes	no
GlucCer synth inh						yes	yes		yes			

See text for references or available on demand

Cysts are mostly of proximal tubular origin in the Han:SPRD rat and of distal/collecting duct origin in most other models.