ABSTRACT
Polycystic kidney disease (PKD) is a leading cause of ESRD worldwide. In PKD, excessive cell proliferation and fluid secretion, pathogenic interactions of mutated epithelial cells with an abnormal extracellular matrix and alternatively activated interstitial macrophages, and the disruption of mechanisms controlling tubular diameter contribute to cyst formation. Studies with animal models suggest that several diverse pathophysiologic mechanisms, including dysregulation of intracellular calcium levels and cAMP signaling, mediate these cystogenic mechanisms. This article reviews the evidence implicating calcium and cAMP as central players in a network of signaling pathways underlying the pathogenesis of PKD and considers the therapeutic relevance of treatment strategies targeting cAMP signaling.

DISRUPTION OF INTRACELLULAR CALCIUM HOMEOSTASIS AND PKD
Polycystin-1, polycystin-2, and fibrocystin, localized in the primary ciliature, are required for induction of calcium transients in response to ciliary bending. The three proteins are found at low levels in tubular epithelial cells, but are abundant in urinary exosomes. Polycystin-1 resembles a receptor or adhesion protein and is also found at the plasma membrane and, possibly, in the endoplasmic reticulum. Polycystin-1 interacts with the inositol 1,4,5-trisphosphate receptor (IP3R). Polycystin-2 is a transient receptor potential (TRP) channel that is mainly located in the endoplasmic reticulum, where it functions as a calcium release channel, and, possibly, located in the plasma membrane. Polycystin-1 and fibrocystin interact with, and modulate the function of, polycystin-2. Polycystin-2 also interacts with other calcium channel proteins (IP3R, ryanodine receptor, TRPC1, TRPC4, and TRPV4).

INCREASED CAMP LEVELS IN CYSTIC TISSUES
Tissue levels of cAMP are increased in numerous animal models of PKD not only in the kidney but also in...
cholangiocytes, vascular smooth muscle cells, and choroid plexus. Tissue levels of cAMP are determined by the activities of membrane-bound (under the positive or negative control of G protein–coupled receptors [GPCRs] and extracellular ligands) and soluble adenylyl cyclases (ACs) and cAMP phosphodiesterases (PDEs), which are also subject to complex regulatory mechanisms.

Several hypotheses may explain the increased levels of cAMP (Figure 1). (1) Reduced calcium activates calcium-inhibitable AC6, directly inhibits calcium/calmodulin-dependent PDE1 (by also increasing the levels of cGMP), and indirectly inhibits cAMP-inhibitable PDE3. (2) Dysfunction occurs in a ciliary protein complex (comprising A-kinase anchoring protein 150, AC5/6, polycystin-2, PDE4C, and PKA), which normally restrains cAMP signaling through inhibition of AC5/6 by polycystin-2–mediated calcium entry and degradation of cAMP by PDE4C transcriptionally controlled by HNF1β. (3) Depletion of the endoplasmic reticulum calcium stores trigger oligomerization and translocation of STIM1 to the plasma membrane, where it recruits and activates AC6. (4) Other contributory factors include disruption of PC1 binding to heterotrimeric G proteins, upregulation of the vasopressin V2 receptor, and increased levels of circulating vasopressin or accumulation of forskolin, liposomatoplastic acid, ATP, or other adenylyl cyclase agonists in the cyst fluid. Increased cAMP levels disrupt tubulogenesis, stimulate chloride and fluid secretion, and activate proliferative signaling pathways, including mitogen-activated protein kinase/extracellularly-regulated kinase (in an Src- and Ras-dependent manner), mTOR, and β-catenin signaling. Activated mTOR transcriptionally stimulates aerobic glycolysis, increasing ATP synthesis and lowering AMP levels, which together with B-Raf–dependent activation of LKB1, inhibits AMPK, further enhancing mTOR activity and CFTR-driven chloride and fluid secretion. PKA signaling also activates a number of transcription factors, including STAT3. Activated STAT3 induces the transcription of cytokines, chemokines, and growth factors that, in turn, activates STAT3 on interstitial alternatively activated (M2) macrophages, which results in a feedforward loop between cyst-lining cells and M2 macrophages. Abluent integrin–extracellular membrane interaction and cAMP signaling within focal adhesion complexes may contribute to the increased adhesion of cyst-derived cells to laminin-322 and collagen. AC-VI, adenylyl cyclase 6; AMPK, AMP kinase; AVP, arginine vasopressin; B-Raf, B rapidly accelerated fibrosarcoma kinase; CDK, cyclin-dependent kinase; cGMP, cyclic guanosine monophosphate; CREB, cAMP response element binding transcription factor; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase 3β; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; Pax2, paired box gene 2; PC1, polycystin-1; PC2, polycystin-2; SST, somatostatin; SSTR, somatostatin receptor; STIM1, stromal interaction molecule 1.
Depletion of the endoplasmic reticulum calcium stores trigger oligomerization and translocation of stromal interaction molecule 1 to the plasma membrane, where it recruits and activates AC6. Other contributory factors include disruption of PCI binding to heterotrimeric G proteins, upregulation of the vasopressin V2 receptor, and increased levels of circulating vasopressin or accumulation of forskolin, lysophosphatidic acid, ATP, or other adenylyl cyclase agonists in the presence of forskolin, lysophosphatidic acid, ATP, or other adenylyl cyclase agonists. 

Table 1. Intracellular calcium homeostasis in PKD microdissected tissues or cultured cells

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Cells</th>
<th>Comparison</th>
<th>Resting ([\text{Ca}^{2+}]_i)</th>
<th>ER (SR) ([\text{Ca}^{2+}]_i) Store</th>
<th>CCE</th>
<th>Flow-Induced ([\text{Ca}^{2+}]_i) Entry</th>
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</thead>
<tbody>
<tr>
<td>Nauli et al. (2003)</td>
<td>E15.5 kidney cells</td>
<td>Pkd1&lt;sup&gt;dlc24a/lcb34&lt;/sup&gt; versus WT</td>
<td>↓</td>
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<td>Qian et al. (2003)</td>
<td>Aortic VSMC</td>
<td>Pkd2&lt;sup&gt;+/+&lt;/sup&gt; versus WT</td>
<td>↓</td>
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<tr>
<td>Nauli et al. (2006)</td>
<td>Cultured kidney cells</td>
<td>Pkd1&lt;sup&gt;-/-&lt;/sup&gt; versus WT</td>
<td>↓</td>
<td>↓</td>
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<td>Yamaguchi et al. (2006)</td>
<td>Immortalized kidney cells</td>
<td>PKD1c versus WT</td>
<td>↓</td>
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<tr>
<td>Geng et al. (2008)</td>
<td>Cultured kidney cells</td>
<td>Pkd1&lt;sup&gt;-/-&lt;/sup&gt; versus Pkd1&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>↓</td>
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<td>Xu et al. (2007)</td>
<td>Cultured kidney cells</td>
<td>PKD1 versus NHK</td>
<td>↓</td>
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<tr>
<td>Ahrabi et al. (2007)</td>
<td>Isolated collecting ducts</td>
<td>Pkd1&lt;sup&gt;-/-&lt;/sup&gt; versus WT</td>
<td>↓</td>
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<tr>
<td>Anyatwau et al. (2007)</td>
<td>E17.5 cardiac myocytes</td>
<td>Pkd2&lt;sup&gt;-/-&lt;/sup&gt; versus WT</td>
<td>↓</td>
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<tr>
<td>Morel et al. (2009)</td>
<td>Aortic VSMC</td>
<td>Pkd1&lt;sup&gt;-/-&lt;/sup&gt; versus WT</td>
<td>↓</td>
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<td>Banales et al. (2009)</td>
<td>Cholangiocytes</td>
<td>PCK versus WT</td>
<td>↓</td>
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<td>Spirli et al. (2012)</td>
<td>Cholangiocytes</td>
<td>Pkd2&lt;sup&gt;-/-&lt;/sup&gt; versus WT</td>
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<tr>
<td>Zaika et al. (2013)</td>
<td>Microdissected cysts and split-open CDs</td>
<td>PCK versus WT</td>
<td>↓</td>
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ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; CCE, capacity calcium entry; WT, wild-type; VSMC, vascular smooth muscle cell; NHK, normal human kidney; CD, collecting duct.

**EPITHELIAL TUBULOBGENESIS AND REGULATION OF TUBULAR DIAMETER**

Epithelial tubulogenesis requires canonical Wnt/β-catenin signaling at early inductive stages and noncanonical Wnt/planar cell polarity (PCP) signaling later. Primary cilia control the switch of canonical to noncanonical Wnt signaling. Convergent extension (process of cell intercalation by which cells elongate along an axis perpendicular to the proximal–distal axis of the tubule by actively crawling between one another to produce a narrower, longer tubule) and oriented cell division (alignment of the mitotic spindle axis and cell division with the proximal–distal axis of the tubule) establish and maintain the normal tubular diameter under the control of PCP signaling. Sustained activation of canonical Wnt signaling, downregulation of noncanonical/PCP signaling, and disruption of ciliogenesis or knock-out of the ciliary protein inversin are all associated with cystic disease.

Enhanced CAMP and PKA signaling disrupts tubulogenesis. PKA is known to enhance Wnt/β-catenin signaling through phosphorylation of glycogen synthase kinase 3β (stabilizing β-catenin) and phosphorylation of β-catenin (promoting its transcriptional activity) (Figure 1). Sustained PKA-dependent canonical Wnt signaling blocks a post-epithelialization morphogenetic step (conversion of the renal vesicle to the S-shaped body) in spinal cord-induced metanephric mesenchyme, resulting in disorganized epithelial clusters and large dilations. Overexpression of constitutively active PKA catalytic subunits can also act as a negative regulator of PCP signaling and block convergent extension during Xenopus gastrulation. Thus, it is conceivable that hyperactivation of CAMP/PKA signaling may interfere with the control of tubular diameter in PKD.

**Cell Proliferation**

Increased cell proliferation is necessary, but not sufficient, for cystogenesis. Analysis of conditional Pkd1 models has shown that the developmental and proliferative activity of the tubular epithelial cells at the time of Pkd1 inactivation determines the rate of development and severity of the cystic disease. The rate of epithelial cell proliferation may also account for cyst migration in humans and animal models of PKD as the kidney develops and matures from predominately proximal to predominantly distal...
and collecting duct. Immature early tubules (S-shaped bodies) exhibit very high rates of proliferation. Later, when the epithelium differentiates into nephron segments recognizable by light microscopy, proliferative indices become very low in the proximal tubule but remain elevated in the distal nephron and collecting duct. In pediatric and adult kidneys, proliferative indices are very low in all tubular segments but remain higher in collecting ducts compared with those indices in proximal tubules.

cAMP exerts opposite effects on cell proliferation in different cell types. cAMP and PKA signaling enhances several proproliferative pathways (extracellular signal-regulated kinase [ERK]) in cells derived from polycystic kidneys while inhibiting proliferation in cells derived from normal human kidney cortex. Treatment of normal human kidney or murine collecting duct cells with calcium channel blockers (gadolinium, 25 μM; nifedipine, 0.1 μM; or verapamil, 1 μM) replicates the proliferative response of the ADPKD cells to cAMP, thus linking this response to the reduction in intracellular calcium that results from disrupting the polycystin pathway. Conversely, treatment of ADPKD or ARPKD cyst-derived cells with calcium channel activators or calcium ionophores restores the normal antiproliferative response to cAMP. Inhibition of calcium-dependent phosphatidylinositol 3-kinase activity with downstream inhibition of Akt, allowing B rapidly accelerated fibrosarcoma (B-Raf) and ERK to be activated in a PKA-, Src-, and Ras-dependent manner, has been proposed to underlie the proliferative response to cAMP in the setting of reduced calcium (Figure 1). Activation of mammalian target of rapamycin (mTOR) signaling downstream from PKA through ERK-mediated phosphorylation of tuberin has been linked to transcriptional activation of aerobic glycolysis, increased levels of ATP, and together with ERK-dependent inhibition of liver kinase B1, inhibition of AMP kinase, which may further enhance mTOR signaling. Phosphorylation and inhibition of glycogen synthase kinase 3β and direct phosphorylation and stabilization of β-catenin by PKA enhance Wnt/β-catenin signaling. PKA-dependent upregulation of cAMP response element binding transcription factor, paired box gene 2 (Pax2), and signal transducer and activator of transcription 3 (STAT3) also contribute to the proliferative phenotype of the cystic epithelium. Pax2 haploinsufficiency inhibits renal cystogenesis in homozygous Pkd1 mutant or cpk mutant mice. The knockout of SOCS-1, a negative regulator of STAT3 expression, induces a cystic disease phenotype.

Although the proliferative effect of cAMP on the renal cystic epithelium seems to be exclusively PKA-mediated, both PKA and exchange protein activated by cAMP (Epac) activations enhance cholangiocyte proliferation and hepatic cystogenesis the mitogen ERK kinase (MEK)/ERK1/2 pathway in PCK rats.

Fluid Secretion
By the time that the renal cysts reach a diameter of approximately 2 mm, they become disconnected from the tubular segment from which they derived. Additional growth depends on transpithelial fluid secretion. A study in 1977 using tritiated water shows a high turnover of fluid fluid in ADPKD (>100 ml in 24 hours for a 10-ml cyst) thought to be secondary to active solute transport and therefore, susceptible to pharmacotherapy. Studies using polarized normal human kidney and ADPKD cells on permeable supports and microcysts in hydrated collagen confirm a model of fluid transport as previously shown in MDCK cells and other secretory epithelia. Intact cysts excised from ADPKD kidneys secrete fluid when treated with forskolin, confirming that intact cyst epithelia secrete fluid by mechanisms regulated by cAMP. The driving force is active transport of chloride from the basolateral to the apical side. The energy is generated by the sodium pump to bring potassium and chloride into the cells. PKA-induced phosphorylation of the cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane opens the channel and allows the flow of chloride ions down an electrochemical gradient into the cyst, generating increased transepithelial electron activity that, in turn, drives sodium ions through parallel pathways. The role of CFTR in PKD is supported by whole-cell patch-clamp studies of cyst-derived epithelial cells, inhibition of fluid secretion by CFTR antisense oligonucleotides, inhibition of cyst growth in vitro and in vivo models of cystogenesis, and the milder cystic phenotype in patients affected by both ADPKD and cystic fibrosis. However, an additional patient with coexisting ADPKD and cystic fibrosis progressed to ESRD after a lung transplant, possibly because of cyclosporin toxicity, and the disease severity of bpk CFTR double mutant mice was not less than the disease severity of bpk mice, a rapidly progressive model of PKD.

CELL, BASEMENT MEMBRANE, AND EXTRACELLULAR MATRIX INTERACTIONS
Evidence indicates that alterations in focal adhesion complexes, basement membranes, and extracellular matrix contribute to the pathogenesis of PKD. focal adhesion complexes contain integrin αβ heterodimer receptors, which link the actin cytoskeleton to the basement membrane laminin αβγ heterotrimers and collagens and multiple structural and signaling molecules, including polycystin-1. Laminin binding integrins (α3β1, α6β1, and α6β4) are expressed in the branching ureteric bud, whereas both laminin binding and collagen binding (α1β1 and α2β1) integrins are expressed in the differentiating collecting duct. Their ligands laminin-511 (α5β1γ1 or laminin 10) and laminin-332 (α3β3γ2 or laminin-5) are also expressed in the ureteric bud and the differentiated collecting duct. Integrin B4 and -β1 are overexpressed in cyst cells and may mediate their

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increased adhesion to laminin-322 and collagen, respectively, which are also overexpressed. Periostin, an extracellular matrix protein and its receptor αv integrin, as well as α1 and α2 integrins are also overexpressed in cystic tissues. Laminin-322 and periostin stimulate, whereas antibodies to laminin-322 and αv integrin inhibit cyst formation in three-dimensional gel culture. β1 integrin knockout and laminin α5 hypomorphic mutant mice develop renal cystic disease, the latter associated with overexpression of laminin-322.

The finding that cAMP signaling plays a role in the altered cell basement membrane interactions in PKD is a sound but untested hypothesis. Integrin binding to specific ligands induces clustering of integrin heterodimers and formation of a multiprotein complex, which includes G-proteins. Although integrins are not conventional GPCRs, they interact with heterotrimeric G proteins through non-canonical pathways to activate or inhibit cAMP signaling in an extracellular matrix- and cell type-specific manner (Figure 1). Integrins may also regulate the activity of PDEs in compartmentalized cAMP pools. Polycystin-1 and possibly, polycystin-2 could function in the focal adhesion complexes to regulate these PDEs pools, which is described in other subcellular compartment like the cilia. In turn, PKA and Epac signaling, downstream from cAMP, modulate integrin-mediated cell adhesion and adhesion-associated events, such as actin cytoskeletal dynamics and migration, which has described in other cells. For example, PKA and/or Epac promote adhesion of human vascular endothelial and vascular smooth muscle cells to fibronectin in an integrin-specific manner. It is, therefore, conceivable that increased cAMP signaling contributes to the increased adhesion to laminin-322 and collagen exhibited by cyst-derived cells.

INTERSTITIAL INFLAMMATION AND FIBROSIS

Almost three decades ago, the observation was made that a germ-free environment inhibits cyst development in CFWwd mice. This observation was confirmed in a chemical model of PKD induced by nordihydroguaiaretic acid. Administration of endotoxin rescued the cystic phenotype, which included marked inflammatory cell infiltrates in close opposition to the cysts. Chemo- kines and cytokines were found at high concentrations in cyst fluids and were found to be produced by cyst-lining epithelial cells. More recently, many alternatively activated macrophages aligned along cyst walls have been detected in polycystic kidneys from conditional Pkd1-knockout and Pkd2WS25/ mice. Macrophage depletion by intraperitoneal liposomal clodronate administration inhibits epithelial cell proliferation and cyst growth and improves renal function. These observations have led to the hypothesis that alternatively activated M2 macrophages contribute to cell proliferation in PKD, as has been described in cancer and during the recovery from AKI. After renal ischemia-reperfusion, macrophages infiltrate the kidney and undergo a phenotypic switch from classically activated proinflammatory M1 to alternatively activated M2 macrophages that promote tubular epithelial cell proliferation, tissue remodeling, and fibrogenesis. STAT3 activation in PKD, as previously described in cancer, plays a critical role in the development and maintenance of an inflammatory microenvironment. In cancer cells and likely, cyst-lining cells, activated STAT3 induces the transcription of cytokines, chemokines, and growth factors that, in turn, activate STAT3 on tumor-associated or M2 macrophages, which results in feed-forward loop between tumor or cyst-lining cells and tumor-associated or M2 macrophages (Figure 1). The trigger for the initial STAT3 activation in PKD is unclear. STAT3 is typically activated through phosphorylation at tyrosine 705 by intrinsic tyrosine kinase activity of activated growth factor receptors or cytokine receptor-associated Janus kinase, but GasPCR adenyl cyclase/cAMP/PKA signaling can also activate STAT3. For example, G-protein α-subunit–activating mutations in sporadic benign and malignant liver tumors are characterized by an inflammatory phenotype associated with STAT3 activation. The vasopressin V2 receptor agonist 1-deamino-8-d-arginine vasopressin increases, whereas absence of circulating vasopressin and the V2 receptor antagonist OPC-31260 inhibits STAT3 activation and cystogenesis in rodent models of PKD (J. Talbott and T. Weimbs, personal communication and unpublished observations). Therefore, upregulation of cAMP signaling may contribute to STAT3 activation and interstitial inflammation in PKD.

TREATMENT STRATEGIES TARGETING CAMP

The central role of cAMP in the pathogenesis of PKD provides a strong rationale for strategies to lower its levels in cystic tissues. Clinical trials of GasPCR antagonists (i.e., vasopressin V2 receptor antagonists) and GiPCR agonists (i.e., somatostatin analogs) have shown encouraging results.

V2 RECEPTOR ANTAGONISTS: RATIONALE AND PRECLINICAL TRIALS

Vasopressin acting on V2 receptors is the most powerful agonist for cAMP generation in freshly isolated collecting ducts. Nearly exclusive localization of V2 receptor (V2R) on collecting ducts, connecting tubules, and thick ascending limbs of Henle, the main sites of cystogenesis, minimizes off-target size effects and improves tolerability. Vasopressin is continuously present in the circulation, likely at a higher level in PKD to compensate for a urinary concentrating defect.

V2 receptor antagonists (mozavaptan and/or tolvaptan) attenuate the progression of PKD in pck mice and rodent models of nephronphthisis (pck mouse), ARPKD (PCP rat), and PKD-2 (Pkd2WS25/− mouse). Mozavaptan is also effective in a conditional Pkd1 knockout
when treatment is started early after gene deletion. Cyst development is markedly inhibited in PK rats lacking circulating vasopressin (generated by crosses of PK and Brattleboro rats), whereas administration of the V2R agonist 1-deamino-8-d-arginine vasopressin fully rescues the cystic phenotype. Satavaptan (V2R antagonist) blocks tubular expression of secreted frizzled related protein 4 that is overexpressed in polycystic kidneys and promotes cystogenesis of zebrafish pronephros. Tolvaptan also inhibits vasopressin-induced cell proliferation, chloride secretion, and in vitro cyst growth of human ADPKD cells.

CLINICAL TRIALS OF V2R ANTAGONISTS IN ADPKD

Preliminary dosing studies showed that two times per day administration of tolvaptan was necessary to maintain urine hypotonicity (a surrogate for V2R blockade) throughout a 24-hour period. Two phase 2, open-label, uncontrolled, 3-year clinical trials ascertained its long-term safety and tolerability. Forty-six patients in the United States were randomized to one of two daily split doses (45/15 and 60/30 mg) of tolvaptan chosen after an analysis of efficacy (Uosm persistently <300 mOsm/kg in 70% and 77% of patients, respectively) and self-reported tolerability (for rest of life in 96% and 61% of patients, respectively) during a dose escalation phase (Figure 2). Seventeen patients in Japan received a 15/15-mg split dose. Adverse events were mainly related to the aquaretic effect; 12 of 63 (19%) patients withdrew from the study, and, in six cases, the withdrawal was because of adverse events. Changes in kidney volume (determined by magnetic resonance imaging [MRI] and estimated GFR) were compared with historical controls from the Consortium of Radiologic Imaging Study of PKD and the Modification of Diet in Renal Disease studies. Kidney volume increased 5.8% versus 1.7% per year. Annualized estimated GFR declined −2.1 versus −0.71 ml/min per 1.73 m² per year. Limitations of the study were the small number of patients and the use of non-contemporary controls with unmatched ethnicities.

Because slight elevations in serum creatinine, rapidly reversible after halting tolvaptan administration, were observed in the phase 2 clinical trials, 20 ADPKD patients underwent renal clearance and MRI studies before and after the 45/15-mg split daily dose of tolvaptan for 1 week. Tolvaptan-induced aquarexis was accompanied by significant reduction in iothalamate clearance and increase in serum uric acid caused by decreased uric acid clearance without change in renal blood flow. Post hoc, blinded analysis of renal MRIs showed a 3.1% reduction in kidney volume and the volume of individual cysts, which was likely because of an acute effect on fluid secretion.

The results of a phase 3, global, multicenter, randomized, double-blind, placebo-controlled, parallel-arm trial of tolvaptan in ADPKD (Tolvaptan Efficacy and Safety in Management of ADPKD and its Outcomes 3:4 [TEMPO3:4]; NCT00428948) have been recently published. ADPKD subjects (n=1445) with rapid disease progression reflected by kidney volumes of at least 750 ml at a relatively young age between 18 and 50 years, but still having preserved renal function (estimated creatinine clearance >60 ml/min), were randomized two to one with tolvaptan to placebo. Daily split 45/15-mg doses were titrated at weekly intervals to 60/30 and 90/30 mg. The maximally tolerated dose was maintained for 3 years. Participants were instructed to drink enough water to prevent thirst. Serum creatinine and laboratory parameters were measured every 4 months, and renal MRIs were obtained yearly; 23% of tolvaptan-treated subjects withdrew from the trial, with 15% of subjects withdrawing because of adverse events (including aquarexis-related symptoms in 8%). Comparatively, 14% of placebo subjects.

Figure 2. Tolerability and efficacy during the titration phase of TEMPO2:4. In the initial 2 months of the TEMPO2:4 study, a split-dose regimen of oral tolvaptan (8:00 AM/4:00 PM) was uptitrated (15/15, 30/15, 45/15, 60/30, and 90/30 mg/d) until tolerability was reached. Tolerability was defined as self-reported tolerance of a specific dose regimen by responding yes to the question: “Could you tolerate taking this dose of tolvaptan for the rest of your life?” Efficacy was defined by the capacity to suppress the action of vasopressin on the kidney reflected by sustained urine hypotonicity (Uosm<300 mOsm/kg). Reprinted from Higashihara et al., with permission.

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withdrew from the trial, with 5% of subjects withdrawing because of adverse events (including aquareasis-related symptoms in 0.4%). Of the subjects randomized to tolvaptan completing 3 years of treatment, 24%, 21%, and 55% were tolerating 45/15, 60/30, and 90/30 mg, respectively, at the end of the study; 17% of subjects randomized to placebo were unable to tolerate the 90/30-mg dose.

Tolvaptan reduced the rate of kidney growth by 50% (from 5.5% to 2.8% per year) (Figure 3A). The treatment effect was greatest from baseline to year 1, but also, it was significant from year 1 to year 2 and from year 2 to year 3. The analysis of time to development or progression of multiple clinical events (worsening kidney function, severe kidney pain, hypertension, and albuminuria) showed fewer clinical events for tolvaptan-treated subjects. Tolvaptan reduced the rate of kidney growth by 50% (from 5.5% to 2.8% per year) (Figure 3A). The treatment effect was greatest from baseline to year 1, but also, it was significant from year 1 to year 2 and from year 2 to year 3. The analysis of time to development or progression of multiple clinical events (worsening kidney function, severe kidney pain, hypertension, and albuminuria) showed fewer clinical events for tolvaptan compared with placebo, with a hazard ratio of 0.87 (95% confidence interval, 0.72 to 0.94). This result was driven by a 61% lower risk of 25% reductions in reciprocal serum creatinine and a 36% lower risk of kidney pain events (Figure 3B). Tolvaptan also reduced the rate of decline of reciprocal serum creatinine from 3.81 to 2.61 per year (Figure 3C).

Frequencies of adverse events were similar in both groups; those events related to aquareasis, such as polyuria, thirst, and nocturia, were more common in the tolvaptan group, whereas those events related to ADPKD, such as kidney pain, hematuria, and urinary tract infection, were more common in the placebo group. Increases in serum sodium and uric acid were more frequently seen in tolvaptan-treated subjects. Tolvaptan-treated subjects had more frequent elevations of liver enzymes exceeding three or five times the upper limits of normal, leading to discontinuation of tolvaptan in 1.8% of subjects.

At the present time, tolvaptan is not approved for the indication of ADPKD and should not be administered outside of an approved research study. Its value and the outcome of those trials that have been completed are summarized in Table 2. The published studies have shown similar results. Kidney growth is halted during the first year of treatment and then resumes, possibly at a lower rate than without treatment. Liver volume decreases by 4%–6% during the first year of treatment, and this reduction is sustained during the second year. Observation periods have been too short to assess an effect on renal function. The addition of everolimus to treatment with octreotide does not provide added benefit. Octreotide and lanreotide are overall well tolerated. Self-resolving abdominal cramps and loose stools are common in the first few days after the injections. Other adverse effects include injection site granuloma and pain, cholelithiasis, steatorrhea, weight loss, and rarely, hair loss. The adverse event profile of pasireotide in patients with ADPKD may include hyperglycemia, because pasireotide inhibits insulin more taking tolvaptan should be able to maintain adequate hydration. Levels of plasma sodium, uric acid, and liver enzymes should be monitored. Patients in TEMPO3:4 had relatively preserved renal function. Efficacy in more advanced stages of the disease has not been thoroughly ascertained.

**Somatostatin Analogs: Rationale and Preclinical Trials**

Somatostatin acts on five GPCRs (SSTR1 to 5). Binding to these receptors inhibits AC and mitogen-activated protein kinase, cell proliferation, and secretion of several hormones (growth hormone, insulin, glucagon, gastrin, cholecystokinin, vasoactive intestinal peptide and secretion of thyroid stimulating hormone, and adrenocorticotropic hormone) and growth factors (IGF I and vascular endothelial growth factor). All five SSTRs are expressed in renal tubular epithelial cells and cholangiocytes. SSTR1 and 2 are expressed in the thick ascending limb of Henle, distal tubule, and collecting duct, and SSTR3, -4, and -5 are expressed in proximal tubules.

Somatostatin inhibits cAMP generation in MDCK cells and rat collecting ducts and antagonizes vasopressin effects in the toad urinary bladder and dog collecting ducts. All five SSTRs are expressed in renal tubular epithelial cells and cholangiocytes. SSTR1 and 2 are expressed in the thick ascending limb of Henle, distal tubule, and collecting duct, and SSTR3, -4, and -5 are expressed in proximal tubules.

Because somatostatin has a half-life of approximately 3 minutes, more stable synthetic peptides (octreotide, lanreotide, and pasireotide) have been developed for clinical use. They differ in stability and affinity to the different SSTRs. Half-lives in the circulation are 2 hours for octreotide and lanreotide, and 12 hours for pasireotide. Octreotide and lanreotide bind with high affinity to SSTR2 and -3, bind with moderate affinity to SSTR5, and have no affinity to SSTR1 and -4. Pasireotide binds with high affinity to SSTR1, -2, -3, and -5.

In preclinical studies, octreotide and pasireotide reduce cAMP levels and proliferation of cholangiocytes in vitro, expansion of liver cysts in three-dimensional collagen culture, and development of kidney and liver cysts and fibrosis in PCK rats and Pkd2<sup>W525C</sup><sup>−</sup> mice. In agreement with the longer half-life and higher affinity to a broader range of SSTRs, the effects of pasireotide are consistently more potent than the effects of octreotide.
potently than glucagon secretion, whereas the contrary is true for octreotide and pasireotide. Currently, ongoing studies of longer duration with a larger number of patients are needed to better ascertain the potential long-term benefit of somatostatin analogs in ADPKD and PKD.

**HIGH WATER INTAKE IN ADPKD**

Another way to decrease the effect of arginine vasopressin (AVP) on the kidney is to suppress its secretion by increasing fluid ingestion and continuously maintaining the osmolality of the urine < 250–300 mOsm/kg H$_2$O. This strategy has been tested directly in the PCK rat, where high fluid intake sufficient to achieve a 3.5-fold increase in urine output by adding 5% glucose to drinking water reduced AVP excretion, renal expression of V2R, cAMP-dependent activation of the B-Raf/MEK/ERK, and attenuation of PKD. It must be noted that the effects of increased water intake and V2R antagonists are not equivalent, even if they both reduce cAMP in the distal nephron and collecting ducts. Increased hydration decreases, whereas the administration of a V2R antagonist increases the circulating levels of vasopressin. Compliance with increased hydration to achieve persistent urine hypotonicity is difficult to achieve, may increase the risk for hyponatremia, and may be less effective in blocking V2R-mediated signaling. However, the administration of a V2R antagonist increases the risk for dehydration, hyperuricemia, and theoretically, nonspecific effects mediated by increased...

**Figure 3.** Effect of tolvaptan on primary (change in kidney volume) and secondary (time to multiple events of ADPKD and change in kidney function) results. (A) Slopes of total kidney volume growth (percent change from baseline). (B) Hazard ratio effects of tolvaptan on composite time to multiple events of ADPKD and its components. (C) Slopes of kidney function estimated by reciprocal serum creatinine. TOL denotes tolvaptan (blue). PBO denotes placebo (red). Reprinted from Torres et al., with permission.

**Table**: Events/100

<table>
<thead>
<tr>
<th>Endpoint/endpoint component</th>
<th>Group</th>
<th>Subjects</th>
<th>Events</th>
<th>Years</th>
<th>P-value</th>
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<tr>
<td>ADPKD composite</td>
<td>TOL</td>
<td>961</td>
<td>1049</td>
<td>44</td>
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<td></td>
<td>PBO</td>
<td>483</td>
<td>665</td>
<td>50</td>
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<tr>
<td>Worsening hypertension</td>
<td>TOL</td>
<td>961</td>
<td>734</td>
<td>31</td>
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<tr>
<td></td>
<td>PBO</td>
<td>483</td>
<td>426</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Worsening albuminuria</td>
<td>TOL</td>
<td>961</td>
<td>195</td>
<td>8</td>
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<tr>
<td></td>
<td>PBO</td>
<td>483</td>
<td>103</td>
<td>8</td>
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<tr>
<td>Kidney pain requiring intervention</td>
<td>TOL</td>
<td>961</td>
<td>113</td>
<td>5</td>
<td>0.007</td>
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<tr>
<td></td>
<td>PBO</td>
<td>483</td>
<td>97</td>
<td>7</td>
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<tr>
<td>Worsening kidney function</td>
<td>TOL</td>
<td>918</td>
<td>44</td>
<td>2</td>
<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>PBO</td>
<td>476</td>
<td>64</td>
<td>5</td>
<td></td>
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</table>

Hazard ratio for event(s) (95% confidence intervals)
<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Entry Criteria/Sample Size</th>
<th>Baseline Values</th>
<th>Status/Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergamo phase 2</td>
<td>Octreotide; R/DB/PC/CO; 6 mo</td>
<td>≥18 yr; SCR 1–3 mg/dl; 14 (ADPKD)</td>
<td>KV; GFR; LV</td>
<td>KV: 71±10.7 ml (O) versus 162±114 ml (P), P&lt;0.05; no change in GFR; LV: −71±57 ml (O) versus +14±85 ml (P), P&lt;0.05</td>
</tr>
<tr>
<td>LOCKCYST phases 2 and 3 (NCT00565097)</td>
<td>Lanreotide; R/DB/PC; 6 mo</td>
<td>≥18 yr; &gt;20 liver cysts; 54 not on dialysis (32 ADPKD, 22 PLD)</td>
<td>LV (MRI); KV; QOL</td>
<td>LV: −2.9% (L) versus +1.6% (P); P=0.01; KV: −1.5% (L) versus +3.4% (P), P=0.02; improved health perception</td>
</tr>
<tr>
<td>LOCKCYST extension</td>
<td>Lanreotide; OL; 12 mo</td>
<td>Same; 41 (25 ADPKD, 16 PLD)</td>
<td>KV (MRI); QOL</td>
<td>LV: −4%; KV: −1%; 6 mo after discontinuation of L, LV: +4%; KV: +2%</td>
</tr>
<tr>
<td>Mayo PLD1 phases 2 and 3 (NCT00426153)</td>
<td>Octreotide; R/DB/PC; 24 mo</td>
<td>≥18 yr; LV&gt;4000 ml or symptoms; 42 (34 ADPKD; 8 PLD)</td>
<td>LV (MRI); KV; QOL</td>
<td>LV: −4.95% (O) versus +0.92% (P), P&lt;0.05; KV: +0.25% (O) versus +8.61% (P), P&lt;0.05; GFR: −5.1 (O) versus −7.1 ml/min per 1.73 m² (P), NS; improved perception of bodily pain and physical activity scores</td>
</tr>
<tr>
<td>Mayo PLD1 extension</td>
<td>Octreotide; OL; ongoing</td>
<td>Same; 41</td>
<td>KV (MRI); QOL</td>
<td>On O for 2 yr; LV: −5.78%; KV: +6.49%; after change from P to O at 2 years: LV: −7.66%; KV: −0.41%</td>
</tr>
<tr>
<td>ELATE phase 2</td>
<td>Octreotide + sirolimus versus octreotide; R/OL; 12 mo</td>
<td>18–70 yr; &gt;20 liver cysts; eGFR=60 ml/min per 1.73 m²; 44 (29 PLD, 15 ADPKD)</td>
<td>LV (CT); QOL</td>
<td>LV: −3.5% (O) versus −3.8% (O + S); no difference in KV change or gastrointestinal symptoms or general health questionnaires</td>
</tr>
<tr>
<td>ALADIN phase 3</td>
<td>Octreotide; R/SB/PC; 36 mo</td>
<td>18–75 yr; eGFR=40 ml/min per 1.73 m²; 78 ADPKD</td>
<td>KV (MRI)</td>
<td>Completed; results pending</td>
</tr>
<tr>
<td>ALADIN 2 phase 3</td>
<td>Octreotide; R/DB/PC; 36 mo</td>
<td>18–75 yr; eGFR=15–40 ml/min per 1.73 m²; 80 ADPKD</td>
<td>KV (CT); GFR</td>
<td>2011–2015; recruiting</td>
</tr>
<tr>
<td>RESOLVE (NCT01354405)</td>
<td>Lanreotide; treated cohort; 6 mo</td>
<td>18–70 yr; &gt;20 liver cysts; eGFR&gt;40 ml/min per 1.73 m²; 43</td>
<td>LV (CT); KV</td>
<td>2011–2012; recruiting</td>
</tr>
<tr>
<td>DIPAK phase 3</td>
<td>Lanreotide; R/OL; 32 mo</td>
<td>18–60 yr; eGFR=30–60 ml/min per 1.73 m²; 300</td>
<td>eGFR; LV; QOL</td>
<td>2012–2017; recruiting</td>
</tr>
<tr>
<td>Mayo PLD2 phase 2</td>
<td>Pasireotide; R/DB/PC; 36 mo</td>
<td>≥18 yr; LV&gt;4000 ml or symptoms</td>
<td>48; LV (MRI); KV; QOL</td>
<td>2012–2016; recruiting</td>
</tr>
</tbody>
</table>

R, randomized; DB, double-blind; PC, placebo controlled; CO, crossover; SCR, serum creatinine; KV, kidney volume; LV, liver volume; O, octreotide; P, placebo; PLD, polycystic liver disease; QOL, quality of life; L, lanreotide; OL, open label; NS, not significant; eGFR, estimated GFR; CT, computed tomography; S, sirolimus.

*Footnote.
endogenous vasopressin levels through V1a and V1b receptors. A small, prospective, case-controlled, 1-year clinical trial of high water intake (NCT01348035) is in progress. Lacking clinical trial results, recommendations for water intake should rest on a critical analysis of potential benefits (reduction in the rate of disease progression) and risks (hypotension if solute intake or GFR is too low or if drugs limiting the ability to dilute the urine are administered, as well as negative effects of polyuria or nocturia). Patients with ADPKD are capable to dilute the urine even at moderately advanced stages of the disease. Therefore, those patients with normal or moderately reduced GFR who follow a diet not severely restricted in protein (<0.6 g/kg ideal body wt per day) or sodium (<60 mEq/d), are not edematous or volume contracted, do not take medications that interfere with the reabsorption of sodium chloride in diluting segments of the nephron (e.g., loop diuretics or thiazides) or enhance the release or effect of AVP (e.g., serotonin reuptake inhibitors and tricyclic antidepressants), and have normal voiding mechanics can handle moderate increases in urine volume (4 L daily) without untoward effect. A reasonable goal is to drink fluids as evenly as possible throughout waking hours and immediately before going to bed. More than likely, patients will experience nocturia at least one time, and, if tolerated, water should be drunk after voiding. Monitoring plasma sodium concentration should be advised. The intake should be of nonmineralized water, with no addition of sugar or caffeine.

**Other Treatment Targets in PKD**

The identification of the genes mutated in ADPKD and ARPKD in 1994, 1996, and 2002 has made possible a greater understanding of the cellular pathophysiologic mechanisms and laid the foundation for potential therapies (Table 3). In addition to vasopressin V2 receptor antagonists and somatostatin analogs, mTOR (sirolimus and everolimus), Src (botulinum), and mutityrosine kinase (KD019) inhibitors have been or are currently tested in clinical trials. Others have been effective in preclinical studies. In summary, advances in molecular biology and genetics have made possible greater understanding of cellular pathophysiologic mechanisms responsible for the development and progression of PKD and laid the foundation for the development of potential new therapies. Most 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. Many therapies have proven efficacious in animal models of PKD but may be limited by toxicity. Because effective treatments for PKD are likely to be long term (possibly lifelong), low toxicity and safe profile are of the utmost importance. Therapies targeting Gs (tolvaptan) or Gi (somatostatin analogs) protein–coupled hormonal receptors with relative tissue and cell specificity have been used in clinical trials with relative safety. Repurposing of drugs with a relatively safe profile that are currently used for other indications (e.g., metformin and peroxisome proliferator–activated receptor γ agonists) deserves attention. Identification of synergisms between different classes of drugs may increase their efficiency and safety. Not all patients with PKD will require treatment. Patient selection and determination of the optimal timing for intervention deserve consideration. After the long wait, ADPKD may become a treatable disease.

### Table 3. Therapeutic targets for PKD supported by preclinical trials

<table>
<thead>
<tr>
<th>Category</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium signaling</td>
<td>Polycystin-2 channel activator</td>
</tr>
<tr>
<td></td>
<td>TRPV4 channel activator</td>
</tr>
<tr>
<td></td>
<td>Calcium-sensing receptor activator</td>
</tr>
<tr>
<td>cAMP signaling</td>
<td>Vasopressin V2 receptor antagonist</td>
</tr>
<tr>
<td></td>
<td>Somatostatin receptor agonist</td>
</tr>
<tr>
<td></td>
<td>PGE2 receptor (EP2 or EP4) antagonist</td>
</tr>
<tr>
<td></td>
<td>Catechol-O-methyl transferase</td>
</tr>
<tr>
<td></td>
<td>Phosphodiesterase activator</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>Receptor tyrosine kinase inhibitor (Erbb1, ErbB2, IGF1, VEGF, cMET)</td>
</tr>
<tr>
<td></td>
<td>Nonreceptor tyrosine kinase inhibitor (Src)</td>
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<td>Serine-threonine kinase inhibitor (B-Raf, MEK1/2, p38MAPK, mTOR) or activator (AMPK)</td>
</tr>
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<td>Transcription factor agonists (PPARγ) or inhibitors (STAT3, STAT6)</td>
</tr>
<tr>
<td></td>
<td>Histone deacetylase (HDAC1, HDAC5, HDAC6) inhibitors</td>
</tr>
<tr>
<td></td>
<td>Cyclin-dependent kinase and Cdk25 phosphatase inhibitors</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Caspase inhibitors</td>
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<td>Fluid secretion</td>
<td>CFTR channel blocker</td>
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<td>KCa3.1 channel blocker</td>
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<tr>
<td>Other mechanisms</td>
<td>Proteasome inhibitors</td>
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<tr>
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<td>Glycosyl ceramide synthase inhibitors</td>
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<td>Lovastatin</td>
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<tr>
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<td>ACE inhibitors</td>
</tr>
<tr>
<td></td>
<td>20-HETE synthase inhibitors</td>
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<td></td>
<td>Protein restriction</td>
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<tr>
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<td>Soy, flax diets</td>
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<td>Citrate</td>
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</table>

References available on request. VEGF, vascular endothelial growth factor; B-Raf, B rapidly accelerated fibrosarcoma kinase; MAPK, mitogen-activated protein kinase; AMPK, AMP kinase; PPARγ, peroxisome proliferator–activated receptor γ; ACE, angiotensin-converting enzyme.

**DISCLOSURES**

None.
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