Department of Defense Peer Reviewed Medical Research Program

Investigational Medicinal Product
Metformin

CLINICAL PROTOCOL

TAME-PKD: Trial of Administration of Metformin – Autosomal Dominant Polycystic Kidney Disease

CONFIDENTIAL – PROPRIETARY INFORMATION

Version 8
Date: June 1, 2018

Table of Contents

List of Abbreviations:

ACC	acetyl co-enzyme A carboxylase
ACE-I	angiotensin-converting-enzyme inhibitor
ADPKD	autosomal dominant polycystic kidney disease
AKI	acute kidney injury
AMPK A	AMP-activated protein kinase
ARB	angiotensin II receptor blocker
BP	blood pressure
bid	twice daily
BUN	blood urea nitrogen
CBC	complete blood count
CDMRP	Congressionally Directed Medical Research Programs
CRHC-DC	Center for Research on Health Care Data Center
CKD	chronic kidney disease
CKDepi	Chronic Kidney Disease Epidemiology Collaboration
CFTR	cystic fibrosis transmembrane conductance regulator
CRISP	Consortium for Radiologic Imaging Studies of PKD
CTRC	Clinical and Translational Research Center at Tufts
DCC	data coordinating center
DCIAC	Data Coordinating and Image Analysis Center
DSMB	data and safety monitoring board
ESC	evaluation of signed consent
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rates
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assays
ESRD	end-stage renal disease
GCRC	General Clinical Research Center at UMMC
GSRS	gastrointestinal symptoms rating scale
HRQOL	health-related quality of life

Ht	height adjusted
IAC	Imaging Analysis Center
IRB	institutional review board
ITT	intention-to-treat
KCV	kidney cyst volume
LCV	liver cyst volume
LDHA	lactate dehydrogenase A
MOP	manual of procedures
MRI	magnetic resonance imaging
mTOR	mammalian target of rapamycin
p70S6K	p70 ribosomal protein 6 kinase
PCC	participating clinical center
PCOS	polycystic ovary syndrome
PDK1	pyruvate dehydrogenase kinase 1
PI	principal investigator
PKD1	polycystin 1
PKD2	polycystin 2
PKDF	Polycystic Kidney Disease Foundation
PKM2	pyruvate kinase M2 isoform
p.o.	orally
RCT	randomized clinical trial
SAE	serious adverse events
SF-36	Medical Outcomes Short Form 36
TCA	tricarboxylic acid
TKV	total kidney volume
TLV	total liver volume
UMMC	University of Maryland Medical Center

INTRODUCTION

BACKGROUND

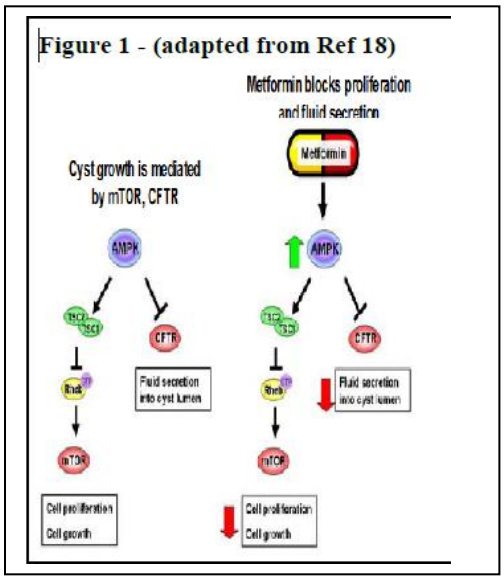
A. ADPKD Background and Pathogenesis: Autosomal dominant polycystic kidney disease (ADPKD) affects 1:700 – 1:1,000 individuals, making it the most commonly inherited kidney disease. Over time, half of ADPKD patients will progress to end-stage renal disease (ESRD). To date, no targeted therapies have been shown to safely and effectively halt progression of ADPKD. ADPKD is caused by a mutation in the *PKD1* or *PKD2* genes, which encode polycystin 1 (PKD1) and polycystin 2 (PKD2), respectively, two proteins that together form a receptor channel complex. ADPKD can be considered, in large part, as a disease in which normally non-proliferating and absorptive cells of the kidney tubule take on a proliferative and largely secretory phenotype, manifesting as progressive cyst development. The pathological processes that produce enlarged cysts are thought to result from specific cellular abnormalities: 1) increased fluid secretion into the cyst lumen; and 2) inappropriately increased cell proliferation of the cyst epithelium (1).

Grantham and colleagues demonstrated that the rate of fluid secretion into the lumen of cultured ADPKD cysts is proportional to the quantity of cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel present at the apical membranes of the cyst-lining epithelial cells (2). These data are consistent with the hypothesis that active fluid secretion plays a critical role in driving cyst growth. The evidence that CFTR acts as a significant contributor to cyst growth has led to preclinical explorations of the utility of CFTR-inhibitors in the treatment of ADPKD (3, 4).

A number of groups have also demonstrated increased proliferation rates in the cells of ADPKD cysts using cell lines derived from animal models (5). These observations suggest that secretion does not account for the entire process of cyst enlargement. Rather, the cyst lining cells have a hyper-proliferative phenotype (6), which results in expansion of the cyst wall surface area required to enclose the increasing cyst volume. Intriguingly, it has been shown that mammalian target of rapamycin (mTOR) activity is elevated in murine models of PKD (7). mTOR, a serine/threonine kinase that regulates cell growth and proliferation, transcription, and protein synthesis, is inhibited by rapamycin (8, 9). Indeed, treatment with rapamycin (sirolimus) has been shown to improve renal cystic indices in mouse and rat models of ADPKD (10).

B. Prior Relevant Clinical Trials for ADPKD: Because of the promising results obtained in rodent ADPKD models, the mTOR inhibitors sirolimus and everolimus were recently tested in patients for the treatment of ADPKD (11, 12). However, these studies did not show significant benefit and there were dose-limiting side effects in the drug treatment groups that may have diminished their effectiveness. In the TEMPO study the vasopressin receptor 2 antagonist tolvaptan slowed the rate of GFR loss by 30% and decreased the growth of total kidney volume (TKV) over three years (2.8% per year vs. 5.5% per year in the placebo arm) (13). However, the tolvaptan group had an increased risk for hepatotoxicity, which led the FDA to issue a black box warning against its use in patients with liver disease and use for more than 30 days. In addition, there were tolerability problems caused by polyuria, which led to a significantly greater percentage of discontinuation of the study in the tolvaptan group (23% vs. 14% of placebo patients). On April 24, 2018, the FDA approved Tolvaptan as a medication to slow kidney decline in some adults with ADPKD, specifically those at risk for rapid progression. However, patients electing such treatment will be required to register in a FDA-mandated Risk Evaluation and Mitigation Strategy (REMS) including frequent laboratory monitoring for liver toxicity. There remains an unmet need for additional therapeutic options to reduce progression in ADPKD that would be safe and well-tolerated.

C. Preclinical Data with Metformin: There is growing evidence that metformin, a drug widely used for the treatment of type 2 diabetes and polycystic ovary syndrome, may serve as a novel therapy for individuals in the early stages of ADPKD by activating the metabolic sensor AMP-activated protein kinase (AMPK). AMPK is activated under conditions of metabolic and other cellular stresses. Through its actions on downstream mediators, AMPK activation during low energy states decreases cellular energy consumption while stimulating energy generating pathways. We have shown that AMPK phosphorylates and inhibits CFTR, thus suppressing epithelial fluid and electrolyte secretion (14, 15). Similarly, AMPK phosphorylates the tuberin protein, leading to indirect inhibition of the mTOR pathway. Thus, AMPK inhibits both CFTR (15) and mTOR (16), suggesting that targeted activation of this kinase by metformin may provide a therapeutic benefit in ADPKD (17). We have shown that metformin treatment of kidney epithelial cells leads to stimulation of AMPK and subsequent inhibition of both mTOR and CFTR activity. We have also shown that metformin slows cystogenesis in animal models of PKD, supporting the potential of this drug in ADPKD treatment (18) (see **Fig. 1**).



D. Byproducts of Perturbed Metabolism as Biomarkers of PKD Progression and Therapeutic Efficacy: One of the major challenges in the field of PKD research is that monitoring disease severity, progression over time and the potential response to novel therapies is challenging because the disease is slowly progressive over decades with patients often remaining asymptomatic for extended periods. Moreover, besides serum creatinine, which is often a late marker of significant renal function decline, current monitoring methodologies involve expensive kidney imaging procedures like serial volumetric MRI measurements. Thus, the availability of biomarkers that are informative, reliable, inexpensive and widely available would be invaluable for the management of patients with ADPKD. This need for diagnostic and prognostic biomarkers will become increasingly important with new potential drug therapies for ADPKD on the horizon, such as metformin proposed in this application and others that are currently being tested in clinical trials. To date, there have been several recent studies investigating potential urinary biomarkers for ADPKD, including a recent multi-center trial that identified a negative correlation between peptides largely derived from collagen and other structural proteins and height-adjusted total kidney volume (and thus disease severity) (19) and another that performed NMR spectroscopic small molecule fingerprinting of the urine in a cross-section of ADPKD patients and healthy controls (20). However, major limitations of such biomarkers are their reliance on the monitoring of multiple simultaneous biomarkers (i.e., patterns) using sophisticated analytic equipment, their frequently lower sensitivity and specificity early in disease, and their ability to provide useful prognostic information.

Recent work by Boletta and colleagues has suggested that the *Pkd1* mutation in kidney cells alters energy metabolism and oxygen sensing by inducing aerobic glycolysis and inhibiting oxidative metabolism (the “Warburg effect” seen in many tumor cells (21)), which in turn has the deleterious effect of inhibiting AMPK activity in ADPKD (22). Of note, studies derived from the cancer literature suggest that metformin treatment may prove beneficial in cancer therapy, and one potential

mechanism behind its beneficial effect may be via reversal of the Warburg effect on metabolism. AMPK has been shown to negatively regulate the Warburg effect and tumor growth in vivo (23). Moreover, metformin decreased HIF-1α levels in a breast cancer model (24), exhibiting an antiproliferative, anti-Warburg effect, probably via AMPK activation. Metformin also inhibited cell growth and division by inhibiting expression of fatty acid synthase (25) and favoring fatty acid oxidation in colon cancer cells (26). In summary, based on the recent literature including our own preclinical work (18), we hypothesize that metformin will slow cyst growth in ADPKD and may improve dysregulated metabolism in the disease. Moreover, as metformin activates AMPK, targeting this fundamental ADPKD disease mechanism of dysregulated metabolism, we believe that the identification of metabolomic biomarkers will be particularly useful in the setting of metformin treatment in human patients.

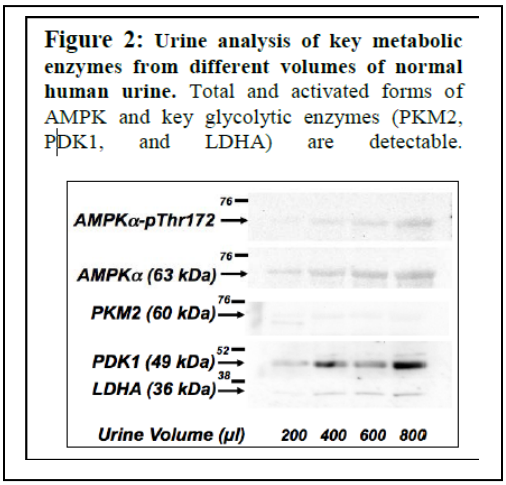
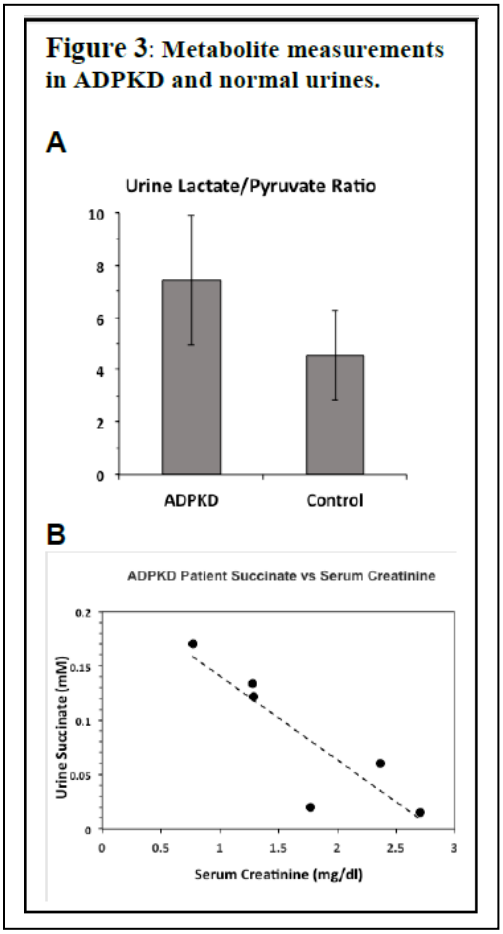


Figure 2: Urine analysis of key metabolic enzymes from different volumes of normal human urine. Total and activated forms of AMPK and key glycolytic enzymes (PKM2, PDK1, and LDHA) are detectable.

E. Preliminary Metabolomic Biomarker Data: We will measure activation of the AMPK pathway in urine as a potential readout of metformin effects in patients by quantitating the band densities on immunoblots corresponding to the ratios of phosphorylated to total AMPK, acetyl-CoA carboxylase (ACC), and P70 ribosomal S6 kinase (P70S6K). Regarding biomarkers, we will measure the following glycolytic enzymes: lactate dehydrogenase A (LDHA), pyruvate dehydrogenase kinase 1 (PDK1), and pyruvate kinase M2 isoform (PKM2), as the levels of each of these enzymes appeared to positively correlate with disease severity in human patients and mice (22). We can detect these enzymes in the urine of control human subjects via immunoblots performed following methanol-chloroform precipitation (**Fig. 2**). For the studies proposed in this application, however, we will utilize more sensitive and quantitative commercially available enzyme-linked immunosorbent assays (ELISAs) to quantitate their levels in urine (normalized by creatinine concentration) to compare across patients and at various treatment times within patients, as described in Aim 2. In addition, we will use colorimetric enzymatic activity assay kits to quantitate levels of various key glycolytic and oxidative metabolites (lactate, pyruvate, succinate and cAMP). Interestingly, preliminary measurements of the lactate/pyruvate ratio and succinate levels in several fasting urine specimens obtained from a cross-section of ADPKD patients enrolled in the Baltimore PKD Research and Clinical Core Center suggest that the lactate/pyruvate ratio, indicative of enhanced glycolytic flux, may be elevated in ADPKD patients as compared with normal volunteers (albeit significant intersample variability exists) (**Fig. 3A**). Conversely, urine succinate levels (a marker of oxidative metabolism via the TCA cycle) appear to fall with rising serum creatinine (i.e., worsening renal function) (**Fig. 3B**). Thus, our preliminary data measuring metabolomic profiles in urine from ADPKD patients not only support the feasibility of these measurements in our hands, but are consistent with a metabolic reprogramming from mitochondrial oxidative respiration to aerobic glycolysis in ADPKD.



F. Synergy of this Study with Preclinical Work: We expect that our efforts may allow us to identify biomarkers whose levels can be used as indicators that the pathways targeted by metformin have been successfully modulated and may correlate with ADPKD disease severity and progression over time. Of note, one of the co-PIs of this proposal (Hallows) is also a partnering PI on a currently proposed Department of Defense Investigator-Initiated grant application to study metformin along with other AMPK activators in preclinical models, which include ADPKD 3-dimensional cell cultures and a progressive disease model in mice. In addition to screening mouse kidney tissue and urine samples for the same biomarkers to be assayed in this clinical study, a variety of additional glycolytic, TCA cycle, amino acid and nucleotide metabolites will be assayed by mass spectrometry in a targeted platform in collaboration with S. Pennathur at the Univ. of Michigan (see attached letter and biosketch). Any novel pharmacodynamic biomarker targets identified through the work in this preclinical study could also be interrogated in banked urine samples from this study on patients treated with the study drug over time to test whether new biomarker correlations with metformin therapy identified in animal models are also observed in ADPKD patients during experimental metformin therapy. Successful completion of this work could thus produce an additional justification to support bringing metformin and other new drugs to the later stages of drug development and reveal biomarkers that will be valuable in future larger clinical trials.

G. Metformin Safety: Metformin has an extensive and long track record for safety. Originally described in 1922, the first clinical trial for metformin occurred in 1957 in patients with diabetes. This biguanide drug that decreases hyperglycemia has subsequently become a worldwide, first-line treatment for type 2 diabetes mellitus as well as a treatment for polycystic ovary syndrome. The most common side effect is gastrointestinal distress, but this can be mitigated by titration of the dose. Moreover, unlike other diabetic agents, it generally does not cause hypoglycemia (27, 28). Metformin, which is not metabolized, gets cleared by the kidneys. While metformin treatment may induce lactic acidosis in patients with severely decreased renal function, this risk is exceedingly rare for patients with an eGFR >30 ml/min (29, 30).

HYPOTHESIS AND SPECIFIC AIMS

Based on our preclinical data as well as the literature, we hypothesize that metformin, an agent that is widely used to treat type 2 diabetes mellitus and polycystic ovary syndrome, may serve as a novel therapy for individuals with early stage ADPKD by activating the metabolic sensor AMPK.

Aim 1: We will perform a double-blind, multicenter parallel-group randomized clinical trial to assess tolerability and safety of treating ADPKD patients with early disease (eGFR ≥50 ml/min) with metformin.

We hypothesize that metformin, an agent that is widely used to treat type 2 diabetes mellitus and polycystic ovary syndrome, may serve as a novel therapy for individuals with early stage autosomal dominant polycystic kidney disease (ADPKD) by activating the metabolic sensor AMPK. To test this hypothesis, we propose a pilot-level double-blind, phase 2, parallel-group, placebo-controlled multicenter clinical trial to evaluate the safety and tolerability of metformin in ADPKD patients over a two-year period. In addition, we will evaluate the effect of metformin therapy on the rate of change in height-adjusted total kidney volume (htTKV) and estimated glomerular filtration rate (eGFR) as well as on metabolomic biomarkers of disease severity and progression. We will enroll 96 adult patients, ages 18-60 years with ADPKD, and eGFR ≥50 ml/min/1.73m^2. We will restrict enrollment to individuals with eGFR ≥50 cc/min/1.73m^2 in order to avoid excess risk of metformin adverse events that might be associated with more advanced renal impairment. In addition, interventions are more likely to be effective if applied early in ADPKD, before there is irreversible renal scarring, as is commonly seen with more advanced renal function impairment. It is anticipated that each clinical site (Tufts and UMMC) will recruit 48 participants (assumes 15% loss of information) who will be randomized in a 1:1 ratio to receive either placebo or metformin, titrated to 1000 mg twice daily, or maximal tolerated dose, over a 6-week period.

The primary outcome of the trial is the safety and tolerability of metformin, which will be evaluated at baseline, 1 and 3 months and every 3 months thereafter. Safety will be assessed through ascertainment of symptoms, laboratory measures (CBC, basic chemistries, liver function tests, hemoglobin A1C and plasma lactate levels), vital signs and physical exam. Other adverse and serious adverse event rates will also be collected. Tolerability will be assessed by a validated instrument, the Gastrointestinal Symptoms Rating Scale (GSRS), used to evaluate the most common anticipated side effects, which are GI-related symptoms. As well, at in-person visits, participants will be asked, “Can you tolerate this dose of the study drug for the rest of your life?” We will also examine rates of study drug `discontinuation, reasons for discontinuation, and adherence with study drug as assessed through pill counts. Efficacy endpoints include a) change in kidney, kidney cyst, liver and liver cyst volumes on MRI; b) change in glomerular filtration rate, estimated from serum creatinine, using the CKD-Epi equation (31); c) the incidence of kidney pain as reported on the TAME Pain Questionnaire; and d) the Medical Outcomes Short Form 36 (SF-36) questionnaire for measuring health-related quality of life (HRQOL). Urine will be collected from participants at each study visit to measure biomarkers that have been shown to correlate with ADPKD disease severity and response to metformin. In addition, and with participant consent, optional blood, sera and urine samples will be collected for biobanking and DNA extraction and banking. An independent Data and Safety Monitoring Board (DSMB) will monitor participants through interim analyses of adverse events.

Aim 2: We will perform metabolomic and metabolite measurements on urine samples from our study patients both before and after initiation of the study drug to evaluate potentially relevant biomarkers that correlate with ADPKD disease severity and response to metformin.

We hypothesize that metformin treatment will ameliorate ADPKD severity and progression in patients and that this beneficial effect occurs in large part through changes in the cellular bioenergetics and proliferative capacity of ADPKD kidney epithelial cells. Moreover, Boletta and colleagues recently identified a Warburg effect-like shift to excessive aerobic glycolysis, as evidenced by increased levels of certain key metabolic enzymes (e.g., lactate dehydrogenase A (LDHA), pyruvate dehydrogenase (PDH) kinase 1 (PDK1), and the pyruvate kinase M2 isoform (PKM2)), and a decrease in signaling through the

AMPK pathway that occurs in ADPKD cystic epithelial cells as compared with normal kidney epithelial cells (22). Metformin promotes cellular AMPK activation (55), which may confer beneficial effects in the treatment of diseases such as the metabolic syndrome, diabetes, and polycystic ovary syndrome (PCOS) (56). Our previously published data demonstrate that metformin, via AMPK activation, inhibits both epithelial cellular proliferation through the mTOR pathway and CFTR-dependent fluid secretion into PKD cysts (Fig. 1), resulting in decreased cystogenesis and disease severity in vivo in rapidly progressing mouse models of ADPKD (18). For the studies proposed in Aim 2, we will investigate candidate metabolic biomarkers (Table 3) in urine based on the work of Boletta and colleagues and others (22). We hypothesize that glycolytic pathway enzymes will be progressively increased and markers of AMPK pathway activation will be progressively reduced with worsening ADPKD severity in patients. We further hypothesize that treatment of patients with metformin will tend to reverse these effects, which are indicative of the metabolic disturbance in ADPKD, and potentially thereby represent a beneficial therapeutic effect.

KNOWN POTENTIAL RISKS AND BENEFITS

ADPKD is the most commonly inherited kidney disease with over half of the gene carriers developing end-stage renal disease. The risks a patient is willing to accept are based on his/her personal and/or familial relationship with ADPKD. The risks of this research are well explained and documented in the consent process, and patients are encouraged to weigh the risks against any potential benefits.

1. Foreseeable Risks

Foreseeable risks of treatment with metformin include rare but serious adverse effects of lactic acidosis, hypoglycemia, leukocytoclastic vasculitis with pneumonitis, or Vitamin B12 deficiency induced neuropathy or megaloblastic anemia. More commonly reported but less serious adverse effects include abdominal discomfort of bloating or dyspepsia, metallic taste, anorexia, nausea, soft stools or diarrhea, headache and myalgias. Potential risks associated with phlebotomy are not greater than minimal and include bruising and bleeding. Potential risks associated with MRI are also minimal, and include: mild physical discomfort from lying still in the scanning bed, claustrophobia; and (very rare) pain and injury from movement of implanted ferromagnetic material/devices. Of note, we will not be utilizing gadolinium-based contrast agents in this trial. As with all human subject research, there is also the potential for loss of privacy and confidentiality.

2. Risk management and emergency response

The research monitor, who is an endocrinologist with vast experience in the use of metformin in clinical care, will be available to the study team to discuss investigation and management (including whether or not to discontinue drug) for any side effect that the PI believes could be related to study drug. In the event of potential serious adverse events that are believed to be study related, investigators will have a low threshold to discontinue the study drug until the situation is clarified. Drugs that interact with metformin will be carefully screened for and – where medically appropriate – we will recommend their providers to discontinue these medications prior to study enrollment. These drugs include nifedipine, furosemide, amiloride, ranitidine, triamterene digoxin, procainamide, quinidine, vancomycin, and trimethoprim. Inability to discontinue one of these medications will be an exclusion criterion. Patients will be given written and verbal instruction about the signs and symptoms of the serious side effects of metformin and will be instructed to monitor for these and to alert the study team immediately should they experience one or more of these. Precautions to reduce the likelihood of a serious adverse event will be taken as discussed in each specific SAE below.

Lactic acidosis is a rare but life-threatening complication of metformin use; the incidence in a diabetic population is estimated at 0.03 per 1000 patient years of follow-up, and the incidence of fatal lactic acidosis is estimated at 0.015 per 1000 patient years. The incidence increases with declining eGFR though and is believed to be extremely low at eGFR >30 mls/min/1.73m^2. Nonetheless, given the clear relationship of this life-threatening outcome to kidney function, we will be monitoring eGFR at least every 3 months (and more often during study drug titration) and if eGFR falls below 45 mls/min/1.73m^2 throughout the trial, the following changes will be made: If GFR is between 30-44 mls/min/1.73m^2, the metformin dose will be reduced by one-half and the frequency of bloodwork (including lactic acid level) will be increased to monthly bloodwork. If eGFR is <30 mls/min/1.73m^2, metformin will be discontinued. Progressive decline in eGFR to <30 ml/min/1.73m^2 over 2 years is unlikely in an ADPKD population with initial eGFR ≥50 mls/min/1.73m^2, with the average rate of decline being 3-5 ml/min/1.73m^2/year. A more likely scenario in which GFR would decline to <30 ml/min/1.73m^2 would be in the setting of AKI. AKI is most likely during hospitalization and with exposure to IV contrast, and patients will be instructed about these associations and that they should discontinue metformin if they are admitted to a hospital or if they will be receiving iodinated contrast. The metformin should not be resumed until eGFR has been confirmed to be >30 mls/min/1.73m^2 (and eGFR is stable) after hospitalization or iodinated contrast exposure. The risk of lactic acidosis is increased with medical / surgical problems and this would be another reason to discontinue metformin during hospitalization. This approach to drug titration and temporary discontinuation is consistent with recently revised US FDA prescribing guidelines. (58)

The onset of lactic acidosis is often subtle and accompanied only by nonspecific symptoms such as malaise, myalgias, increasing somnolence, dyspnea and nonspecific abdominal distress. Study staff and patients will be educated to monitor for these symptoms. Once a patient is stabilized on any dose level of metformin, GI symptoms, which are common during initiation of therapy, are unlikely to be drug related. Later occurrence of GI symptoms could be caused by lactic acidosis and patients will be told to promptly communicate with the PCC should they experience such symptoms. In the event of any of these symptoms, the study coordinator will inform the PI immediately, and metformin may be withdrawn (depending on symptom severity), per the PI's discretion, until the situation is clarified – this may include measurement of serum electrolytes and lactate levels where appropriate. If lactic acid levels are not elevated, then the drug will be resumed, with close-follow-up (within 3 days) by telephone to assess the patients' symptoms. In the event lactic levels are >5 mmol/l or the patient describes more severe symptoms, the patient will be instructed to go to the closest Emergency Room immediately for further management. Further management consists of supportive care and in severe cases, dialysis.

Hypoglycemia is an infrequent complication of metformin therapy in patient populations with or without diabetes. For example, there were no reported episodes of hypoglycemia in the Diabetes Prevention Trial in a patient population with prediabetes (59). Under usual circumstances of use in populations with diabetes, it does not occur in patients receiving metformin alone but could occur if caloric intake was deficient, when strenuous exercise was not compensated by caloric supplementation or during alcohol intoxication. Patients will be told to avoid the aforementioned circumstances that could precipitate hypoglycemia. Patients will be closely monitored with serum glucose levels checked bi- weekly during titration and every 3 months thereafter in individuals with GFR >50 ml/min/1.73m^2, and monthly for individuals with GFR 30-44 ml/min/1.73m^2. Study drug will be discontinued at eGFR <30 ml/min/1.73m^2, as noted earlier. While there are established values of fasting blood glucose levels for defining abnormal glucose tolerance, the lower limit of normal for fasting glucose in individuals without diabetes is not as clear. For this reason, we will modify the current protocol to define hypoglycemia as a blood glucose <70 mg/dl if it is accompanied by symptoms and signs of hypoglycemia. Staff and patients will be alerted to symptoms of hypoglycemia (E.g. shakiness, lightheadedness, tachycardia, blurred vision, weakness, diaphoresis, somnolence) and patients will be told to immediately drink a sugar

containing beverage (E.g. fruit juice or regular soda) and contact the PCC if one or more of these symptoms occur.

- A blood glucose >60 and <70 mg/dl noted on laboratory examination that is not accompanied by one or more of the described hypoglycemia symptoms will be considered an alert value that does not require modification of dosing of study medication or increased frequency of glucose monitoring,
- A blood glucose >60 and <70 mg/dl on laboratory examination that is accompanied by symptoms of hypoglycemia will prompt a discussion between the investigator and the research monitor. Management will typically consist of modification of dosing of the study medication as well as an increase in frequency of glucose monitoring.
- A blood glucose <60 mg/dl) will be considered as defining hypoglycemia, independent of the presence or absence of symptoms. The investigator must discuss with the research monitor but management will typically consist of reducing the dose or discontinuation of the study medication permanently.

These recommendations are consistent with the Endocrine Society Clinical Practice Guideline for Evaluation and Management of Adult Hypoglycemic Disorders [2], which recommends " . . .evaluation and management of hypoglycemia only in patients in whom Whipple's triad—symptoms, signs, or both consistent with hypoglycemia, a low plasma glucose concentration, and resolution of those symptoms or signs after the plasma glucose concentration is raised—is documented." (60)

Vitamin B12 Deficiency Related Complications. It is estimated that 30% of diabetics treated with metformin malabsorb Vitamin B12 (Vit B12) [Ting RZ *Arch Intern Med* 2006; 166:1975–1979]. Complications of Vitamin B12 deficiency – where severe and persistent – may include irreversible neuropathy and/or megaloblastic anemia. To reduce the risk of these serious adverse events, Vitamin B12 levels will be checked prior to enrollment and Vitamin B12 deficiency will be an exclusion criterion. The risk of adverse effects from metformin-induced vitamin B12 malabsorption is extremely unlikely in this 2-year trial of metformin-naïve patients who are not Vitamin B12 deficient at baseline, as it takes twelve to fifteen years to totally deplete pre-existing Vitamin B12 stores in individuals with B12 deficiency [Deller *Q J Med* 1962;31:71–88] . Nonetheless we will measure Vitamin B12 levels annually in patients and if Vitamin B12 deficiency is found, the participant will be referred to appropriate medical care for B12 replenishment. Study drug will be continued provided that the patient receives B12 replacement therapy with monitoring every three (3) months until the B12 value is >250 pg/ml.

Leukocytoclastic vasculitis /pneumonitis was reported in 1 case. Patients will be instructed to alert the PCC immediately should they develop a rash. Drug will be discontinued if rash is typical of vasculitis (palpable purpura), and patient will be referred for dermatology consultation where clinically appropriate; if the rash is not consistent with a drug-related leukocytoclastic vasculitis, the study drug may be restarted, with careful monitoring for symptoms. The potential for pneumonitis will be considered if a patient presents with dyspnea or respiratory symptoms without fever or other upper respiratory infection symptoms, though other diagnoses are much more likely.

GI symptoms are common, especially during drug titration, and usually resolve over time. Symptom burden can be reduced by titrating slowly and administering drug with food and these measures will be instituted to facilitate initiation, up titration and maintenance of therapy. Patients will be followed very closely, every 2 weeks during drug titration phase, and will be assessed for GI symptoms burden using the Review of Symptoms checklist, the GSRS, a standardized and validated instrument, and the response to the question: "Are you able to tolerate this dose of the study drug for the rest of your life?" Study drug will not be up-titrated in patients with persistent or distressing symptoms.

Risks associated with phlebotomy will be minimized by having skilled and certified phlebotomists performing all blood draws.

Risks associated with MRI scans will be minimized by careful MRI safety screening using a standardized MRI safety checklist. Specifically, those patients with implanted ferromagnetic devices which preclude safe MRI scanning will be excluded from participation and enrollment. Likewise, any patient with severe claustrophobia or who has been otherwise unable to tolerate an MRI procedure will be ineligible. This safety screening will be administered prior to each participants' MRI acquisition during follow-up in this trial. As current standard of care for ADPKD patients includes abdominal MRI, in most cases in this trial it will be relatively straightforward to ascertain an individual's previous ability to safely tolerate such scanning.

3) Potential Benefits

Patients may receive direct benefits by being in this study. These benefits include close follow-up of blood pressure and kidney disease, the latter assessed through eGFR and TKV. Longitudinal information about eGFR and TKV may help to determine the rate of progression of ADPKD. Repeated standardized measurement of kidney growth (size) by MRI is not normally performed in clinical practice. The study will test treatments that are thought to play an important role in slowing kidney growth and loss of kidney function early in the disease. The knowledge gained from this study will benefit all patients and families with PKD.

OVERALL TRIAL DESIGN, AIM 1

1. Study Population/Eligibility

Study participants will include N=96 adults aged 18-60 with ADPKD as defined by Pei-Ravine criteria (32). Exclusion criteria are selected to identify participants for whom metformin therapy would pose potential excess risk, or for whom randomization to placebo control would be inappropriate or infeasible. Inclusion and exclusion criteria are described in Table 1 below.

Concurrent use of tolvaptan will not be permitted in this trial, due to the lack of information regarding the safety of combined use of tolvaptan with metformin in ADPKD. Further, the large number of tolvaptan associated side effects will make it impossible to ascertain the tolerability and safety of metformin which is the primary endpoint of the present study.Also, among the specific aims of this randomized trial are to estimate the effects of metformin vs. placebo on change in kidney volume and renal function, measures which are affected (often acutely) by tolvaptan. However, participant will be informed through the informed consent process and documentation that tolvaptan has been approved by the FDA for some patients with ADPKD, and that they should discuss with their treating nephrologist whether this clinical trial is appropriate for them, and that tolvaptan use will be prohibited during this clinical trial.

Table 1

Inclusion Criteria	**Rationale**
Autosomal Dominant Polycystic Kidney Disease as defined by Pei-Ravine criteria	Standard clinical definition of this inherited renal disorder; distinguishes ADPKD from acquired cystic renal disease
Age 18-60	
Fluent English-Speaking	Instructions and patient-reported outcomes are in English language

Able to provide written informed consent	Must have cognitive capacity to provide informed consent for participation
Exclusion criterion	**Rationale**
Estimated GFR<50 cc/min/1.73m^2 (estimated from serum creatinine using the CKD-Epi equation)	Excess risk of metformin adverse drug reactions including lactic acidosis with more advanced renal impairment
Diabetes (currently diagnosed, or fasting glucose ≥126 mg/dL, or non-fasting glucose > 200), or HbA1C≥6.5	Patients may require metformin or other oral hypoglycemic agent for clinical care
Pregnancy or lactation or intending to become pregnant within the next three years	Risk of metformin to fetus/infant
Unstable or unclipped (>7mm) cerebral aneurysm	High risk of acute intracranial hemorrhage, which is often fatal
Implanted ferromagnetic object (e.g., pacemaker) or severe claustrophobia	Contraindication to renal MRI
Systemic disease (other than hypertension) likely to contribute to kidney disease (e.g., lupus)	Exclude patients with renal diseases other than ADPKD
Vitamin B12 deficiency	Metformin may reduce B12 levels leading to worsening deficiency
Active coronary artery disease, which will be defined as presence of stable or unstable angina	Exclude patients expected to suffer acute cardiovascular events during 2-year follow-up
Use of medications that may interact with metformin: nifedipine furosemide Cationic drugs (amiloride, ranitidine, triamterene digoxin, procainamide, quinidine, vancomycin, trimethoprim)	Minimize drug-drug interactions that can increase potential toxicity of metformin. If patients are taking these drugs and wish to participate, they must have discontinued these drugs at least 2 weeks prior to the baseline visit, based on supervision by their primary physicians.
Current or Recent (within 2 weeks) use of tolvaptan (Jynarque or Samsca)	Concurrent use of tolvaptan and metformin has not been previously examined, and its safety is uncertain. The large number of tolvaptan associated side effects will make it impossible to ascertain the tolerability and safety of metformin which is the primary endpoint of the present study. Furthermore, the aims of this trial are to estimate the effects of metformin on change in kidney volume and renal function, measures which are affected by tolvaptan.
Participation in a clinical trial with study medications	Potential interaction between metformin and investigational drugs on primary and secondary outcomes
Active military personnel	DoD regulations regarding enhanced protections of active duty military personnel
Any solid organ transplant	
History of non-compliance	
Allergy or intolerance to metformin	
Solitary kidney	

Enrollment criteria note: We considered whether to define a minimal kidney size for eligibility in this trial. Although specific identification of patients with large kidney volumes may select those at greater risk for disease progression, requiring MRI scanning and TKV quantification as part of screening would significantly prolong and complicate the screening process, adding additional cost and making it less likely to reach our pre-specified accrual goals. Nevertheless, even without specific TKV eligibility criteria, we expect the vast majority of eligible participants to have significantly enlarged kidneys with volumes greater than 750 cc. Based on prior data from the CRISP study, participants had a mean baseline TKV of 1060 ± 642 ml and a mean eGFR exceeding 99 ml/min/1.73 m^2 (f Ref. (33) Table 1).

2. Study Time Line

The protocol and study procedures will be refined during the first 6 months of the award. At month 6, recruitment will begin and continue for 24 months. The last patient will be enrolled by 30 months. All study participants will complete the main protocol by Month 56. During the last 6 months, participants will complete close-out visits, and the data will be analyzed and prepared for publication.

3. Recruitment Goals

We propose that each clinical site (Tufts and UMMC) will enroll 48 participants over a two-year period. The recruitment goal of 48 participants per site assumes a 15% loss of information (dropout, lost to follow up). We anticipate that sites will be able to obtain and review serum creatinine measurements and clinic letters prior to the screening visit so as to minimize screen failures. We anticipate 10% screen failures per site (i.e., 5 screen failures/site).

4. Recruitment

Although recruitment is always challenging, ADPKD patients are highly motivated to participate in research that may benefit either themselves or their children. In fact, there was a marked over-representation of ADPKD patients in the MDRD (Modification of Diet in Renal Disease) Study, at 25% of the study population, as compared with the prevalence of ADPKD among all causes of ESRD at 10% (34). Potential study participants will be identified from among several sources:

a. PKD specialty clinics at UMMC and Tufts, staffed by site PIs Drs. Terry Watnick and Ronald Perrone, respectively, and identified after review of their medical records performed under an IRB-approved HIPAA waiver. Dr. Watnick has been in charge of an inherited renal disease clinic for more than 15 years. This clinic draws patients from the entire mid-Atlantic region including Maryland, Virginia, Washington DC, southeastern Pennsylvania and Delaware. Her site was one of the highest recruiting sites in the Otsuka-sponsored tolvaptan (TEMPO) study. Similarly, Dr. Perrone has a well-established clinic and he has successfully recruited participants for a number of large studies including TEMPO 3:4 and HALT PKD, and several smaller studies including TEMPO 271, TEMPO 285, TEMPO 290, and TEMPO 291. Dr. Perrone's clinic draws patients from a wide geographic distribution including international referrals, but the majority of patients are from the northeastern U.S., particularly New England and the greater Boston area. Both UMMC and Tufts serve as tertiary referral centers in their respective geographic regions and therefore have a large population from which to recruit. Of note, both sites are currently recruiting for the REPRISE study, which evaluates tolvaptan in PKD patients with eGFRs 25-65.

b. PKD patient registries maintained at UMMC and Tufts of patients who have expressed interest in participation in interventional clinical trials. Drs. Watnick and Seliger maintain registries of well-characterized patients who are enthusiastic about participation in clinical trials. Any patient seen at UMMC is asked for permission to be contacted about participation in clinical studies. The UMMC registry is about to expand to an online format that should make recruitment easier. Dr. Perrone established an IRB-approved PKD Research Registry (IRB protocol #8891) in 3/2006; any patient with ADPKD seen at Tufts is asked for permission to be included in this research

registry and informed consent is obtained. By signing the informed consent, Dr. Perrone and his research team are given permission to contact potential subjects about participation in clinical studies and to store clinical data elements. As of 9/23/2014, 334 subjects have been consented to participate in this database.

c. PKD patients from the greater Baltimore-Washington and Boston metropolitan regions who respond to recruitment materials distributed through printed media or to study descriptions on clinicaltrials.gov. Potentially eligible patients who express interest in participation will be referred to the study coordinator and/or site investigators, who will discuss details of the study protocol with them.
d. The Polycystic Kidney Disease Foundation (PKDF) has played an important role in recruiting participants for many clinical trials. The mission of the foundation is to "promote programs of research, advocacy, education, support and awareness in order to discover treatments and a cure for PKD". They maintain a tab on their website (http://www.pkdcure.org/research/clinical-trials) that lists all clinical trials that are recruiting participants. We propose to list this clinical trial on the PKDF website if funded. We also note that Investigator Dr. Perrone is a past Chair of the Scientific Advisory Committee and Investigator Dr. Watnick is the current chair; both have been active members of the PKDF Scientific Advisory Committee for many years. They have been visible and dedicated members of their local chapters and the national organization. They personally know many of the members and their families and have given numerous educational talks and presentations at annual meetings and other events. Their active involvement is a key strength that will increase the success of recruiting members to this study.
e. Referring Community Nephrologists: The Baltimore PKD Clinical Translational Core has established a network of community nephrologists/practices who have actively referred participants for the PKD Core study. We have carefully nurtured these collaborations and we anticipate that these nephrologists will continue to refer participants for this study. Similarly, Dr. Perrone works closely with referring physicians throughout New England and will communicate the existence of this study to this network.

5. Recruitment Methods

We will use a multifaceted approach to recruitment including 1) communicating directly with patients when they come for clinic visits; 2) mailing invitations to PKD patients identified by collaborating physicians; 3) disseminating information about the study on clinicaltrials.gov and the PKD foundation web site (see above); 4) posting information on the Baltimore PKD Center website, once such activity has been IRB approved; and 5) posting information on the Tufts Medical Center websites, including the research portal and the Division of Nephrology.

6. Study Procedures

All study procedures will take place at the General Clinical Research Center (GCRC) at UMMC and at the Clinical and Translational Research Center (CTRC through the Tufts Clinical and Translational Science Institute) at Tufts Medical Center.

a. Patient Enrollment and Randomization: The data entry process will begin during the online patient enrollment into the study. There will be an Eligibility form, which will generate a participant ID. Screening information will then be completed to assess essential inclusion and exclusion criteria detailed in the protocol. Any information collected that identifies the patient will remain at the clinical center and will not be sent to the data coordinating center (DCC). Once the inclusion criteria have been met, the clinical center will submit the randomization form for the subject, and the Web-based system will return the participant's drug card assignment in a

double-blind fashion. New subjects enrolled will be given IDs that include a clinical center number but will not be sequential to avoid case confusion.

b. Screening Visit (S): After written informed consent is provided, a complete medical history will be obtained, including documentation of comorbid conditions, concomitant medications, and PKD related history. A complete physical examination including vital signs, height and weight will be conducted. Screening labs will include a CBC, renal panel (electrolytes, BUN –including a fasting glucose, and creatinine), hemoglobin A1C, EKG and a urine pregnancy test for women of child-bearing potential. Laboratory studies will be performed at either UMMC or Tufts. Patients with an eGFR <50cc/min/1.73m^2 or any of the other criteria listed in Table 1 will be excluded from enrollment into the trial and will exit the study prior to the baseline visit. Since long-term metformin treatment can result in low Vitamin B12 levels (35), this will be measured and, if deficiency is found, will be repleted by the patient's primary care physician as part of standard medical care prior to randomization. Confirmation of normalization of B12 levels will be required prior to the baseline visit. Participants who meet the entry criteria will proceed to the baseline visit.

c. Baseline Visit (B; within 8 weeks of Screening Visit): The baseline visit will include a review of interval medical history family history, liver history and life-style history and a full set of baseline laboratory studies including CBC, renal panel, liver function tests, fasting glucose, hemoglobin A1C, lactate level, lipid profile and a urine pregnancy test in women of child bearing potential. Urine will also be collected for biomarker studies to be performed at the University of Southern California. With specific patient consent, optional plasma, sera and urine will be collected and frozen at -80°C and shipped or transferred to the UMBiobank at the University of Maryland for long-term banking for future analyses. Optional blood may also be collected in two EDTA-coated tubes and shipped or transferred to the UMBiobank at University of Maryland for DNA isolation and banking for potential future genotyping or other analyses. An unenhanced abdominal MRI study will be obtained at the participating clinical center (either UMMC or Tufts) and, after de-identification, uploaded to the University of Pittsburgh Imaging center via Pitt.BOX, a secure file sharing system, which is supported by the Tufts Medical Center Information Services department and the University of Maryland Medical Center Information Services department and is approved by all of the institutions for sharing data with external collaborators. In the event Pitt.Box is not available, the de-identified images can be recorded onto CDs and FedEx'd to the University of Pittsburgh imaging center. In addition, each participant's scans will be reviewed at their respective clinical center by a certified radiologist for any unexpected findings. Volumetric measurements of the kidney, kidney cysts, liver, and liver cysts will be performed on the MR images. Baseline questionnaires, which include the Review of Symptoms, the TAME-PKD pain questionnaire, the Gastrointestinal Symptom Rating Scale (GSRS) and the Short Form-36 questionnaire will be administered. After these data are collected, participants will be randomized in blinded fashion to metformin or matching placebo, in a 1:1 ratio, as described below.

d. Supply of Study Drug: Study drugs will be dispensed from the Tufts or UMMC investigational drug pharmacy. Supplies will be dispensed at each visit to ensure an adequate number of pills until the next in-person follow-up visit. In order to save costs on drug dispensing and placebo manufacturing, as well as to simplify the drug administration, we will dispense only 500-mg capsules of metformin/placebo. The University of Pittsburgh Investigational Drug Service will encapsulate and bottle the placebo and metformin so that they appear identical then dispense to the sites.

e. Drug Titration Protocol and Study Drug Discontinuation: Participants will be started on 500 mg of Metformin (or matched placebo) once daily for two weeks, with the following scheduled dose titrations:

    - Increase to 500 mg twice daily at the beginning of week 3

- Increase to 1000 mg qAM, 500 mg qPM at the beginning of week 5
- Increase to 1000 mg twice daily at the beginning of week 7

This gradual titration regimen was chosen in an attempt to minimize gastrointestinal side effects. Laboratory studies, including a CBC and renal panel, will be obtained at the PCC or local Quest lab, whichever is most convenient for the patient, at weeks 2, 4, and 6 per the schedule in Table 2. An in-person visit with an extended laboratory evaluation will take place in the middle of the titration period at week 4. A member of the study team will contact the participant by phone at 2 weeks and 6 weeks during the titration period to assess tolerability and to up- or down-titrate the dose, depending on tolerability. Tolerability will be assessed by asking a standardized question "Can you tolerate this dose of the study drug for the rest of your life?" and responses to the Review of Symptoms checklist and the GSRS. Up-titration will occur if the participant's eGFR within the past 2 weeks is ≥50 cc/min/1.73m^2, and if the person answers "yes" to the standardized question re: tolerability of the current dose and has few, if any, adverse symptoms based on responses to the review of symptoms and GSRS. If a participant is unable to tolerate a dose of the study drug, and after discussing the participant's responses with the study doctor, the coordinator will then instruct the participant whether to adjust the dose of the study drug or placebo. If the patient is unable to tolerate a specific dose, then he/she will be down-titrated progressively to the highest tolerated dosage; this dose will be continued for that participant for the remainder of the trial.

Although metformin is considered to be a Category B drug during pregnancy and has been used safely in pregnant women with polycystic ovary syndrome (36), to ensure maximal participant safety and to avoid confounding effects of pregnancy on study outcomes, pregnancy will be considered an exclusion criterion for this study and female participants of child-bearing potential will be required to use contraception during their participation. Furthermore, urine pregnancy tests will be administered during each in-person visit. In the unlikely event that a pregnancy is detected, the study drug will be stopped immediately.

Participants will be instructed at each visit to avoid medications that may raise the level of metformin, including cationic drugs such as amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin. Nifedipine and furosemide have also been shown to elevate the levels of metformin (see package insert). Although the risk of lactic acidosis in individuals with GFR >45 is small (29), participants will be instructed to contact the study coordinator and hold metformin before any surgical procedure that requires restricted intake of food or fluids or if they should require iodinated contrast. Participants will also be warned to avoid excessive alcohol intake since alcohol is known to increase exponentially the effects of metformin on lactate metabolism.

f. Drug Adjustment in Setting of low eGFR: The study drug dose will be reduced to 500 mg twice daily (or maximally tolerated dose less than 500 mg twice daily) if and when eGFR falls to between 30-44 ml/min, and patients will be monitored more closely and have laboratory tests, including lactic acid levels, checked every month. It is conceivable that a participant's eGFR may fall below 30 ml/min during the study period of two years. Progression rate reported in the TEMPO control population, which had a mean eGFR of 82 ml/min/1.73 m^2 at enrollment, was 3.7 ml/min/1.73m^2 per year (13), making it unlikely that disease progression would lead to eGFRs below 30 during the two-year study duration; episodes of AKI rather than disease progression would be a more likely cause of reduced eGFR. If a patient experiences AKI or is found to have eGFR <30 ml/min on a lab test taken outside of the study, the participant will be told to contact the PCC, and monthly lab-only visits will be arranged at the PCC or local Quest lab (location as per patient preference). If a participant's eGFR remains below 30 ml/min/1.73m2 (confirmed on a repeat value), the study drug will be discontinued; however, the participant will continue to be followed per the protocol consistent with intention-to-treat

(ITT) principles. Resolution of the cause of AKI and improvement of eGFR to ≥30 ml/min/1.73 m^2 will permit resumption of the study medication, with dose adjustment if eGFR is <45mL/min/1.73m^2.

g. In-person Follow-up Visits (F): In person follow-up visits will occur at the PCC at Months 1, 3, 6, 9, 12, 15, 18, 21, and 24. These visits will consist of an interval medical history, a review of symptoms, pain evaluation, reconciliation of concomitant medications, adverse event history, and a symptom-directed physical examination. Laboratory evaluation will be similar to the baseline visit, including CBC, renal panel, liver function tests, hemoglobin A1C, lactate level and a urine pregnancy test for women of child-bearing potential. Laboratory tests will be performed at the UMMC or Tufts clinical laboratories. Serum creatinine is measured at both institutions and at Quest Labs by using standardized IDMS-traceable methods (37). The TAME Pain Questionnaire, GSRS and SF-36 will be administered at each of these visits. Pill counts will be conducted to assess adherence to the medication regimen. Repeat Vitamin B12 will be measured at the 12- and 24-month visits. Urine will be collected for biomarkers. Optional plasma, sera and urine will be collected for long-term banking. MRIs will be performed at months 6, 12, 18 and 24. A complete physical examination will be performed at month 24.
h. Laboratory-only Visits (L): Kidney function will be monitored on a monthly basis during the first 6 months of the study protocol. In order to avoid frequent in-person visits to either UMMC or Tufts, safety labs consisting of a CBC and a renal panel can be drawn at a local Quest lab convenient to the participant at and months 2, 4 and 5. Quest uses standardized IDMS-traceable methodologies. If a participant's eGFR falls below 45 ml/min/1.73m^2, then additional Laboratory-only visits will be continued on a monthly basis during those months where there is no in-Person Follow up visit scheduled.
i. Close-out Visit: Study drug will be discontinued at the Month 24 visit. Participants will return for a final Close-out visit within 2 months after stopping the study medication. This visit will be similar to the in-person follow-up visits and will include the review of systems, labwork, GSRS, Tame Pain Questionnaire, SF-36, urine sample for biomarkers and an EKG. Optional blood, sera and urine will be also be collected for transfer to the biorepository.
j. Blood Pressure goals: Blood pressure (BP) management will not be a component of the randomized intervention for this pilot-level metformin trial; however, BP control may impact on outcomes, making it important to manage BP uniformly in all study participants. Every effort will be made to ensure BP is below 130/80. In the event BP is not consistently at goal (<130/80) the study team will communicate with the participant's treating nephrologist to recommend appropriate adjustments or additional anti-hypertensive therapy. Unless contra-indicated by drug intolerance (e.g., hyperkalemia, h/o angioedema), agents that target the renin-angiotensin system (ACE inhibitors or angiotensin receptor blockers) will be recommended for first-line anti-hypertensive therapy.

7. Optional Genetic Samples

An optional blood sample will be collected at the baseline visit in two EDTA-coated (purple-top) tubes and shipped or transferred to the UMBiobank at University of Maryland for DNA isolation and banking for potential future genotyping or other analyses. Patients will be able to opt out of the DNA collection but still participate in this clinical trial. As stated in the consent document, participants will have the option to withdraw their samples from the biorepository at any time by submitting a written request. However measurements already performed on biorepository samples will not be removed from the study database. Samples will be tracked by the University of Pittsburgh at the Data Coordinating Center using standard tracking software. This DNA may be tested for changes in the genes that cause ADPKD, or in other genes that can influence the progression of ADPKD or regulate energy metabolism. Patients will not be provided with the results of these genetic tests as they are research related and are not done as part of routine medical care.

8. Retention

Retention of participants is vital to the success of any study. We will utilize decades of experience at both Tufts and UMMC in conducting studies to ensure retention of participants. We have attempted to minimize participant burden by using local Quest laboratory facilities to monitor safety labs between in-person visits. We will aim to schedule participants at convenient times so as to minimize interruption of their work schedule. In addition, we have planned to pay participants a stipend for gas and parking costs in order to keep the economic burden to a minimum. In the event a patient relocates from one clinical site to the other, the data center will be able to accommodate the participant's retention in the study.

Patients unlikely to understand the importance of maintaining follow-up for the entire duration of the 2-year study, following strict adherence to study medications, or that are unwilling or unable to make the required visits during the screening and baseline periods, will not be enrolled. We have generally found that patient retention at Tufts and UMMC in other PKD clinical trials has been excellent. Patients highly valued the unique opportunity they had to be evaluated by and ask questions of internationally recognized experts in ADPKD at each study visit. Contact between patients and study staff, particularly these clinicians, is frequent in the proposed study, and we believe this will similarly promote high rates of study retention.

9. Treatment Interruption

During the 2-year long trial, it is expected that subjects may have one or more treatment interruptions during the study period. If a subject's study drug is interrupted for safety reasons, laboratory abnormalities, use of a prohibited concomitant medication or recommendation of the PCP, PI or *safety monitor*, the subject's study medication should be resumed as early as the situation allows and with Research Monitor approval, if required. Any study drug interruption of <7 days will be recorded as missed doses rather than as a temporary interruption of study drug. The subject should immediately inform the investigator of any missed doses reaching or expected to reach 2 *consecutive* days or more so that the investigator or coordinator can continue to monitor the subject's treatments. If the subject has a study drug interruption $\geq$7 consecutive days, the subject may be asked to complete a subset of the screening labs to re-evaluate safety before resuming the study drug, as determined by clinical center PI based on the clinical status of the participant and reasons for study drug interruption. The PI may consult with the Study Research Monitor on this to ensure appropriate safety of the participant.

10. Treatment Discontinuation

If a subject stops the study drug for medical or personal reasons, they will be asked to continue their participation in the study until its conclusion at 26 months. If the subject is unwilling or unable to continue in the study, a Close-Out visit should be scheduled within two months to obtain the same measurements that would have been collected at the end of the study.

Patients choosing to initiate treatment with the (non-study) medication tolvaptan after enrollment will be withdrawn from the study permanently (early completion as per protocol) and will not continue with follow-up study visits. The rationale for this discontinuation from follow-up is the expected extreme difficulty of performing regularly scheduled study visits while a participant also requires differently scheduled safety laboratory testing for the clinical use of tolvaptan under the REMS program. Further, the large number of tolvaptan associated side effects will make it impossible to ascertain the tolerability and safety of metformin which is the primary endpoint of the present study.

11. **Outcome Measurements**

The primary outcome is the safety and tolerability of using metformin in ADPKD patients with baseline eGFR ≥50 ml/min.

a. Assessment of Tolerability: Tolerability will be assessed using two standardized patient-reported instruments that will be administered at baseline, at each telephone visit during titration, and at every PCC visit. The most anticipated adverse effects of metformin are gastrointestinal (GI)-related. Accordingly, we will use the GSRS instrument to assess GI symptom burden. A widely used and validated 15-item questionnaire, the GSRS instrument has been used to compare the GI tolerability of different mycophenolate preparations in renal transplant recipients (39). The change in GSRS during follow-up will be compared between metformin and control groups, as described in "Statistical Plan". The second assessment of tolerability will be based on responses (yes or no) to the following question "Can you tolerate this dose of the study drug for the rest of your life?" As described in Section IIIB.7e Drug titration, a negative response will lead to down-titration of drug. We will assess the maximally tolerated dose of metformin and also analyze the rate of discontinuation of study drug and the reasons for discontinuation by study arm.

b. Safety Outcomes: At each study visit (telephone or PCC), patients will be asked if they had an interim hospitalization or medical encounter and if so, the nature of the event. The patient will sign a release of medical information form at the baseline visit and this will be used to obtain discharge summaries or other clinical documentation to determine the nature and severity of adverse events. Serious adverse events (SAE) will be defined as an undesirable experience occurring from the time a participant signs the informed consent (at the screening visit) until the end of the study, meeting 1 or more of the criteria of: 1) Resulting in death, 2) Non-elective hospitalization, 3) Life threatening (if patient continued on study drug would result in death), 4) Persistent or permanent harm or disability, 5) Exceeding the nature, severity or frequency of risk described in the protocol or 6) resulting in Congenital anomaly. Reporting requirements to the DCC and IRBs are described in part 12 SAFETY, Section C. A symptoms checklist (which lists the more common or concerning side-effects of the study drug, but allows for free text entry of other symptoms) will be collected at baseline and at each subsequent PCC visit. Rates of AEs (based on the symptoms checklist) and SAEs will be compared across arms. Hypoglycemia is a potential complication of metformin use in a non-diabetic population. The number of hypoglycemia events (based on lab results) will be compared across arms. Values for other electrolytes, eGFR and liver function tests that are being drawn per the Study Schedule Table 2 will also be compared across arms.

c. Adherence: Adherence with study medication will be assessed through pill counts, which will be performed by the study coordinator at each study visit. Patients that miss their in-person visit

and thus do not refill their study drug will be contacted in a timely fashion by the study coordinator, who will have a record of the missed visit. Adherence with study visits will be assessed by tracking the number of missed study visits. During the drug titration telephone visits and the F1 in-person visit, the missed study visit window will be +/- 1 day; for subsequent visits a missed study visit will be documented as +/- 6 days. Practical measures to minimize inconvenience (parking, stipends if possible), maintaining communication with referring physicians, and other means of maintaining direct communication with the patient (follow-up and thank you cards after visits, birthday and holiday cards, small gifts) should also promote adherence. Finally, regular and frequent follow-up visits should also aid in the retention of study patients.

d. Patient-reported Outcomes: Metformin is hypothesized to reduce TKV growth, which may manifest as a difference in pain and other symptoms relating to organ enlargement. The Medical Outcomes Short Form 36 (SF-36) questionnaire is the most widely used instrument for measuring health-related quality of life (HRQOL), though scores have not been shown to discriminate among ADPKD patients with larger vs. smaller kidneys at eGFR >60 ml/min (40). The TAME Pain Questionnaire is a modified version of the Wisconsin Brief Pain Questionnaire (41) that measures the frequency and intensity of pain and symptoms relating to organ enlargement as well as their impact on everyday living. It was validated in a clinical population of ADPKD patients from a single center observational study and subsequently adapted for use in the HALT Study (42). The frequency of back pain and symptoms related to abdominal distension correlated with htTKV in a cross-sectional analysis of baseline questionnaire results in the HALT-PKD Study A cohort, which is a population with similar eGFR range as expected in the proposed study (43). GI symptoms from metformin may affect HRQOL, and thus we will also examine this at every visit. The GSRS (a widely-used, validated 15-item questionnaire, described earlier) and the SF-36 (as a generic measure of HRQOL) will be administered by study staff (39, 44). The questionnaires will be administered at baseline and at in-person follow-up visits. In addition, on telephone visits during drug titration, the GSRS will be asked to evaluate common gastrointestinal symptoms. Each question is rated on a seven-point Likert scale, from no discomfort to very severe discomfort. For the GSRS the responses on the 15 items are reduced to average scores on 5 specific scales (diarrhea, reflux, constipation, abdominal pain, indigestion), identified from factor analysis. Among U.S. patients with gastro-esophageal reflux, internal reliability of GSRS scores on the outcomes that may be affected by metformin use was moderate to high (Cornbach's alpha: 0.61 to 0.83), and the instrument displayed good construct validity (45). During follow-up GSRS scores will be compared between metformin and control groups, as described in "Statistical Plan" below.

e. Death, ESRD and hospitalizations: At each death, if any, study coordinators will request release of information from next of kin. Supporting materials will include hospital death summary, death certificate, and ESRD Death Notification Form (HCFA Form 2746; the latter if the patient is ESRD). ESRD is unlikely in patients with a starting eGFR of ≥ 50 ml/min, but we will ascertain if this occurs through communication with the patient at telephone and/or in-center PCC visits. Patients will be asked at each in-person or telephone-based study visit about interim medical encounters and specifically about hospitalizations. Discharge summaries will be requested to determine reasons for hospitalization; length of stay will be recorded. After receiving supporting information, the event will be classified by the study team on the appropriate forms (Hospitalization, Death and ESRD Forms) and submitted to the DCC. Such information will be communicated through the study portal, as was done for the HALT PKD study (46).

f. Biomarker & Biobank: For biomarker analyses, urine samples (30cc) taken at the clinical sites will be divided into 10 –ml aliquots before freezing at -80°C. One 10 ml urine sample will be sent to Dr. Kenneth Hallows at the Keck School of Medicine of USC. The remaining optional urine

samples along with the optional blood samples (20 cc), which will be centrifuged before dividing the plasma into 1-ml aliquots and freezing at -80°C, will be transferred or overnight shipped on dry ice to the UMMC Biorepository for long-term storage at -80°C. At the Maryland Clinical Center, this aliquoting and freezing will be performed directly by the UMMC Biorepository. Presently we have no plans for mutation detection, but for patients who also gave consent to have their DNA stored for potential future analyses, the UMMC Biorepository will also isolate the DNA from blood taken at the baseline visit, according to their standard protocols.

g. MR Imaging and Volumetric Measurements: The Image Analysis Center (IAC) has been established to handle the imaging data and to manage all imaging-related issues for the CRISP and HALT studies. The MR Imaging and volumetric measurements established in the CRISP study will be employed in the present study, as results from the CRISP study indicate that this MR protocol will provide an effective means for longitudinal evaluation of kidney, kidney cyst, liver, and liver cyst volumes in ADPKD patients (47). MR images will be obtained at each clinical center and transferred to the IAC at the University of Pittsburgh. Prior to scanning participants, the MR technologist will be trained or will have experience in scanning PKD participants according to the MR study protocol. MR Imaging begins with placing a phased-array surface coil with its center over the inferior costal margin, estimated as the upper margin of the kidney, in each participant. The field of view will be maintained between 30 and 35 cm. The kidneys will be imaged at 3 mm fixed slice thickness in the coronal plane using the T2-weighted single-shot fast spin-echo sequence with fat saturation as well as three-dimensional spoiled gradient interpolated T1-weighted images without fat saturation and during breath-hold. After the acquisition of the images, participant names and identifiers will be removed from the images and replaced with study numbers and accession numbers for participant confidentiality prior to image transmission. The transmitted images will be reviewed by IAC to generate quality control reports for the clinical centers. Images determined to be inadequate for measurement must be reacquired within 30 days.

   Individual whole-kidney volumes (TKV) will be measured from T1-weighted images by means of the stereology method, while T2-weighted images are reviewed simultaneously. The areas of the whole kidney in each image are calculated from the collection of points, and volume measurements are made from a set of contiguous images by summing the products of the areas measured and the slice thickness. The volume of kidney cysts (KCV) in each kidney will be measured using a region-based thresholding method. Cysts are brighter than the renal parenchyma in T2-weight images. Therefore, they could be measured by summing voxels with intensity values greater than those of the background renal parenchyma. On each renal MR image slice, a binary signal-intensity map will be generated. This will be done by determining a threshold signal intensity that visually distinguishes the cyst and renal parenchymal regions. In the binary map, cysts that are brighter than renal parenchyma are represented as white regions, whereas the background renal parenchyma will be designated as black regions. By summing the pixels of white regions, the cystic area will be measured in each slice. The total cyst volume will be calculated from each set of contiguous images. Similarly, the total liver volume (TLV) and liver cyst volume (LCV) will be measured using the region-based thresholding method. Each volumetric measurement will be made by a trained analyst at the IAC and will be reviewed by a radiologist for quality control.

12. Safety

a. Data and Safety Monitoring Board (DSMB): An independent data and safety monitoring board is being appointed by the steering committee. The composition of this committee is described below:

Member	Expertise	Institution
Mary Korytkowski	Endocrinology	University of Pittsburgh
Mark Unruh, MD	Nephrology	University of New Mexico
Silvio Inzucchi, MD	Endocrinology/Diabetology	Yale University
Ben D. Cowley, MD	Nephrology/PKD	University of Oklahoma
Kenneth Wilkins, PhD	Biostatistician	NIH/NIDDK

The DSMB will convene to monitor safety and adverse events every 3 months at the beginning of the study until the first 10 subjects have reached the three-month mark, and then every 6 months thereafter. Consistent with the phase II design of this study, there will be no planned interim analysis of efficacy as the study is not powered for efficacy. Unanticipated serious adverse events, related to study medication will be carefully scrutinized. If significant lactic acidosis occurs in any participant, Dr. Korytkowski, chair of the DSMB and the DoD Research Monitor, will convene the DSMB urgently to determine whether the study will continue. The DSMB will report to the sponsor (DoD) and to the Steering Committee, including each site PI, who will have responsibility for submitting reports to their institutions' IRB for review and acknowledgement

DoD Research Monitor: Mary Korytkowski, MD is Professor of Medicine and Interim Chief, Division of Endocrinology. Dr. Korytkowski is an outstanding clinical diabetologist and is a Professor of Medicine at the University of Pittsburgh. She is the Director of Diabetes QI (Quality Improvement) at UPMC. Her QI program has received support from the Department of Defense, which has adopted several of the protocols developed in Pittsburgh for use in military hospitals. Dr. Korytkowski has significant expertise in the management of diabetes and the use of metformin. Dr. Korytkowski will serve as the DoD Research monitor and as such, will be responsible for monitoring and coordinating all patient safety, data and security measures at all sites. Her role includes: discussing the research protocol with the investigators; the authority to stop a research protocol in progress; to remove individual human subjects from a research protocol, and to take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and the responsibility to promptly report observations and findings to the IRB or other designated official and the HRPO.

b. Safety Monitoring: Study visits and safety laboratory monitoring will occur at an interval of every 3 months or more often (as during titration) throughout this trial, and the frequency will be increased to every month for individuals whose GFR falls below 45 ml/min/1.73^{m2}. Lactic acidosis is a rare but life-threatening complication of metformin use; the incidence in a diabetic population is estimated at 0.03 per 1000 patient years of follow-up. The incidence in non-diabetics is unknown, but we believe it is lower because diabetes, reduced kidney function, and older age predispose to lactic acidosis. The incidence of lactic acidosis increases with declining eGFR but is believed to be low at eGFR >30 ml/min/1.73m^2, and European guidelines have recommended continuation of metformin unless renal function declines below this level (48). Similarly, in the US, prescribing guidelines were recently (April 2016) revised by the Food and Drug Administration; these guidelines recommend discontinuation of metformin if the eGFR falls below 30 ml/min/1.73m^2, and to "assess the benefits and risks of continued treatment" for patients with eGFR 30-44 ml/min/1.73m^2 with regular monitoring of eGFR for these patients (58). To provide a high level of protection against the risk of lactic acidosis for participants in this study, we have developed the following monitoring and dose adjustment procedures:

1) Study drug dose will be reduced to no more than 1000 mg/day, or ½ of the full dose (if patient was tolerating a full dose) when eGFR is between 30-44 ml/min/1.73m^2, and patients will be monitored more closely with monthly blood tests.
2) The study drug will be discontinued when eGFR is <30 ml/min/1.73m^2, although patients will continue to participate in follow-up measurements according to intention-to-treat principles.

As mentioned in Section IIIB.7.f, progressive decline in eGFR to <30 ml/min over 2 years is unlikely in an ADPKD population starting with eGFR ≥50 ml/min, and a more likely risk for lactic acidosis is acute kidney injury (AKI). Patients will be instructed about this risk and that they should alert their provider to the fact that they are in this study if they are admitted to the hospital and that the study drug should be discontinued during hospitalization. The risk of metformin-induced lactic acidosis is well known, but to ensure metformin is not continued when patients have or are at risk for AKI (as is common during hospitalizations), patients will be instructed to contact the PCC if they are admitted, and the PCC staff will communicate this to the hospital provider.

Hypoglycemia is another potential serious risk of metformin use in non-diabetics and this will be monitored by symptoms and with lab work, which will be performed every 2 weeks during titration and every 3 months thereafter, or for individuals with eGFR <45/min/1.73m^2, monthly. Patients will be instructed about symptoms associated with hypoglycemia and appropriate intervention including prompt ingestion of sugar-containing food or liquids.

c. SAE Reporting Requirements: All SAEs must be reported within 24 hours of study personnel learning of the event to the local PI and to the DCC via data-entry of SAE Report Form 60. The DCC will notify the DSMB of SAEs that are drug-related and unanticipated within 5 business days of initial knowledge of the event and all PIs within 5 business days (annually if anticipated). Each site PI will be responsible for informing their IRB of study-related unanticipated SAEs according to their local reporting requirements. Lactic acidosis is an anticipated event but will fall under the same guidelines for reporting as unanticipated events, given the life-threatening nature of the event and the potential for further events in active participants. The DCC will prepare reports of such events for the DSMB at least annually. PIs at the clinical centers are responsible for fulfilling local IRB reporting requirements for anticipated SAEs, which may vary by center.

Statistical Plan and Data Analysis for Aim 1 – See detailed addendum p.33-37: **TAME –PKD Statistical Analysis Plan**

All primary and secondary outcomes will be described using sample means or sample proportions along with 95% confidence intervals, depending on the nature of the outcomes. Volume outcomes from MRI (i.e. total kidney, kidney cyst, liver, liver cyst) that are known to be highly skewed will be assessed to see if suitable transformations are needed (i.e., natural log). Participant demographics and baseline clinical characteristics will be compared between study arms (Metformin versus Placebo) using two-sample t-tests or chi-squared tests of independence. All primary outcomes will be analyzed under intent-to-treat. Every effort will be made to minimize the amount of participant dropout and missing data. We will record specific reasons for attrition as well as implement various data verification measures for missing data. Ultimately, we will compare participants who dropped out with those who completed follow-up as a way to investigate the relationship between attrition and key variables. We will also assess the mechanism and degree of missing data (i.e. missing at random) using established techniques by Little and Rubin (50). If missing data are substantial, we will compare various multiple imputation techniques to see how robust the overall inferences are.

The primary objective of this phase II, multicenter clinical trial is to evaluate the safety and tolerability of metformin versus placebo with a secondary emphasis on efficacy. The safety outcome is defined by the occurrence of adverse events and serious adverse events (as defined in Section IIIB.9.b) throughout the study. We will calculate cumulative incidence between study arms and use logistic

regression to assess the association between study arms and the number of participants affected across various SAEs.

Tolerability will be defined by the GSRS scale, the rate of discontinuation of study drug, and the maximal dose that patients are using at the end of the 2-year study, as discussed in Section IIIB.9.a. Using a linear mixed model, we will compare the change in overall GSRS score as a function of time (months since baseline), the interaction between time and study arm, and clinical site. We will also include a random intercept to allow for participant-level variability of baseline GSRS. Of primary interest is whether the interaction terms are significant, which would indicate the change over time differs between the metformin and placebo arms. Finally, tolerability will also be assessed by comparing drug discontinuation rates between study arms. Chi-squared tests of independence will be utilized to compare discontinuation rates as well as adherence (measured by pill counts) between study arms. In addition, we will also calculate Kaplan-Meier curves to compare time-to-drug discontinuation between arms.

Secondary outcomes: The annual rate of change in height-adjusted total kidney volume (htTKV) will be compared between Metformin and Placebo arms to give an indication of the efficacy of metformin. A Laird and Ware linear mixed model (51) will be fit with the natural log of htTKV (LnTKV) as a function of time, the interaction between time and study arm, and clinical site. In order to account for the participant-level variabilities of baseline LnTKV as well as rate of change, the model intercept and slope will be allowed to vary if needed. A significant interaction between time and study arm could indicate a slowing of PKD progression due to metformin. This same model will be used to evaluate secondary efficacy outcomes such as kidney cyst volume (KCV), total liver volume (TLV), and liver cyst volume (LCV). Renal function, as measured by eGFR, will be compared between metformin and placebo using a similar Laird & Ware model as above. The TAME-PKD Pain questionnaire has over 50 questions. A priori we identify the following items of the TAME PKD Pain questionnaire as being most likely to differ across study arms after 2 years of study drug treatment: back pain frequency, impact of pain on physical activity and sleep, and abdominal distension symptoms. The response to each of these questions is a 5-point Likert scale. The sample size is small in the present study and we are likely to have low frequencies in each of the 5 categories. Thus, we will group the lowest 3 levels together with the top 2 levels when comparing these items across study arms.

Power and Sample Size: The proposed study is a phase II trial assessing safety and tolerability. We anticipate enrolling 48 participants per study arm with 15% attrition, resulting in an effective sample size of 40 per arm. We are not powered to detect meaningful differences in efficacy, but instead focus on 95% confidence interval width (two times the margin-of-error) for relevant point estimates within each study arm. For the primary safety outcome of SAEs, we will have the ability to estimate a CI width no larger than 0.16, assuming the proportion of participants who experience at least one SAE is no more than 6% (similar to the highest rates in the HALT trial (Schrier et al., 2014)). For the primary tolerability outcome of GSRS, we will have the ability to estimate a CI width no greater than 21, assuming standard deviations of the GSRS are below 1.3. With regard to the tolerability outcome of drug discontinuation, our anticipated sample size will allow the ability to estimate a CI width no larger than 0.25, assuming the rate of drug discontinuation is 15% (similar to TEMPO (Torres et al., 2012)). For the efficacy outcome of TKV, we assume the participants taking placebo will see a 5.51% annual increase in TKV (as seen in TEMPO (Torres et al., 2012)) with standard deviations for the slope and error of 0.041 and 0.044 (on the natural log scale), respectively. With our anticipated sample size, we will be able to estimate a margin-of-error of 1.5 percentage points for 95% intervals.

BIOMARKER IDENTIFICATION, AIM 2

1. Rationale: We hypothesize that metformin treatment will ameliorate ADPKD severity and progression in patients and that this beneficial effect occurs in large part through changes in the cellular bioenergetics and proliferative capacity of ADPKD kidney epithelial cells. Moreover, Boletta and colleagues recently identified a Warburg effect-like shift to excessive aerobic glycolysis, as evidenced by increased levels of certain key metabolic enzymes (e.g., lactate dehydrogenase A (LDHA), pyruvate dehydrogenase (PDH) kinase 1 (PDK1), and the pyruvate kinase M2 isoform (PKM2)), and a decrease in signaling through the AMPK pathway that occurs in ADPKD cystic epithelial cells as compared with normal kidney epithelial cells (22). Metformin promotes cellular AMPK activation (55), which may confer beneficial effects in the treatment of diseases such as the metabolic syndrome, diabetes, and polycystic ovary syndrome (PCOS) (56). Our previously published data demonstrate that metformin, via AMPK activation, inhibits both epithelial cellular proliferation through the mTOR pathway and CFTR-dependent fluid secretion into PKD cysts (Fig. 1), resulting in decreased cystogenesis and disease severity in vivo in rapidly progressing mouse models of ADPKD (18). For the studies proposed in Aim 2, we will investigate candidate metabolic biomarkers (Table 3) in urine based on the work of Boletta and colleagues and others (22). We hypothesize that glycolytic pathway enzymes will be progressively increased and markers of AMPK pathway activation will be progressively reduced with worsening ADPKD severity in patients. We further hypothesize that treatment of patients with metformin will tend to reverse these effects, which are indicative of the metabolic disturbance in ADPKD, and potentially thereby represent a beneficial therapeutic effect.
2. Methods and Study Design: We will measure key metabolites that are markers of glycolytic vs. oxidative metabolism and cAMP by enzymatic activity assays (Table 3A), candidate glycolytic pathway enzymes previously reported to be elevated in ADPKD (22) by ELISA assays (Table 3B), and known markers of AMPK pathway activation by immunoblotting (Table 3C). Assays for the various analytes will be performed as described in Table 3 (legend) at the various time points throughout the study specified in Table 2 (baseline at 0 mos., and then 1, 3, 6, 9, 12, 15, 18, 21, and 24 mos. after treatment initiation) for each of the patients (~40 in the metformin treatment group and ~40 in the placebo control group). Enzymatic assays will be fit to a standard curve at an appropriate dilution so that the levels fall within the linear range of the standard fit curve. We will analyze changes in the mean levels of each of the biomarkers over time for each patient and correlate these with metformin treatment status and disease severity/progression over time, as measured by htTKV measurements performed every 6 months and eGFR measured at each visit. In addition, in the cross-section of all of our patients, stratified by treatment group, we will correlate levels of each biomarker with htTKV and eGFR.
3. Statistical Plan and Data Analysis for Aim 2: All variables will be described using sample means or sample proportions along with 95% confidence intervals, depending on the nature of the outcomes. The primary goal of Aim 2 is to evaluate potential biomarkers that correlate with ADPKD disease severity and response to metformin. Our focus will be on both prognostic as well as predictive biomarkers of total kidney volume and eGFR. The analysis for prognostic biomarkers will be restricted to participants taking placebo and will utilize a Laird & Ware linear mixed model with the following predictors: time (months since baseline), a time-dependent covariate for the biomarker of interest, their interaction, and random effects for the intercept and slope. Apart from the ability to see whether a particular biomarker at baseline is related to disease severity and progression, we will also be able to estimate cross-sectional and longitudinal effects on TKV and eGFR among untreated participants.

   The analysis for predictive biomarkers will utilize the same mixed model with the following predictors: time, time-by-study arm interaction, baseline biomarker of interest, time-by-biomarker interaction, and the three-way interaction of these predictors. This model will give us the ability to identify subgroups of participants who may or may not benefit from metformin therapy.

4. Expected Results and Future Directions: In general, we will consider a particular biomarker to be predictive of ADPKD disease severity if it positively correlates with htTKV and negatively correlates with eGFR, our surrogate disease markers. Based on the reported Warburg effect on metabolism in ADPKD (22), we expect to see higher levels of the glycolytic enzymes LDHA, PKM2 and PDK1, indicative of a reliance on aerobic glycolysis, to correlate with ADPKD disease severity and/or progression in patients' urine samples. In addition, we predict that the lactate/pyruvate ratio will positively correlate and the succinate levels will negatively correlate with disease severity. We also predict that metformin will: (1) reduce the lactate/pyruvate ratio and increase succinate levels, which would suggest a shift from aerobic glycolysis to mitochondrial oxidative metabolism, and (2) decrease cAMP levels in urine, as it has been reported that metformin, independently of AMPK activation, inhibits cAMP production in cells (57). Elevated cAMP signaling is a well-established component of ADPKD pathogenesis that drives cystogenesis via enhanced CFTR-dependent fluid secretion into cysts (2). We also predict that treatment with metformin will tend to increase markers of AMPK pathway activation (pAMPK/AMPK ratio and pACC/ACC ratio) and decrease the pP70S6K/P70S6K ratio, a downstream target of mTOR. Finally, we predict that metformin-induced changes in these markers may be associated with a potentially beneficial response to metformin treatment on disease progression.

IMPACTS OF THE STUDY

Potential Short-term and Long-term Impacts of this Study: To test the potential of metformin as a novel pharmaceutical intervention to delay the progression of early ADPKD, we propose a double-blind, phase 2, multicenter parallel-group clinical trial that will evaluate the safety and tolerability of metformin in ADPKD patients with eGFR ≥50 ml/min over a two-year period. We will also evaluate the effect of the drug on TKV and eGFR. Exploratory analysis of urinary metabolomics and AMPK signaling pathway biomarkers related to the predicted effect of metformin will also be assessed. We expect that this study will demonstrate the safety and tolerability of metformin in the setting of treatment of ADPKD patients with only mildly decreased renal function. If this short-term expectation is realized, then a potential long-term impact for this study will be the planning and execution of a much larger phase III clinical trial that will be sufficiently powered to detect potential efficacy of metformin on disease progression.

One major advantage of this drug over other drugs that have been tested recently for ADPKD therapy is that metformin has a very long track record of clinical use and safety and tolerability, which is critical in this population because for any drug to be useful in slowing disease progression, it will likely need to be taken over decades, if not for the rest of these patients' lives. Of note, despite its primary usage in diabetic patients, metformin does not appear to cause clinically relevant hypoglycemia in non-diabetic subjects. Another advantage is that the drug is generic and very inexpensive, so there will likely be a potential for wide distribution to patients at relatively minimal costs to the healthcare system. A third advantage is that the drug is FDA-approved for other indications, so its potential off-label use will be more quickly translated to the clinic. Another short-term benefit is that we expect to identify and confirm the importance of urinary metabolomic biomarkers in ADPKD, which may reflect disease responsiveness to metformin. In the longer term, we expect that the identification of such markers could play a key role in ADPKD disease management as they could provide a relatively inexpensive and non-invasive tool to monitor disease severity, progression potential and response to metformin treatment and potentially other therapies that may be used alone or in combination with metformin.

Table 2: Schedule of Assessments for Screening, Baseline, and Follow-up Visits

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Month		-2	0	0.5		1	1.5	1.5		3	4	5	6	9	12	15	18	21	24	26
2	Week(s) between this visit and the next			1-2		3-4	5-6	7-12			13-24	Follow-up[b]	Follow-up[b]	25-36	37-48	49-60	61-72	73-84	85-96	97-104	Closeout
3	Visit Window-± days				±1	±1	±1	±1	±1	±1	±6	±6	±6	±6	±6	±6	±6	±6	±6	±6	±6
4	Visit Type[a]	Form #	S1-Screen	B0-Baseline	L 0.5	T 0.5	F1	L1.5	T1.5	L2	F3	L4	L5	F6	F9	F12	F15	F18	F21	F24	F26
5	# weeks SINCE Baseline visit				2	2	4	6	6	8	12	16	20	24	36	48	60	72	84	96	104
6	Informed Consent/HIPAA		X																		
7	Demographics	F3	X																		
8	Clinical History	F4	X																		
9	Past Med History-Comorbid Conditions	F5	X																		
10	Review of Symptoms	F6	X	X		X	X		X		X			X	X	X	X	X	X	X	X
11	BP and EKG[a] (*S1 and F26)	F7	X*	X			X				X			X	X	X	X	X	X	X	X*
12	Physical Exam	F8	X																	X	
13	Review of Medications	F9	X	X			X				X			X	X	X	X	X	X	X	X
14	Labwork:																				
15	CBC	F10	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X
16	Renal Panel (BUN, electrolytes, serum creatinine, glucose[f])	F10	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X
17	HbA1C	F10	X	X			X				X			X	X	X	X	X	X	X	X
18	LFTs	F10		X			X				X			X	X	X	X	X	X	X	X
19	Lipid	F10		X			X														
20	Urine Pregnancy[c]	F10	X	X			X				X			X	X	X	X	X	X	X	X
21	Plasma Lactate	F10		X			X				X			X	X	X	X	X	X	X	X
22	Vitamin B12	F10	X													X				X	
23	Baseline Clinical History	F11		X																	
24	Interval Medical History	F12		X		X	X		X		X			X	X	X	X	X	X	X	
25	Study Drugs	F13		X*		X*	X*		X*		X			X	X	X	X	X	X	X	
26	MRI[d]	F14		X										X		X		X		X	
27	GSRS	F16		X		X	X		X		X			X	X	X	X	X	X	X	X
28	SF-36 - TAME Quality of Life	F17		X			X				X			X	X	X	X	X	X	X	X
29	TAME Pain Questionnaire	F18		X			X				X			X	X	X	X	X	X	X	X
30	Urine for Biomarkers USC	F20		X			X				X			X	X	X	X	X	X	X	X
31	Blood and urine samples for Biobank-UM (optional)	F21		X			X				X			X	X	X	X	X	X	X	X
32	Blood for DNA isolation/ Genetic Sample UM (optional)	F21		X																	
33																					
34	a) Visit type: S=Screening B=Baseline/Randomization L=Lab-only follow-up F=in-center follow-up T=Telephone only follow-up																				
35	b) Additional Laboratory-only visits will be required monthly during months from months 7-24 without in-person visits for participants with eGFR<45 ml/min/1.73m2 during follow-up.																				
36	c) Among women of child-bearing potential only																				
37	d) MRI rescan window is 30 days																				
38	e) Titration schedule: 500mg daily → 500 mg bid → 1000mg/500mg → 1000 mg bid																				
39	f) For Screening and Baseline visits, fasting glucose is required. Fasting is defined as ≥ 8 hours																				

Table 3

A. Metabolites (enzymatic assay kits)

i. lactate
ii. pyruvate
iii. succinate
iv. cAMP
v. creatinine

B. Glycolytic Pathway Enzymes (ELISA kits)

i. Lactate Dehydrogenase A (LDHA)
ii. Pyruvate Dehydrogenase Kinase 1 (PDK1)
iii. Pyruvate Kinase M2 isoform (PKM2)

C. AMPK Pathway (Western blots)

i. pAMPK-alpha and total AMPK-αlpha
ii. phosphorylated Acetyl CoA Carboxylase (pACC) and total ACC
iii. phosphorylated ribosomal S6 kinase (P70S6K) and total P70S6K

Metabolomic Biomarkers to be Tested: Metabolites listed in A will be quantitated by enzymatic assays on 96-well plates with colorimetric read-outs. Glycolytic pathway enzymes listed in B will be quantitated by ELISA on 96-well plates with colorimetric read-outs. All samples will be assayed in triplicate as per manufacturers' recommendations. Ratios of phosphorylated levels to total levels of enzymes listed in C, as measured by triplicate samples loaded on SDS-PAGE gels for Western blotting, will be used as indicators of AMPK pathway activity (C). For all specimens, analyte levels will be normalized by the creatinine concentration from the same urine sample so that comparisons can be made across patients, conditions, and time points. For urinary proteins to be measured by immunoblotting (C), equal volumes (≥200 µl) of the urine samples will be first subjected to methanol-chloroform precipitation to concentrate proteins.

MR Imaging protocol for PKD Metformin study

Before each study, the MR scanner will be adjusted for proper shimming.

1. Breath-holding instruction will be provided, and the subject will be coached prior to MR scanning. Administration of oxygen via nasal cannula may help improve the breath-hold capacity, particularly for subjects with limited breath-hold capacity.

2. Subject will be placed supine on the MR table with his or her arms to the side.

3. A phased-array surface coil will be positioned with its center over the inferior costal margin, i.e. over the expected location of the kidneys.

4. Scout scan to locate the scan range of the entire kidneys. A stack of axial images to cover the most anterocaudal and posterocranial aspects of the kidneys is highly recommended.

5. The field-of-view (FOV) should be kept as small as possible (30-35 cm) without producing wrap-around artifacts.

6. (Entire Abdomen covering kidney and liver) Breath-hold, coronal T2 scan (SSFSE/HASTE **with** fat sat) at 9mm fixed slice thickness, usually achievable in a single breath-hold. Please make sure both kidneys and liver are imaged completely without missing any anterior or posterior portions.

7. (Kidney only) Breath-hold coronal T2 scan (SSFSE/HASTE **with** fat sat) at 3mm fixed slice thickness, which may require 1-4 breath-holds depending on the kidney size. Use as few breath-holds as possible. The first scan should cover the posterior aspect of the kidney. Neighboring image groups should be overlapped by a single 3mm slice. To determine correct table position choose the "shift-mean (starting point in GE)" of the second scan for example: the first shift-mean = -60mm, the number of slices in the first set =23, (23-1)x3=66mm, new shift mean =-60+66=6mm.

8. (Kidney only) Breath-hold coronal T1 scan (3D VIBE/FMPSPGR/LAVA **without** fat sat) at 3mm fixed slice thickness (acquisition will be performed at 6mm thickness and then the slice will be interpolated at 3mm, i.e., in GE, ZIP =1 in the slice direction). Keep the flip angle ≤15^{o}. To improve SNR, keep the Bandwidth low (62 kHz or 42 kHz) and/or increase the number of phase-encoding steps (be aware, the acquisition time will increase). In GE LAVA sequence, turning off "optimize flip for CNR" will allow to change the flip angle or bandwidth. **Do NOT** use parallel imaging (no SENSE, ASSET, iPAT or GRAPPA).

9. (Entire Abdomen covering kidney and liver) Breath-hold coronal 2D true-FISP (FIESTA) **without** fat sat at 3mm fixed slice thickness, which may require 2-3 breath-holds depending for the size of the kidney and liver. Use as few breath-holds as possible. The first scan should cover the posterior aspect of the kidney. The last scan covers the anterior liver. **No incomplete coverage or wrap of anterior liver onto posterior kidney**. Neighboring image groups should be overlapped by a single 3mm slice. To determine correct table position choose the "shift-mean (starting point in GE)" of the second scan for example: the first shift-mean = -60mm, the number of slices in the first set =23, (23-1)x3=66mm, new shift mean =-60+66=6mm.

10. (For Liver and Liver cyst volume) Breath-hold coronal T2 scan (SSFSE/HASTE) **without** fat sat of the kidneys with adjusted slice thickness, 3-6 mm, i.e. the slice thickness best attainable with a single breath-hold (The adjusted slice thickness may not remain the same in a follow-up MR scan if there is a change in the subject's breath-hold capacity or kidney size.) Repeat the scan over the liver with the same slice thickness. This scan and the scan for the kidney should share one overlapping liver slice (i.e., the most posterior slice of the liver scan should be identical to the most anterior slice imaging the liver in the kidney scan. If more than two scans are required to cover the anterior liver, again the neighboring scans should be overlapped by one slice.

After Image acquisition, images will be transferred to the Coordinating Center at University of Pittsburgh via CD-ROM. At each clinical site, a staff radiologist with expertise in abdominal MRI will review each scan for unexpected findings. In the event of such findings, the site investigators will notify the participant and –with the participant's permission – the participant's physician.

TAME –PKD Statistical Analysis Plan

Study Design & Objectives. The design of the proposed study is a double-blind, multicenter parallel-group randomized clinical trial. The scientific aims are as follows: to assess tolerability and safety of treating ADPKD patients with early disease (eGFR ≥50 ml/min) with metformin (Aim 1) and to evaluate potentially relevant biomarkers that correlate with ADPKD disease severity and response to metformin. This study will be conducted at 2 institutions in the United States (Tufts University and University of Maryland), and we will enroll adult patients, ages 18-60 years with ADPKD, and eGFR ≥50 ml/min/1.73m2.

Aim 1: We hypothesize that metformin, an agent that is widely used to treat type 2 diabetes mellitus and polycystic ovary syndrome, may serve as a novel therapy for individuals with early stage autosomal dominant polycystic kidney disease (ADPKD) by activating the metabolic sensor AMPK. To test this hypothesis, we propose a pilot-level double-blind, phase 2, parallel-group, placebo-controlled multicenter clinical trial to evaluate the safety and tolerability of metformin in ADPKD patients over a two-year period. In addition, we will evaluate the effect of metformin therapy on the rate of change in height-adjusted total kidney volume (htTKV) and estimated glomerular filtration rate (eGFR) as well as on metabolomic biomarkers of disease severity and progression.

Aim 2: We hypothesize that metformin treatment will ameliorate ADPKD severity and progression in patients and that this beneficial effect occurs in large part through changes in the cellular bioenergetics and proliferative capacity of ADPKD kidney epithelial cells. We will investigate candidate metabolic biomarkers in urine based on the work of Boletta and colleagues and others (22). We hypothesize that glycolytic pathway enzymes will be progressively increased and markers of AMPK pathway activation will be progressively reduced with worsening ADPKD severity in patients. We further hypothesize that treatment of patients with metformin will tend to reverse these effects, which are indicative of the metabolic disturbance in ADPKD, and potentially thereby represent a beneficial therapeutic effect.

Sample Size Calculation. We anticipate enrolling 48 participants per study arm with 15% attrition, resulting in an effective sample size of 40 per arm. We are not powered to detect meaningful differences in efficacy, but instead focus on a 95% confidence interval width (two times the margin-of-error) for relevant point estimates within each study arm. For the primary safety outcome of SAEs, we will have the ability to estimate a CI width no larger than 0.16, assuming the proportion of participants who experience at least one SAE is no more than 6% (similar to the highest rates in the HALT trial (Schrier et al., 2014)). For the primary tolerability outcome of GSRS, we will have the ability to estimate a CI width no greater than 21, assuming standard deviations of the GSRS are below 1.3. With regard to the tolerability outcome of drug discontinuation, our anticipated sample size will allow the ability to estimate a CI width no larger than 0.25, assuming the rate of drug discontinuation is 15% (similar to

TEMPO (Torres et al., 2012)). For the efficacy outcome of TKV, we assume the participants taking placebo will see a 5.51% annual increase in TKV (as seen in TEMPO (Torres et al., 2012)) with standard deviations for the slope and error of 0.041 and 0.044 (on the natural log scale), respectively. With our anticipated sample size, we will be able to estimate a margin-of-error of 1.5 percentage points for 95% intervals.

Interim & Final Analyses. We will not have any planned interim looks for stopping for efficacy, although safety and tolerability data will be monitored by the study DSMB at each meeting. The final analyses will be conducted once study follow-up is complete, after all data is cleaned, and once the study database is locked. We anticipate database lock will happen in September of 2020 with completion of primary analyses occurring in October of the same year.

Hypotheses. The primary study hypotheses are:

1) compared to placebo, participants taking metformin (titrated to 1000 mg twice daily or maximal tolerated dose) will have a better safety profile over 24 months

2) compared to placebo, participants taking metformin will have better tolerability, defined by change in the Gastrointestinal Symptom Rating Scale (GSRS) over 24 months.

The secondary hypotheses are:

3) compared to placebo, participants taking metformin will have a smaller annual % increase in height-adjusted total kidney volume (htTKV) over 24 months

4) compared to placebo, participants taking metformin will have a slower annual rate of decline in estimated glomerular filtration rates (eGFR) over 24 months.

Analysis Sets. The full analysis set will be based on an intention-to-treat (ITT) analysis with a per-protocol (PP) sensitivity analyses. The ITT analysis set will comprise all participants who have been randomized to either study arm, regardless of length of follow-up or actual intervention received. The PP analysis set will include participants who have "adhered" to study intervention, in this case defined as all participants who have successfully completed drug titration (completed at least F3 visit) with a maximum tolerate dose. The safety analysis set will be a subset of the ITT set and include all participants who have at least received and taken metformin or placebo.

Endpoints & Covariates. The primary outcomes are the safety and tolerability of using metformin in ADPKD patients with baseline eGFR ≥50 ml/min.

a. Assessment of Tolerability: Tolerability will be assessed using two standardized patient-reported instruments that will be administered at baseline, at each telephone visit during titration, and at every PCC visit. The most anticipated adverse effects of metformin are gastrointestinal (GI)-related. Accordingly, we will use the GSRS instrument to assess GI symptom burden. A widely used and validated 15-item questionnaire, the GSRS instrument has been used to compare the GI tolerability of different mycophenolate preparations in renal transplant recipients (39). The second assessment of tolerability will be based on responses (yes or no) to the following question "Can you tolerate this dose of the study drug for the rest of your life?" As described in Section 6e (Drug titration), a negative response will lead to down-titration of drug.
b. Safety Outcomes: At each study visit (telephone or PCC), patients will be asked if they had an interim hospitalization or medical encounter and if so, the nature of the event. The patient will sign a release of medical information form at the baseline visit and this will be used to obtain discharge summaries or other clinical documentation to determine the nature and severity of adverse events. Serious adverse events (SAE) will be defined as an undesirable experience occurring from the time a participant signs the informed consent (at the screening visit) until the end of the study, meeting 1 or more of the criteria of: 1) Resulting in death, 2) Non-elective hospitalization, 3) Life threatening (if patient continued on study drug would result in death), 4) Persistent or permanent harm or disability, 5) Exceeding the nature, severity or frequency of risk described in the protocol or 6) resulting in Congenital anomaly. Reporting requirements to the DCC and IRBs are described in Section 12 (Safety). A symptoms checklist (which lists the more common or concerning side-effects of the study drug, but allows for free text entry of other symptoms) will be collected at baseline and at each subsequent PCC visit. Hypoglycemia is a potential complication of metformin use in a non-diabetic population.
c. Adherence: Adherence with study medication will be assessed through pill counts, which will be performed by the study coordinator at each study visit. Patients that miss their in-person visit and thus do not refill their study drug will be contacted in a timely fashion by the study coordinator, who will have a record of the missed visit. Adherence with study visits will be assessed by tracking the number of missed study visits. During the drug titration telephone visits and the F1 in-person visit, the missed study visit window will be +/- 1 day; for subsequent visits a missed study visit will be documented as +/- 6 days. Practical measures to minimize inconvenience (parking, stipends if possible), maintaining communication with referring physicians, and other means of maintaining direct communication with the patient (follow-up and thank you cards after visits, birthday and holiday cards, small gifts) should also promote adherence. Finally, regular and frequent follow-up visits should also aid in the retention of study patients.
d. Patient-reported Outcomes: The Medical Outcomes Short Form 36 (SF-36) questionnaire is the most widely used instrument for measuring health-related quality of life (HRQOL), though scores have not been shown to discriminate among ADPKD patients with larger vs. smaller kidneys at eGFR >60 ml/min (40). The TAME Pain Questionnaire is a modified version of the Wisconsin Brief Pain Questionnaire (41) that measures the frequency and intensity of pain and symptoms relating to organ enlargement as well as their impact on everyday living. It was validated in a clinical population of ADPKD patients from a single center observational study and subsequently adapted for use in the HALT Study (42). The frequency of back pain and symptoms related to abdominal distension correlated with htTKV in a cross-sectional analysis of baseline questionnaire results in the HALT-PKD Study A cohort, which is a population with similar eGFR range as expected in the proposed study (43). GI symptoms from metformin may affect HRQOL, and thus we will also examine this at every visit. The GSRS (a widely-used, validated 15-item questionnaire, described earlier) and the SF-36 (as a generic measure of HRQOL) will be

administered by study staff (39, 44). The questionnaires will be administered at baseline and at in-person follow-up visits. In addition, on telephone visits during drug titration, the GSRS will be asked to evaluate common gastrointestinal symptoms. Each question is rated on a seven-point Likert scale, from no discomfort to very severe discomfort. For the GSRS the responses on the 15 items are reduced to average scores on 5 specific scales (diarrhea, reflux, constipation, abdominal pain, indigestion), identified from factor analysis. Among U.S. patients with gastro-esophageal reflux, internal reliability of GSRS scores on the outcomes that may be affected by metformin use was moderate to high (Cronbach's alpha: 0.61 to 0.83), and the instrument displayed good construct validity (45).

Handling of Missing Values. As a preventive measure, we will make every attempt to document all reasons for missing data. In addition, baseline characteristics will be compared between participants who do and do not withdraw from the study as a way to assess the impact of missing information and attrition. We will also compare the rates of lost-to-follow-up (LTF) between study arms.

Our general assumption will be that missing data is of the Missing at Random (MAR) mechanism. In this case, the use of linear mixed models for the primary analysis will be sufficient in reducing the impact that missing data has on biasing the primary results. Additionally, we will conduct a sensitivity analysis using multiple imputation via chained equations (MICE) to see how robust the overall inferences are.

There is the potential to have non-ignorable missingness (NMAR) on key secondary outcomes of htTKV and eGFR if there is substantial LTF/dropout or death. We will assess this by using shared parameter models to jointly estimate annual rate of change and time-to-event.

Statistical Analyses. All primary and secondary outcomes will be described using sample means or sample proportions along with 95% confidence intervals, depending on the nature of the outcomes. Volume outcomes from MRI (i.e. total kidney, kidney cyst, liver, liver cyst) that are known to be highly skewed will be assessed to see if suitable transformations are needed (i.e., natural log). Participant demographics and baseline clinical characteristics will be compared between study arms (Metformin versus Placebo) using two-sample t-tests or chi-squared tests of independence. All primary outcomes will be analyzed under intent-to-treat.

The primary objective of this phase II, multicenter clinical trial is to evaluate the safety and tolerability of metformin versus placebo with a secondary emphasis on efficacy. The safety outcome is defined by the occurrence of adverse events and serious adverse events (as defined in Section 11b) throughout the study. We will calculate cumulative incidence between study arms and use logistic regression to assess the association between study arms and the proportion of participants affected across various SAEs.

Tolerability will be defined by the GSRS scale. Using a linear mixed model, we will compare the change in overall GSRS score as a function of time (months since baseline), the interaction between time and study arm, and clinical site. We will also include a random intercept to allow for participant-level variability of baseline GSRS. Of primary interest is whether the interaction terms are significant, which would indicate the change over time differs between the metformin and placebo arms. We will compare drug discontinuation rates between study arms using Chi-squared tests of independence. Adherence (defined as >=80% of proportion of pills taken) will be compared between study arms using Chi-squared tests of independence. In addition, we will also utilize Kaplan-Meier curves, along with logrank tests for significance, to compare time-to-drug discontinuation between arms.

Secondary analyses: The annual rate of change in height-adjusted total kidney volume (htTKV) will be compared between Metformin and Placebo arms to give an indication of the efficacy of metformin. A Laird and Ware linear mixed model (51) will be fit with the natural log of htTKV (LnTKV) as a function of time, the interaction between time and study arm, and clinical site. In order to account for the participant-level variabilities of baseline LnTKV as well as rate of change, the model intercept and slope will be allowed to vary if needed. A significant interaction between time and study arm could indicate a slowing of PKD progression due to metformin. This same model will be used to evaluate secondary efficacy outcomes such as kidney cyst volume (KCV), total liver volume (TLV), and liver cyst volume (LCV). Renal function, as measured by eGFR, will be compared between metformin and placebo using a similar Laird & Ware model as above. The TAME-PKD Pain questionnaire has over 50 questions. A priori we identify the following items of the TAME PKD Pain questionnaire as being most likely to differ across study arms after 2 years of study drug treatment: back pain frequency, impact of pain on physical activity and sleep, and abdominal distension symptoms. The response to each of these questions is a 5-point Likert scale. The sample size is small in the present study and we are likely to have low frequencies in each of the 5 categories. Thus, we will group the lowest 3 levels together with the top 2 levels when comparing these items across study arms.

Exploratory Aim 2: The primary goal is to evaluate potential biomarkers that correlate with ADPKD disease severity and response to metformin. Our focus will be on both prognostic as well as predictive biomarkers of total kidney volume and eGFR. The analysis for prognostic biomarkers will be restricted to participants taking placebo and will utilize a Laird & Ware linear mixed model with the following predictors: time (months since baseline), a time-dependent covariate for the biomarker of interest, their interaction, and random effects for the intercept and slope. Apart from the ability to see whether a particular biomarker at baseline is related to disease severity and progression, we will also be able to estimate cross-sectional and longitudinal effects on TKV and eGFR among untreated participants. The analysis for predictive biomarkers will utilize the same mixed model with the following predictors: time, time-by-study arm interaction, baseline biomarker of interest, time-by-biomarker interaction, and the three-way interaction of these predictors. This model will give us the ability to identify subgroups of participants who may or may not benefit from metformin therapy.

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