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TITLE:
Metformin versus Tolvaptan in adults with Autosomal Dominant Polycystic Kidney Disease (ADPKD): a phase 3a, independent, multi-centre, 2 parallel arms randomized controlled trial

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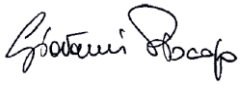
MEDICAL WRITING	Name: Prof. Loreto Gesualdo;  Name: Dr. Giovanni Piscopo 

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A. LIST OF ABBREVIATIONS

ADPKD	Autosomal dominant polycystic kidney disease
ABG	Arterial-blood gas
AEs	Adverse Events
ALI	Acute liver injury
ALT	Alanine aminotransferase
AMPK	Adenosine Monophosphate activated Protein Kinase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the concentration time curve
AVP	Serum vasopression levels
cAMP	Cyclic adenosine monophosphate
cAMP	Cyclic adenosine monophosphate
CBC	Complete blood count
CFTR	Cystic fibrosis transmembrane conductance regulator
CKD	Chronic kidney disease
CKD1	Cyclin-dependent kinase1
CKD-EPI	Chronic kidney disease-Epidemiology
Cmax	Maximum concentration
CRISP	Consortium for Radiologic Imaging Study of Polycystic Kidney Disease
CT	Computed tomography
CTEP	Cancer Therapy Evaluation Program
CYP	Cytochrome
EGF	Epidermal growth factor
eGFR	Estimated Glomerular Filtration Rate
EMA	European Medicines Agency
EoTx	End of Treatment
ESRD	End-stage renal disease
EU	European Union
GCP	Good Clinical Practice
HBV	Hepatitis B infection
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hazard Ratio
ICF	Informed consent forms
ICH	Conference on Harmonization
ID	Identification number

IEC	Indipendent ethics committee
IMP	Investigational medicinal product
IRE	Irreversible electroporation
IVRS	Interactive voice response system
MALA	Metformin associated lactic acidosis
MCP1	Monocyte chemoattractant protein 1
MPR	Multi-planar Reconstructions
MRI	Magnetic Resonance Imaging
mTOR	Mammalian target of rapamycin
mTORi	Mammalian target of Rapamycin inhibitors
NCI CTCAE	Cancer Institute Common Terminology Criteria for Adverse Events
NICE	National Institute for Health and Clinical Excellence
NSAID	Nonsteroidal anti-inflammatory drugs
OCT	Organic Cations Transporter
PC	Polycystins
PCOS	Polycistic Ovary Syndrome
PK	Pharmacokinetics
PKD1	Polycystin 1
PKD2	Polycystin 2
pVBGA	Peripheral venous blood gas analysis
QALY	Quality-adjusted life years
RAAS	The renin–angiotensin system
RCTs	Randomized controlled trials
RNA	Ribonucleic acid
SAE	Serious adverse event
SIADH	The syndrome of inappropriate antidiuretic hormone secretion
SmPC	Summary of product characteristics
SSN	Sistema Sanitario Nazionale
TKV	Total kidney volume
uEGF	Urinary epidermal growth factor
uMCP-1	Urinary monocite chemotactic peptide-1
UNL	Upper Normal Limit
USA	United States of America
VR	Volume rendering
WOCBP	Women of childbearing potential
EDC	Electronic Data Capture

1. SYNOPSIS

TITLE	Metformin versus Tolvaptan in adults with Autosomal Dominant Polycystic Kidney Disease (ADPKD): a phase 3a, independent, multi-centre, 2 parallel arms randomized controlled trial
SPONSOR	Università degli Studi di Bari Dipartimento dell'Emergenza e dei Trapianti di organi (D.E.T.O.)
PHASE	III
RATIONALE	Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder occurring in 1:400–1:1.000 live births. It affects 4 to 6 million persons worldwide and about 205.000 people in Europe (EU). This figure is equivalent to 4 in 10.000 people and thus below the prevalence threshold of 5 in 10.000 used to designate a disease as rare in EU. Renal cyst development and expansion in ADPKD involves both fluid secretion and abnormal proliferation of cyst-lining epithelial cells. The chloride channel of the cystic fibrosis transmembrane conductance regulator (CFTR) participates in secretion of cyst fluid, and the mammalian target of rapamycin (mTOR) pathway may drive proliferation of cyst epithelial cells. CFTR and mTOR are both negatively regulated by AMP-activated protein kinase (AMPK). Metformin, a drug widely used, is a pharmacological activator of AMPK. We found that metformin stimulates AMPK, resulting in inhibition of both CFTR and the mTOR pathways. Metformin induces significant arrest of cystic growth in both in vitro and ex vivo models of renal cystogenesis. In addition, metformin administration produces a significant decrease in the cystic index in two mouse models of ADPKD. These results suggest a possible role for AMPK activation in slowing renal cystogenesis as well as the potential for therapeutic application of metformin in the context of ADPKD
OBJECTIVES	Objective of the study is to assess if a two-year course of 1500 mg oral metformin is effective and safe in treatment of ADPKD, as compared to the actual gold-standard therapy, tolvaptan (Jinarc®)
ENDPOINTS	Primary outcome of the study is to evaluate the difference between Metformin and Tolvaptan in annualized slope of eGFR (CKD-EPI) for individual subjects, that will be calculated using an appropriate baseline and post-randomization assessment. The key secondary endpoint is the percent change from baseline in htTKV as measured by CT-scan at 24 months. The safety endpoin include: - changes from baseline in creatinine; - vital signs; - laboratory values including liver function tests, rate

	of aquaretic AEs, thus including serum sodium, rate of Metformin Associated Lactic Acidosis, blood insulin and glucose levels, HOMA test in both treatment groups
STUDY DESIGN	This is a phase 3a, independent, multi-centre, parallel arms, randomized controlled trial comparing efficacy and safety of metformin and tolvaptan in ADPKD
STUDY POPULATION	This trial will enroll approximately 150 tolvaptan and metformin naïve subjects affected by Type I-truncating ADPKD, as they have grater probability of progression
STUDY TREATMENT	The trial contemplates a 2 weeks screening period (included in a 9 months total recruitment period) during which 3 visits have to be collected (the 1st and the 2nd in three days, and the 3rd after biochemical analyses performed during the first two visits have been reviewed). The subject's eligibility for the trial will be confirmed by the mean of eGFR calculated from the 2 pre-treatment, central-lab, serum creatinine assessments. Longer screening periods (up to 4 additional weeks) are acceptable for subjects needing stabilization after changing or discontinuing other treatments, especially anti-hypertensives and diuretics. Once eligibility is assessed, patients will undergo non-contrast enhanced CT-scan of kidneys (if not performed within six months prior to randomization). Randomization visit will occur on Day -29. During this visit patients will be randomized (in a ratio 1:1 tolvaptan:metformin) to each arm of treatment and will start an IMP titration period (3 weeks from -28 to -8). Subjects not tolerating the minimum IMP dose will be considered "Titration failures" and will complete End of Treatment (EoTx) visit assessments and will be followed up after 7 days by phone call to assess any ongoing AEs. Subjects tolerating the minimum IMP dose enter the unblind run-in period (1 week from Day -7 to -1). During the "Run-in" phase, subjects will continue on a stable IMP dose to confirm tolerability over a longer period. At the end of the run-in period (Day -1), subjects not tolerating the minimum IMP dose will be considered "Run-in failures" and will complete EoTx visit assessments and will be followed up after 7 days by phone call to assess any ongoing AEs. Subjects completing the run-in will start the open-label 24 months treatment period, during which visits will be collected three-monthly. At the end of the 24th month, and in case of early treatment cessation, follow-up period (3 weeks from +8 to +21) will start, during which 2 visits have to be collected. No IMP will be administered during this period
NUMBER OF SUBJECTS AND SITES	This trial will enroll approximately 150 tolvaptan and metformin naïve subjects and will be conducted in about 11 Italian Hospital Nephrology Departments

INCLUSION CRITERIA	1) Men and women aged between 18 and 50 years 2) eGFR (CKD-EPI) ≥ 45 ml/min/1,73 m2 3) Genetic Diagnosis of Type I ADPKD truncating mutation 4) Signed and dated informed consent
EXCLUSION CRITERIA	1) Women of childbearing potential (WOCBP) who do not agree to practice 2 different methods of birth control or remain abstinent during the trial and for 30 days after the last dose of IMP. If employing birth control, 2 of the following precautions must be used: vasectomy of partner, tubal ligation, vaginal diaphragm, intrauterine device, birth control implant, condom, or sponge with spermicide. Non-childbearing potential in women is defined as female subjects who are surgically sterile (ie, have undergone bilateral oophorectomy or hysterectomy) or female subjects who have been postmenopausal for at least 12 consecutive months. 2) Women who are breast-feeding and/or who have a positive pregnancy test result prior to receiving investigational medical product (IMP). 3) Treatment with acarbose, guar gum, cimetidin, phenprocoumon, oral anticoagulants, thrombolytic drugs, diuretics, ranolazin, cephalexin. 4) Evidence of active systemic or localized major infection at the time of screening. 5) Hepatic impairment or liver function abnormalities other than that expected for ADPKD with typical cystic liver disease during the screening period as defined by: o AST O ALT >8x UNL o AST O ALT >5x UNL >2 WEEKS o AST O ALT >3x UNL E BT >2x UNL OR INR >1,5 o AST O ALT >3x UNL E SIGNS AND SYMPTOMS OF LIVER DAMAGE (fatigue, anorexy, nausea, vomiting, right hypocondrium pain, fever, jaundice, skin rash, itching) 6) Acute or chronic disease causing tissue hypoxia (e.g.: myocardial failure, severe arythmias, myocardial infarction, respiratory failure, liver failure, alcohol acute intoxication, alcoholism, dehydration). 7) Previously diagnosed diabetes already in treatment with other hypoglycemic drugs. 8) Ongoing breast feeding. 9) Use of any other investigational drug or treatment up to 4 weeks before enrollment and during the treatment phase. 10) Known hypersensitivity to metformin and its derivatives. 11) Psychiatric disorders and any condition that might prevent full comprehension of the purposes and risks of the study. 12) Malignancies within three years before enrolment in the

	study. 13) HIV, HBV, HCV infection. 14) Urinary tract obstruction.
INVESTIGATIONAL PRODUCT(S)	Metformin (Zuglimet®) and Tolvaptan (Jinarc®)
STATISTICAL ANALYSIS	We plan to recruit a total of 150 patients which are currently within reach of the network coordinated by the proponent and composed by 11 Nephrology Centres. This network treats a total of 1500 (already genetically studied) patients of which we expect (based on standard response rates recognized in the population) acceptance to participate in the study to a value of approximately 40% of patients. These will be then allocated to the experimental and control intervention. The selected sample is adequate to evaluate a significant reduction in the slope of eGFR at 2 years by 10%, which is a clinically relevant piece of information at the current state of knowledge, as well as a complete assessment of the benefits-harms trade-off of the two interventions.
STUDY DURATION	**Study Duration:** The trial has a 36 months overall duration, that include a 9 months recruitment period **Screening Period:** Has a 2 weeks duration, it is included in the 9 months recruitment period **Treatment Period:** It has a 25 months duration. Each month lasts 28 days. It includes the 3 weeks titration period and the 1 week run-in period. **Post-Treatment Follow-up Period:** It lasts 21 days **Total Study Duration:** About 3 years

2. INTRODUCTION AND RATIONALE

2.1 Disease Background

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder occurring in 1:400–1:1.000 live births. It affects 4 to 6 million persons worldwide and about 205.000 people in Europe (EU). This figure is equivalent to 4 in 10.000 people and thus below the prevalence threshold of 5 in 10.000 used to designate a disease as rare in EU (1).

ADPKD is a heterogeneous disorder existing in two types: type I is caused by mutations in the *PKD1* gene (encoding for Polycystin 1) and accounts for 85 to 90 percent of cases (2); type II is caused by mutations in the *PKD2* gene (encoding for Polycystin 2) accounting for 10 to 15 percent (3).

Tremendous cystic enlargement of both kidneys is characteristic of ADPKD and even if relatively oligosymptomatic during the first three decades of life, renal insufficiencyimpaired renal function usually occurs suddenly after the 4th decade. Even with normal renal function, patients present with hypertension, hematuria, polyuria, flank pain, renal stones, and are prone to recurrent urinary tract infections.

In addition to renal cysts, clinically relevant cysts are also common in liver (particularly in women), pancreas, intestine, seminal vesicles, arachnoid membrane and spinal meninges. Patients with ADPKD have an increased risk of aortic aneurysms and heart-valve defects. Cardiovascular complications are the most common cause of morbidity and mortality (4). Other complications comprise abdominal wall hernias and colon diverticula, and some kindred have five-fold increased risk of sudden death from ruptured intracerebral aneurysms compared to the general population (5).

The mechanism of cysts formation in ADPKD has been comprehensively analyzed in recent years, and several preclinical studies and clinical trials have been carried out in order to assess specific drugs' efficacy and safety in treatment of ADPKD.

The polycystins (PC) constitute a subfamily of protein channels and are thought to regulate intracellular calcium signaling. They are expressed in many tissues, including renal tubular epithelia, hepatic bile ducts, and pancreatic ducts. PC-1 is localized in the primary cilium and structures and is involved in cell-cell contacts (e.g., tight junctions). PC-1 probably functions as a receptor and/or adhesion molecule, whereas PC-2, a calcium-permeable nonselective cation channel, is found on the primary cilium, endoplasmic reticulum, and the plasma membrane. These PCs interact to form the PC complex, which is localized in the primary cilia and plays a role in intracellular calcium regulation.

Cystogenesis in ADPKD is not fully understood, but mutations in PKD1 or PKD2 lead to a reduction in intracellular calcium, an increase in cyclic adenosine monophosphate (cAMP), activation of protein kinase A, and an increase in sensitivity of collecting duct principal cells to the constant tonic effect of vasopressin. The disruption in calcium signaling, coupled with enhanced cAMP signaling, activates downstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion, and interstitial inflammation.

Abnormal epithelial chloride secretion occurs through the cAMP-dependent transporter encoded by the CFTR gene and plays an important role in generating and maintaining fluid-filled cysts in ADPKD. Other pathogenic pathways may include activation of mTOR, Wnt, or hedgehog signaling; direct effects of PC-1 fragments on gene transcription; and increased aerobic glycolysis (6). Interestingly, both these pathogenic pathways may be influenced by metformin (7).

To date, only Tolvaptan (Jinarc®) has been proved as effective in reducing cystogenesis in specific subsets of ADPKD patients and therefore approved (in 2013 by EMA and in 2017 by AIFA) for the treatment of this disease. However, it requires additional monthly monitoring, because of potential liver toxicity. Moreover it causes discomfort due to significant polyuria. AIFA, also according to the Società Italiana di Nefrologia (SIN) position statement (8), stated that, despite Jinarc® is indicated for all patients affected by ADPKD and chronic kidney disease (CKD) stages I-II-III, only those with evidence of fast progressing disease can benefit from Sistema Sanitario Nazionale (SSN) reimbursement of the treatment costs.

AIFA Reimbursement criteria indicate those patients that may benefit the most from the therapy.. These criteria are the following:

- CKD stage II and IIIa (based on GFR estimated using CKD-EPI or measured)
- Nephromegaly as defined by:
    - Kidney length > 16,7 cm or
    - Total Kidney Volume (TKV) > 750 ml or
    - Heigh normalized TKV (htTKV) > 600 ml/m
- Rapidly progressing disease as defined by:
    - GFR loss > 5 ml/min/1,73m^2/year within 12 months or
    - GFR loss > 2,5 ml/min/1,73m^2/year within 5 years or
    - TKV increase ≥ 5%/year as recorded by repeated CT or RMN measurement (at least 3) or
    - htTKV Class 1C-1D-1E according to Mayo Classificator (9) or
    - PROPKD score > 6 (10)

A 12 months retrospective study conducted (11) on 243 patients in Northern Europe enrolled between April and December 2014 showed that employment rates were lowest

among dialysis patients; only 18% of those aged < 65 years were employed. The overall work productivity loss ranged between 9% in CKD stage 1–3 to 42% in dialysis patients.

The average total annual costs amounted to €9,919 in CKD stage 1–3, €16,761 in CKD stage 4–5, €74,015 in dialysis patients and € 31,496 in transplant recipients (p<0.0001). Productivity loss was a major driver of costs across all stages of disease, reaching 72% of total costs in CKD stage 1–3 and ranging from €8,339 in CKD stage 1–3 to €19,598 in dialysis patients.

Direct medical costs were substantial among dialysis patients, with maintenance dialysis alone accounting for 58% of total costs. Costs associated with ADPKD increase substantially as the patient progresses to dialysis. Interventions that can slow the progression of the disease have the potential to lead to substantial reductions in costs and patient burden.

On the other hand, as reported by Erickson et al. costs analysis (12) of the TEMPO trial (13), initiating therapy with tolvaptan in 40 year-olds with ADPKD and eGFR of 80 ml/min/1.73m^2 delayed the median age of developing ESRD by 6.3 years in women and 6.8 years in men. Tolvaptan therapy yielded an average increase in life expectancy of 2.8 years in women and 2.3 years in men. Therapy with tolvaptan yielded an increase of 1.2 discounted quality-adjusted life years (QALY) in women and 1.1 discounted QALYs in men. Total lifetime medical costs were substantially higher in patients receiving tolvaptan ($858,300 higher in women and $830,100 higher in men). Combining health benefits and costs, for this patient group, tolvaptan therapy cost $720,600 per QALY gained in women and $769,500 in men. In a balanced cohort of women and men, tolvaptan increased median time to ESRD by 6.5 years, increased life expectancy by 2.6 years, and cost $744,100 per QALY gained.

Tolvaptan therapy was less cost-effective when given to patients with slower rates of eGFR decline. When tolvaptan is given to patients with a rate of eGFR decline of 2.4 ml/min/1.73m^2/year (the rate observed in a large cohort of ADPKD patients) tolvaptan cost $1,215,200 per QALY gained. Because, for a given eGFR when starting tolvaptan, older patients are more likely to die from other causes before experiencing CKD progression, tolvaptan was less cost-effective in older patients. For instance, tolvaptan cost 54% more per QALY gained in 65 year-olds ($1,147,800 per QALY gained) compared to 40 year-olds. The cost per QALY gained also depended on the eGFR at which tolvaptan was begun. The cost per QALY gained in 40 year-olds was 16% lower when tolvaptan is started at an eGFR of 75 ml/min/1.73m^2 ($626,938 per QALY) compared to the base case with a starting eGFR of 80 ml/min/1.73m^2.

Tolvaptan therapy would be more cost-effective if it were offered at a lower price. At a willingness to pay threshold of $100,000 per QALY gained, therapy with tolvaptan would be cost-effective in both men and women if offered at or below $1,155 per month (80% below the base case price and 94% below the current price per milligram). At a willingness to pay threshold of $50,000 per QALY gained, therapy with tolvaptan would be cost-effective in both men and women if offered at or below $805 per month (86% below the base case price and 96% below the current price per milligram).

A daily 1500 mg dose of metformin may cost about €6 per month (not taking into account potential costs deriving from potential side effects), thus yielding a substantial money sparing for National Health System. Moreover, as metformin has been shown to be largely safe in different sub-sets of population (as non-diabetic obese patients, pregnant women and fetuses, and diabetics affected by CKD, even in elderly), we do not envisage negative implications in ADPKD .

2.2 Investigational Products

Metformin is a biguanide, able to forcedly activate AMPK (AMP dependent kinase),It is used in form of tablets orally administered to treat diabetes and insulin resistance.

The mechanism of action of metformin is to acutely decrease hepatic glucose production, mostly through transient inhibition of the mitochondrial respiratory-chain complex 1, circulating insulin and intestinal glucose absorption, and improving peripheral tissue utilization of glucose, without causing hypoglicemia.

The resulting decrease in hepatic energy status activates the AMP-activated protein kinase (AMPK), a cellular metabolic sensor, providing a generally accepted mechanism for metformin action on hepatic gluconeogenic activity. Direct, albeit transient and mild mitochondrial effects of metformin may explain theoretical risk of lactic acidosis in diabetic patients.

Metformin is now indicated only for the treatment of Type 2 Diabetes Mellitus and no studies have been completed using metformin for the treatment of ADPKD in human, both "*in vivo"* and *"in vitro"*, nor the effect of metformin on glucose metabolism of non diabetic patients has been widely studied.

Nonetheless a number of studies showed the existence of insulin resistance in ADPKD (14) and the same CKD induces insulin-resistance and causes Type 2 Diabetes Mellitus. Moreover, a large amount of evidences indicate that metformin is widely recognized for improving insulin resistance among patients affected by PolyCystic Ovary Syndrome (15) and that metformin may be safe also in non diabetic patients (16,17).

Tolvaptan is a Vasopressin V2 receptor antagonist, mainly used (in form of tablets orally administered) to treat hyponatremia related to SIADH. It has been recently approved in

Italy for the treatment of ADPKD (Jinarc®) and in this trial Jinarc® will be used in accordance with its marketing authorization.

2.2.1 Non-clinical Data

Recently, new insights in ADPKD pathogenesis and therapy derived from some preclinical studies (7,18). They found that metformin stimulates AMPK, resulting in inhibition of both CFTR and mTOR pathways (that drive respectively intracystic fluid secretion e cell proliferation) and significant arrest of cystic enlargement in "*in vitro"* canine models and in "*ex vivo*" and "*in vivo*" murine models of renal cystogenesis.

2.2.2 Clinical Data and Potential Risks and Benefits

At this time, we have data from several clinical trials: three tested mammalian Target Of Rapamycin inhibitors (mTORi), one tested Octreotide-LAR, a long acting somatostatin analogue, and another tested Pravastatin. None of these drugs has been found effective in reversing or at least slowing its progression.

Tolvaptan was clinically effective in delaying decline of renal function, as determined by changes in serum creatinine concentrations over 3 years, in an international, multicenter, clinical trial and in its open-label extension in subjects with CKD stage 1 to 3 due to ADPKD (19,20). Recently, Tolvaptan has been shown to be effective up to stage 4 (21).

At the moment, Metformin is only indicated for the treatment of Diabetes Mellitus but it has been safely used in several clinical trials in non diabetic patients. Metformin is widely and safely used for the treatment of diabetes in CKD patients, but we have no clinical data regarding its efficacy in slowing disease progression in ADPKD, except those from retrospective analyses.

Metformin in Pregnancy

Metformin easily passes through the placental filter, reaching therapeutic concentrations in fetal blood.

Several trials have been conducted in order to evaluate metformin in non diabetic pregnant women affected by insulin resistance.

The EMPOWaR (22) study enrolled 449 pregnant women aged over 16, who were randomized (1:1) to take Metformin (at a dose from 500 mg a day to 2500 mg a day) or placebo, since the 12th or 16th week of gestation till the end of gestation. Primary outcome of the study was the fetus weight at birth, secondary outcome was the Maternal Index of Insulin Resistance at the end of gestation. No statistical differences were observed between the two groups. Actually, follow-up of the newborns is ongoing.

A British phase 2/3 trial (17) – NCT01273584 – enrolled 450 pregnant obese women (BMI>35) aged more than 18, between the 12th and the 18th gestational week. They were randomized (1:1) to be treated with Metformin (3000 mg a day) or placebo till the end of pregnancy. No differences were observed in newborns weight (primary outcome) and in fetal or neonatal adverse events incidence. Despite this, among pregnants treated with metformin there was a lower incidence of eclampsy.

The Norwegian phase 3 trial (PregMet) (23) – NCT00159536 – enrolled 257 pregnant women, affected by PCOS (Polycistic Ovary Syndrome), during the first trimester of pregnancy. They were randomized (1:1) to take Metformin (2000 mg a day) or placebo.

No differences were found in the primary outcome (a composite of gestational diabetes, eclampsia, preterm birth) nor in the fetal birth weight. Moreover, a post-hoc analysis of this study showed lower blood insulin levels in pregnant treated with metformin, but non difference was found in blood from fetal umbilical vein and artery, thus demonstrating that metformin does not influence glycemic control in non-diabetic healthy individuals (24).

In another study (25), 40 pregnant women affected by PCOS, some of whom affected by diabetes, were randomized (1:1) to receive Metformin (1700 mg a day) or placebo since 19th week to delivery. No differences were found in terms of glucose homeostasis.

A small randomized clinical trial (26) conducted in diabetic pregnants (treated both with metformin or insulin) showed that insulin blood levels are not able to influence metformin pharmacokinetic and OCT-2 mediated metformin transport.

Metformin in Polycystic Ovary Syndrome (PCOS)

PCOS is characterized by ovarian micro-polycistosis, hyperandrogenism, hirsutism, insulin-resistance.

Recently, a clinical trial (27) randomize (1:1) 40 women affected by PCOS to take Metformina (1500 mg a day) or placebo for 3 months. Women treated with metformin had significantly lower blood pressure, but no differences were found in terms of glycemic control.

Similarly, another phase 2 trial (28) – NCT00151411 – randomized (1:1) 114 women affected by PCOS to take Metformin (2000 mg a day) or placebo for six months and showed no differences in terms of insulin-sensitivity or glycemic control (secondary outcome).

A British cross-over trial (29) conducted on 30 PCOS affected patients treated with Metformin or placebo, did not prove any advantage in terms of glycemic control but showed a better arterial stiffness and blood pressure control in women treated with metformin.

Conversely, an Argentine trial (30) – NCT00679679 – showed that metformin treatment (1500 mg a day), if associated with a correct lifestyle, may improve glycemic control in women affected by PCOS, without differences in terms of adverse events.

Discrepancies between these studies may be explained by the post-hoc analysis of a cross-over trial (31), that metformin treatment seemed to influence glycemic control and insulin resistance only in obese women affected by PCOS, whilst no difference was found in incidence of adverse events.

On the other hand, three other trials (32–34) revealed that metformin treatment in women affected by PCOS, even not obese, may improve insulin resistance, without causing adverse events.

These evidences suggest that Metformin treatment in non diabetic individuals can not cause hypoglycemia, even if interfering on glycemic control, in a manner dependent on consistency of insulin resistance (35).

Finally, a recent clinical trial conducted on women affected by PCOS (36) – NCT01389778 – suggested that metformin may improve “Glucose Effectiveness”, that is the glucose capacity to stimulate itself uptake or to suppress itself production, under basal insulin levels.

Metformin in Obesity

Several randomized clinical trials conducted in non diabetic obese patients showed that metformin (at a dosage variable from 100 mg to 2000 mg a day), can improve insulin resistance compared to placebo, especially if associated with physical exercise, without causing hypoglycemia or severe adverse events (37–41).

Metformin in Neoplastic disease

Several clinical trials (mainly of phase 2) (42–55) explored the efficacy of metformin (compared to placebo) at different dosages, in addition to conventional chemotherapy.

These studies were inconclusive in showing metformin efficacy in reducing tumoral cell proliferation and prolonging disease-free survival. Moreover, only one trial showed al little

increase of lactate production in tumor tissue, but none of them showed an increased incidence of Metformin Associated Lactic Acidosis (MALA) (56).

Metfomin in CKD

Currently metformin is widely used in diabetic patients affected by CKD, and consequently in ADPKD, nevertheless no study has been published directly evaluating this drug efficacy in slowing or possibly reverting ADPKD.

The concerns over metformin and renal impairment arise from the perceived risk of lactic acidosis in such patients. Lactic acid is produced when there is tissue hypoperfusion with resulting hypoxia. The condition occurs when the production of lactate exceeds its metabolism and removal. Risk factors for lactic acidosis include sepsis, shock, myocardial infarction, cardiac, respiratory or hepatic failure and hypoxemia.

Metformin associated lactic acidosis (MALA) has been reported, with a frequency ranging from 1 to 47 cases per 100,000 person years and a recent meta-analysis showed that the incidence of lactic acidosis wasn’t higher among patients treated with metformin compared to those treated with other antidiabetics (57). On the other hand, as the mortality rate is around 50% this is a feared complication (58).

One study examined metformin levels in elderly patients with eGFR of 30-60 ml/min, or >60 ml/min, at doses of 850mg per day and 1700mg per day respectively. Metformin levels and plasma lactate levels were unchanged in both treatment groups, suggesting that metformin usage was safe in elderly patients with significant renal impairment (58).

A recent large cohort study conducted in the USA attempted to quantify the association between metformin use and hospitalization with acidosis across the range of eGFR, accounting for change in eGFR stage over time.

In the primary cohort (n = 75 413), mean (SD) patient age was 60.4 (15.5) years, and 51% (n = 38 480) of the participants were female. There were 2335 hospitalizations with acidosis over a median follow-up of 5.7 years (interquartile range, 2.5-9.9 years). Compared with alternative diabetes management, time-dependent metformin use was not associated with incident acidosis overall (adjusted hazard ratio [HR], 0.98; 95% CI, 0.89-1.08) or in patients with eGFR 45 to 59 mL/min/1.73 m2 (adjusted HR, 1.16; 95% CI, 0.95-1.41) and eGFR 30 to 44 mL/min/1.73 m2 (adjusted HR, 1.09; 95% CI, 0.83-1.44). On the other hand, metformin use was associated with an increased risk of acidosis at eGFR less than 30 mL/min/1.73 m2 (adjusted HR, 2.07; 95% CI, 1.33-3.22). Results were consistent when new metformin users were compared with new sulfonylurea users (adjusted HR for eGFR 30-44 mL/min/1.73 m2, 0.77; 95% CI, 0.29-2.05), in a propensity-matched cohort (adjusted HR for eGFR 30-44 mL/min/1.73 m2, 0.71; 95% CI, 0.45-1.12), when baseline insulin users were excluded (adjusted HR for eGFR 30-44 mL/min/1.73 m2, 1.16; 95% CI, 0.87-1.57), and in the replication cohort (adjusted HR for eGFR 30-44 mL/min/1.73 m2, 0.86; 95% CI, 0.37-2.01).

These results suggest that metformin use is associated with acidosis only at eGFR less than 30 mL/min/1.73 m2. Caution should be exercised when using metformin in patients with type 2 diabetes and low eGFR (59).

In conclusion, lactic acidosis seems to be a rare event in patients affected by CKD treated with metformin. Guidelines from the UK National Institute for Health and Clinical Excellence (NICE) and European Medicines Agency (EMA) suggests that metformin dose should be reduced by 1/3 at an estimated glomerular filtration rate (eGFR) of 45 ml/min (in this case avoiding a dosage greater than 1000 mg a day), and stopped at an eGFR of 30 ml/min.

In this trial we decided not to associate metformin with tolvaptan, but to directly compare metformin versus the gold standard tolvaptan for the following reasons:

- The “on-treatment” period has a duration of two years and, as showed by the TEMPO 3/4 trial (13), tolvaptan seems to be effective only after the first year of treatment;
- The open label extension, the TEMPO 4/4 (20), showed that those patients still on tolvaptan after the first 3 years of treatment had kidney volume growth rate grater than those who received placebo during the first three years of the trial. On the other hand, no advantages were found in terms of eGFR declining rate in those who started tolvaptan later;
- Even if rarely, tolvaptan treatment may cause liver injury, that may virtually increase the risk of MALA;
- Tolvaptan, being an aquaretic drug, may cause dehydration and a virtual increase of the MALA risk;
- Preclinical studies conducted in rodents models of diabetes insipidus induced with tolvaptan, indicate that metformin may significantly interfere with tolvaptan pharmacodynamics, thus limiting its effects.

For all these reasons we can argue that metformin treatment, alone, may be safe and effective in slowing ADPKD progression, at the same extent of tolvaptan. If not, patients not receiving tolvaptan for two years, would not be detrimentally deprived of treatment, as tolvaptan starts to be effective after the first year, and its effects are not additional year by year (20).

2.2.3 Pharmacokynetics and Pharmacodynamics

Metformin has a poor oral bioavailability in the horse. Instead, oral bioavailability in the cat is similar to that in human. In fact, a dosage of 2 mg/Kg in the cat (equivalent to that

commonly used in human) allows to enrich therapeutic plasma levels (60). Furthermore, no pharmacokinetics differences have been found between diabetic and non diabetic cats, in whom metformin effects on glycemic control depend on basal insulin levels (61).

Absorption

After an oral dose of metformin, T_{max} is reached in 2.5 h. Absolute bioavailability of a 500 mg metformin tablet is approximately 50-60 % in healthy subjects. After an oral dose, the non-absorbed fraction recovered in faeces was 20-30 %.

After oral administration, metformin absorption is saturable and incomplete. It is assumed that the pharmacokinetics of metformin absorption is non-linear. At the usual metformin doses and dosing schedules, steady state plasma concentrations are reached within 24-48 h and are generally less than 1 µg/mL. In controlled clinical trials, maximum metformin plasma levels (C_{max}) did not exceed 5 µg/mL, even at maximum doses.

Food decreases the extent and slightly delays the absorption of metformin. Following administration of a dose of 850 mg, a 40 % lower plasma peak concentration, a 25 % decrease in AUC and a 35 min prolongation of time to peak plasma concentration was observed. The clinical relevance of this decrease is unknown.

Distribution

Plasma protein binding is negligible. Metformin partitions into erythrocytes. The blood peak is lower than the plasma peak and appears at approximately the same time. The red blood cells most likely represent a secondary compartment of distribution. The mean Vd ranged between 63 – 276 L.

Biotransformation

Metformin is excreted unchanged in the urine. No metabolites have been identified in humans.

Elimination

Renal clearance of metformin is > 400 mL/min, indicating that metformin is eliminated by glomerular filtration and tubular secretion. Following an oral dose, the apparent terminal elimination half-life is approximately 6.5 h. When renal function is impaired, renal clearance is decreased in proportion to that of creatinine and thus the elimination half-life is prolonged, leading to increased levels of metformin in plasma.

In humans, oral bioavailability of metformin does not differ between diabetics and non diabetics, nor between men and women; it is reduced by concomitant assumption of food and it does not proportionally increase as dosage increases (61).

Plasma levels of metformin are mainly influenced by age and eGFR (62–64), than by OCT (Organic Cations Transporter) polymorphisms. In fact, Duong and coll. (63) have demonstrated that, in order to avoid MALA onset, metformin plasma levels have to be lower than 2,5 mg/l, thus it is useful to adapt metformin dosage to eGFR, avoiding to overcome these ratios: 500 mg of metformin if eGFR lower than 15 ml/min, 1,000 mg if eGFR <30 ml/min, 2,000 mg if <60 ml/min, 3,000 mg if <120 ml/min.

Diuretics (65,66), even if able to induce eGFR reduction because of dehydration, are not able “per sè” to reduce metformin clearance, unlike proton pump inhibitors. As they block OCT (67).

Even if metformin is excreted un-modified by the kidney, liver failure can itself reduce metformin excretion, thus increasing metformin toxicity (68).

Metformin effects on glycemic control are directly proportional to basal glucose and insulin levels; in fact in healthy individuals metformin has no influence on fasting glucose levels, but significantly reduces after-meal insulin levels (69).

Metformin activates AMPK (AMP dependent Protein Kinase) in a dose dependent manner, thus causing forced inhibition of mTOR (mammalian Target of Rapamycin) and CFTR (Cystic Fibrosis Transmembrane conductance Regulator).

Very recent published data suggests that the mechanism of action of metformin in vivo may involve pathways other than those related to AMPK. It is critically important to determine whether the potential therapeutic effects of metformin that has been identified in the context of ADPKD are due to its capacity to activate AMPK or are, instead, due to its effects on other targets.

Recent research indicates that metformin can decrease cellular levels of cAMP. This is especially relevant in the setting of ADPKD because a substantial body of research has demonstrated that elevation of cAMP promotes cyst growth in vitro and in vivo. Furthermore, drug therapies that reduce cAMP are able to slow cyst growth both in mouse models of ADPKD as well as in human ADPKD patients (70).

A recent paper from the group of Dr. Alessandra Boletta reported that cells homozygous for ADPKD-causing mutations feature substantial perturbations in energy production (71). These cells exhibit very high levels of glycolysis and low levels of oxidative metabolism, reminiscent of the Warburg effect that is seen in tumor cells. As a result of the very high levels of glycolysis the cytoplasmic levels of ATP are very high and levels of active AMPK are consequently very low. These data further support the idea that small molecule AMPK-activators may have therapeutic benefit in ADPKD.

2.2.4 Justification for the route of administration, dosage and dosage regimens

Dose choice has been done on the basis of Takiar et al. study (7). They examined *"in vivo"* only one dose known to activate AMPK (daily i.p. injections of metformin 300 $mg \cdot kg^{-1} \cdot d^{-1}$ dissolved in a 5% dextrose solution). When considered on a simple milligram per kilogram body weight basis, this dose appears considerably higher than the current maximum dose

prescribed for patients with diabetes or polycystic ovary syndrome. However, human-equivalent dose extrapolation is calculated more accurately based on body surface area than on weight. When this calculation is performed for a 60-kg adult, the dose used in our mouse studies extrapolates to a daily dose of ~1,500 mg (7), well within the range in which metformin is safely used in humans.

After oral administration, metformin absorption is saturable and incomplete. It is assumed that the pharmacokinetics of metformin absorption is non-linear. For this reason, a split doses regimen has been chosen for metformin administration (one cap every eight hours).

Patients randomized to tolvaptan, will start the treatment according to AIFA indications (45 mg followed, 8 hours later, by 15 mg), then they will up-titrate (every week, if tolerated) to 60 mg + 30 mg and then to 90 mg + 30 mg.

Patients on metformin, will start to take metformin once a day (500 mg), between meals. Every week they will up-titrate to 500 mg twice a day (if tolerated) and then to 500 mg thrice a day.

2.3 Rationale of the study

Renal cyst development and expansion in ADPKD involves both fluid secretion and abnormal proliferation of cyst-lining epithelial cells. The chloride channel of the cystic fibrosis transmembrane conductance regulator (CFTR) participates in secretion of cyst fluid, and the mammalian target of rapamycin (mTOR) pathway may drive proliferation of cyst epithelial cells. CFTR and mTOR are both negatively regulated by AMP-activated protein kinase (AMPK). Metformin, a drug widely used, is a pharmacological activator of AMPK. We found that metformin stimulates AMPK, resulting in inhibition of both CFTR and the mTOR pathways. Metformin induces significant arrest of cystic growth in both in vitro

and ex vivo models of renal cystogenesis. In addition, metformin administration produces a significant decrease in the cystic index in two mouse models of ADPKD. These results suggest a possible role for AMPK activation in slowing renal cystogenesis as well as the potential for therapeutic application of metformin in the context of ADPKD.

Currently three randomized clinical trial are evaluating Metformin effectiveness in slowing ADPKD progression.

A phase II, double-blinded randomized placebo-controlled trial ("TAME" study – NCT02656017) of 26 months duration will include non diabetic adults (n = 96) aged 18-60 years, with an eGFR ≥50 mL/min/1.73 m2 and ADPKD, recruited from university-based practices in Baltimore and Boston. Participants will be randomized in 1:1 ratio to metformin or placebo at 500 mg once daily, increased every 2 weeks to a maximum of 1,000 mg twice daily as tolerated. Dose is decreased if eGFR falls to 30-45 mL/min/1.73 m2 and discontinued at eGFR < 30 mL/min/1.73 m2.

The primary outcomes are safety, assessed by the rates of hypoglycemia, elevated lactic acid levels, adverse events, and tolerability assessed by the Gastrointestinal Severity Rating Scale and maximum tolerated dose of study medication. Secondary outcomes include changes in total kidney and liver volumes, pain, and health-related quality of life, and changes in urinary metabolomics biomarkers (72).

Another phase II double-blinded randomized placebo-controlled trial – NCT02903511 –of 12 months duration will include non diabetic adults (n = 50) aged 30-60 years, with an estimated glomerular filtration rate (eGFR) between 50-80 mL/min/1.73 m^2 and ADPKD, recruited from university-based practices in Denver. Participants will be randomized in 1:1 ratio to metformin or placebo at 500 mg once daily, increased every 2 weeks to a maximum of 1,000 mg twice daily as tolerated.

The primary outcomes are safety, assessed as percentage of participants who at the end of 12 months are still prescribed the full-randomized dose of metformin, and the percentage of participants who are prescribed at least 50% of the randomized dose. Secondary outcomes include changes in total kidney volume measured by MRI at baseline and 12 months, Change in kidney function calculated from serum creatinine measurements at baseline and after 3, 6, 9 and 12 months, Rate of Serious Adverse Events (73).

Moreover a Dutch cross-over clinical trial will evaluate metformin efficacy in attenuating aquaretic effects of tolvaptan (74)

2.4 Population of the Study

This trial will enroll approximately 150 tolvaptan and metformin naïve subjects affected by Type I-truncating ADPKD, as they have grater probability of progression (10). Moreover data from Tempo trials (3/4 and 4/4) (19,75) and from its post-hoc analysis (76) revealed that patient with faster disease progression had greater beneficial effects from therapy.

Male and female adults will be enrolled, from 18-50 years of age with eGFR greater than 45 ml/min/1,73 m^2

The study will be conducted in 11 Hospital Nephrology Departments. Patients will be selected from local databases and subjects will be enrolled till the estimated sample size is achieved.

Only subjects tolerating a run-in period of tolvaptan or metformin will enter the on-treatment period, in order to limit subsequent withdrawal due to lack of tolerability.

3. OBJECTIVES

Objective of the study is to assess if a two-year course of 1500 mg oral metformin is effective and safe in treatment of ADPKD, as compared to the actual gold-standard therapy, tolvaptan (Jinarc®).

3.1 Primary objectives

Currently, drugs' efficacy in ADPKD has been evaluated by means of eGFR and TKV variations. In ADPKD, kidney function may remain preserved until the 4th to 6th decade of life, when advanced renal cystic involvement has occurred. In fact, measured GFR is a poor marker of disease severity and progression, especially in early phases, when it is preserved through compensatory hyperfiltration during a prolonged period (typically decades), but eventually declines sharply. Despite this, to date, eGFR remains the standard to assess kidney function in randomized clinical trials in ADPKD. By contrast, TKV in relation to age can identify patients with progressive disease. TKV is an accurate estimate of kidney cyst burden and associates with pain, hypertension, gross hematuria, proteinuria or albuminuria, and loss of kidney function. TKV increases exponentially in virtually every ADPKD patient, with an average of 5–6%/year in adults. Elevated TKV, particularly when used together with age and kidney function, identifies individuals who are at higher risk for progression to ESRD. Precise measurement of TKV is necessary in clinical trials to assess the impact of therapeutic interventions over short periods of time and can be obtained by planimetry or stereology analysis of CT images.

3.2 Secondary objectives

Main secondary objective is to assess metformin efficacy (as compared to tolvaptan) in reducing the increase of TKV.

Moreover, it will be evaluated the rate of onset of metformin and tolvaptan related Adverse Events (AEs) namely Metfromin Associated Lactic Acidosis (MALA) and Acute Liver Injury (ALI), respectively.

4. TRIAL DESIGN

This is a phase 3a, independent, multi-centre, parallel arms, randomized controlled trial comparing efficacy and safety of metformin and tolvaptan in ADPKD. The overall design is illustrated in figures 1,2,3,4.

Each investigator has both ethical and legal responsibility to ensure that subjects being considered for inclusion in this trial are given a full explanation of the protocol and of their role and responsibilities in the proposed research. This will be documented on a written ICF that shall be approved by the same institutional review board/independent ethics committee (IRB/IEC) responsible for approval of this protocol. In addition, the protocol explanation may include recorded or electronic means of education, which will also meet IRB/IRC approval. Each ICF will include the elements required by the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guideline and local regulatory requirements and must adhere to the ethical principles that have their origin in the Declaration of Helsinki. Subject insurance and the publication policy for the trial will be provided and documented according to DM 14 Luglio 2009.

Written informed consent will be obtained from all subjects (or their guardian or legal representative, as applicable for local laws). Investigators may discuss the availability of the trial and the possibility for entry with a potential subject without first obtaining consent. However, informed consent must be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for this trial,

including withdrawal from current medication(s). When this is done in anticipation of, or in preparation for, the research, it is considered to be part of the research.

Once the appropriate essential information has been provided to the subject and fully explained in layman's language by the investigator (or a qualified designee) and it is felt that the subject understands the implications of participating, the IRB/IEC-approved written ICF shall be personally signed and dated by both the subject and the person obtaining consent (investigator or designee), and by any other parties required by the IRB/IEC. The subject shall be given a copy of the signed ICF; the original shall be kept on file by the investigator. All of the above mentioned activities must be completed prior to the subject's participating in the trial. Every phase of the trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement.

The trial contemplates a **2 weeks screening period** (included in a 9 months total recruitment period) during which 3 visits have to be collected (the 1st and the 2nd in three days, and the 3rd after biochemical analyses performed during the first two visits have been reviewed). The subject's eligibility for the trial will be confirmed by the mean of eGFR calculated from the 2 pre-treatment, central-lab, serum creatinine assessments. Longer screening periods (up to 4 additional weeks) are acceptable for subjects needing stabilization after changing or discontinuing other treatments, especially anti-hypertensives and diuretics. In this case Subjects must sign ICF and then agree to be switched to an alternate form of therapy in order to be eligible. As part of this stabilization or wash-out period different lab safety tests may be performed. Subjects who fail to meet trial requirements during the screening period may be rescreened at a later date.

Once eligibility is assessed, patients will undergo non-contrast enhanced CT-scan of kidneys (if not performed within six months prior to randomization).

Randomization visit will occur on Day -29. During this visit patients will be randomized (in a ratio 1:1 tolvaptan:metformin) to each arm of treatment and will start an **IMP titration period** (3 weeks from -28 to -8). Subjects not tolerating the minimum IMP dose will be considered “Titration failures” and will complete End of Treatment (EoTx) visit assessments and will be followed up after 7 days by phone call to assess any ongoing AEs. Subjects tolerating the minimum IMP dose enter the unblind **run-in period** (1 week from Day -7 to -1). During the “Run-in” phase, subjects will continue on a stable IMP dose to confirm tolerability over a longer period. At the end of the run-in period (Day -1), subjects not tolerating the minimum IMP dose will be considered “Run-in failures” and will complete EoTx visit assessments and will be followed up after 7 days by phone call to assess any ongoing AEs. Subjects completing the run-in will start the open-label **24 months treatment period**, during which visits will be collected three-monthly. At the end of the 24th month, and in case of early treatment cessation, **follow-up period** (3 weeks from +8 to +21) will start, during which 2 visits have to be collected. No IMP will be administered during this period.

Assessments at the Randomization (Day -29), End of IMP Run-in Period (Day -1), End of each three-monthly of on-therapy period will include those for points 2 to 8 of screening period plus IMP dispensation.

Assessments during EoTx Visits (in case of Titration failure, Run-in failure, early treatment cessation) and Follow-up visits will include those for points 2 to 8 of screening period. Non contrast enhanced CT-scan of the kidneys for TKV measurement will be also performed at the end of Month 24.

Patients treated with tolvaptan, will anyhow undergo monthly visits during the first 18 months of therapy (as prescribed by the additional monitoring requested by AIFA), then every three months.

4.1 Screening Period (up to Day -30)

No investigational treatments will be administered during the screening period.

Preliminary eligibility for the trial will be initially assessed using the subjects' historical laboratory or imaging data.

During this period, the subject's eligibility for the trial will be confirmed using historical genetic test data and imaging data of the kidneys to support a diagnosis of ADPKD and to verify the level of CKD primarily due to ADPKD and not to other renal (hypoplasia) or metabolic (diabetic or hypertensive nephropathy) disorders.

Eligibility will be confirmed by the mean of eGFR calculated from the subjects' 2 pre-treatment, central-lab, serum creatinine assessments (collected at least 24 hours apart). The final screening visit on Day -29 will not be scheduled until laboratory results from the second screening visit (V2) are received and evaluated. The eGFR values will be estimated based on the Chronic Kidney Disease-Epidemiology (CKD-EPI) formula. Subjects will be told that they will receive tolvaptan or metformin during the subsequent periods of the trial.

On Day -30, patients will undergo TKV measurement by means of Ct- or MRI-scan (if not already performed within the 6 months before the enrollment). Then, patients will be randomized to receive either metformin or tolvaptan and they will be told to start IMP assumption since the day after randomization.

Those randomized in metfromin arm will receive Zuglimet® 500 mg (2 packages, each of them containing 3 blisters; every blister contains ten 500 mg coated tablets).

Those randomized to tolvaptan arm will receive Jinarc® 45/15 mg (2 packages, each of them containing 4 blisters; every blister contains seven 15 mg tablets of and seven 45 mg tablets).

The screening period will last 1-2 weeks, but may be extended up to an additional 8 weeks for subjects who require modification of medical care or further medical evaluation specifically for this trial. This may include, for example, stabilizing anti-hypertensive regimens for subjects discontinuing diuretics or “wash-out” of other investigational agents.

4.2 Randomization visit (Day -30)

Randomization process will be carried out by a WEB-based, internet-accessible, system (e-trial platform). Investigators willing to include a patient should log-in into the e-trial platform and complete the registration form.

The patient allocation number and the treatment arm will be immediately TRASMITTED on line without blinding.

Any controversial eligibility assessment will be discussed with the Study Chair.

Each patient will be informed via a cover letter including clear instructions on participation, drug use, dosing and prescription.

Randomization visit may coincide with CT-scan visit.

4.3 IMP Titration Period (since Day -28 up to Day -8)

Subjects randomized in tolvaptan arm will be given a split dose of 45/15 mg tolvaptan (unblind) with upward titration every 7 days to 45/15 mg, 60/30 mg, up to a maximum dose

of 90/30 mg per day over 3 weeks. Jinarc® has to be taken between meals; the first dose upon waking and the second dose after eight hours from the first.

Dose up-titration will be done after tolerability check (by phone call the patient will be asked: “Are you having troubles taking this drug? Are you able to tolerate this dose for the rest of your life?”). Up-titration will be done the day after tolerability check, in Day -22 and in Day -15.

Subjects who are unable to tolerate at least 45/15 mg of daily tolvaptan will be considered “Run-in failures” and will complete an EoTx visit and be followed up after 7 days by phone call to assess any ongoing AEs.

Subjects randomized to metformin arm will be given a single dose of 500 mg of metformin for the first week (unblind), then a split dose of 500/500 mg metformin with upward titration after 7 days to 500/500/500 mg, (that is the maximum trial dose of metformin per day). Zuglimet® has to be taken between meals; the first dose upon waking, the second and the third daily dose after eight hours from the previous dose.

Dose up-titration will be done after tolerability check (by phone call the patient will be asked: “Are you having troubles taking this drug? Are you able to tolerate this dose for the rest of your life?”). Up-titration will be done the day after tolerability check, in Day -22 and in Day -15.

Subjects who are unable to tolerate at least 500/500 mg of daily metformim will be considered “Run-in failures” and will complete an EoTx visit and be followed up after 7 days by phone call to assess any ongoing AEs. Subjects tolerating the minimum IMP dose will enter the unblind run-in period (1 week from Day -7 to -1).

Assessments during up-titration phone call will include:

1) Assess IMP tolerability

2) Assess AEs, if reported

4.4 IMP Run-in Period (since Day -7 up to Day -1)

Subjects tolerating at least 45/15 mg tolvaptan or 500/500 mg metformin may enter the unblind, run-in period (1 week duration). Subjects will continue on a stable IMP dose to confirm tolerability over a longer period.

At the end of the run-in period, subjects not tolerating at least 45/15 mg tolvaptan or 500/500 mg metfromin will be considered “Run-in failures”. They will complete EoTx visit assessments and be followed up after 7 days by phone call to assess any ongoing AEs.

Subjects found ineligible to be randomized and continue in this trial must have a 7-day follow-up visit.

4.5 Unblind Treatment Period (Day 0 to Month 24)

Only subjects completing the IMP run-in period tolerating at least 45/15 mg of tolvaptan and 500/500 mg of metformin will be allowed upon entry to this unblind period.

In the metformin arm, during the treatment period visits will be scheduled every 3 months (each month has 28 days duration), within 3 days of the end of the trimester.

In tolvaptan treated patients, visits will be performed every month for the first 18 months, then every three months (each month has 28 days duration), within 3 days of the end of the month or the trimester (as prescribed by the additional monitoring requested by AIFA).

Beside the clinic visit and dispensing new bottles of IMP, subjects will have blood drawn on the last day of this period for efficacy and safety measures.

The treatment duration of these subjects will be 24 months from the end of IMP run-in. Subjects not continuing in this trial will complete EoTx visit assessments and be followed for 7 days to assess any ongoing AEs.

From that point forward, every effort to maintain adherence and continuation of the subjects until the end of the trial should be undertaken. If continued tolerability becomes an issue, subjects may temporarily interrupt treatment as needed.

All subjects have the right to withdraw their consent from further participation in the trial at any time without prejudice.

Regardless of discontinuation of IMP, the subjects are expected to complete all monthly visits and assessments including the Month 24 visit.

4.6 Follow-up Period (up to Day +21 since Month 24 or End of Treatment)

Randomized subjects will enter the follow-up period after they complete the unblind, randomized treatment period, or after their EoTx visit, if they discontinued IMP. The follow-up period will be for 21 days in duration.

There will be no scheduled visits/assessments during the first week of the follow-up period. After the first week, 2 visits should be scheduled during the remaining 2 weeks of the follow-up period (between Day +8 and Day +21). The first visit should be scheduled on approximately Day +8, the second visit on approximately Day +21. These visits will be clinic visit.

4.7 End of Treatment End of Trial

The end of trial date is defined as the last date of last contact with the subject. This does not refer to overall trial duration. The end of trial date and timing for follow-up assessments will be individualized for each subject.

Randomized subjects will have their last scheduled treatment 25 months from their date of randomization. If a subject discontinues IMP before Month 25, the last date that the subject received IMP will be recorded as EoTx.

4.8 Measures to Minimize/Avoid Bias

Only subjects who reach Day -1 and are who have indicated that they would likely be able to tolerate the minimum dose of IMP “for the rest of their lives” at a level of 45/15 mg for tolvaptan and of 500/500 mg for metformin will be eligible to enter the un-blind, randomized treatment period.

Immediately prior to randomization, and at all subsequent visits or site-subject contacts, the subject will be reminded of the importance of their commitment to continue participation in the trial.

Randomization will be 1:1, tolvaptan:metformin. Randomization process will be carried out by a WEB-based, internet-accessible, system (e-trial platform). Investigators willing to include a patient should log-in into the e-trial platform and complete the registration form.

Each patient will be informed via a cover letter (that is the informed consent form) including clear instructions on participation, drug use, dosing and prescription.

5. POPULATION OF THE STUDY

5.1 Number of Subjects and Sites

This trial will enroll approximately 150 tolvaptan and metformin naïve subjects affected by Type I-truncating ADPKD, as they have grater probability of progression (10). Moreover data from Tempo trials (3/4 and 4/4) (19,75) and from its post-hoc analysis (76) revealed that patient with faster disease progression had greater beneficial effects from therapy.

Male and female adults will be enrolled, from 18-50 years of age with eGFR greater than 45 ml/min/1,73 m^2

The study will be conducted in about 11 Italian Hospital Nephrology Departments. Patients will be selected from local databases and subjects will be enrolled till the estimated sample size is achieved. Only subjects tolerating a run-in period of tolvaptan or metformin will enter the on-treatment period, in order to limit subsequent withdrawal due to lack of tolerability.

5.2 Inclusion Criteria

1) Men and women aged between 18 and 50 years

2) eGFR (CKD-EPI) ≥ 45 ml/min/1,73 m2

3) Genetic Diagnosis of Type I ADPKD truncating mutation

4) Signed and dated informed consent

5.3 Exclusion Criteria

1) Women of childbearing potential (WOCBP) who do not agree to practice 2 different methods of birth control or remain abstinent during the trial and for 30 days after the last

dose of IMP. If employing birth control, 2 of the following precautions must be used: vasectomy of partner, tubal ligation, vaginal diaphragm, intrauterine device, birth control implant, condom, or sponge with spermicide. Non-childbearing potential in women is defined as female subjects who are surgically sterile (ie, have undergone bilateral oophorectomy or hysterectomy) or female subjects who have been postmenopausal for at least 12 consecutive months.

2) Women who are breast-feeding and/or who have a positive pregnancy test result prior to receiving investigational medical product (IMP).

3) Treatment with acarbose, guar gum, cimetidin, phenprocoumon, oral anticoagulants, thrombolytic drugs, diuretics, ranolazin, cephalexin.

4) Evidence of active systemic or localized major infection at the time of screening.

5) Hepatic impairment or liver function abnormalities other than that expected for ADPKD with typical cystic liver disease during the screening period as defined by:

- AST O ALT >8x UNL
- AST O ALT >5x UNL >2 WEEKS
- AST O ALT >3x UNL E BT >2x UNL OR INR >1,5
- AST O ALT >3x UNL E SIGNS AND SYMPTOMS OF LIVER DAMAGE (fatigue, anorexy, nausea, vomiting, right hypocondrium pain, fever, jaundice, skin rash, itching)

6) Acute or chronic disease causing tissue hypoxia (e.g.: myocardial failure, severe arythmias, myocardial infarction, respiratory failure, liver failure, alcohol acute intoxication, alcoholism, dehydration).

7) Previously diagnosed diabetes already in treatment with other hypoglycemic drugs.

8) Ongoing breast feeding.

9) Use of any other investigational drug or treatment up to 4 weeks before enrollment and during the treatment phase.

10) Known hypersensitivity to metformin and its derivatives.

11) Psychiatric disorders and any condition that might prevent full comprehension of the purposes and risks of the study.

12) Malignancies within three years before enrolment in the study.

13) HIV, HBV, HCV infection.

14) Urinary tract obstruction.

5.4. Withdrawal of Consent

All subjects have the right to withdraw their consent from further participation in the trial at any time without prejudice. The investigator can also discontinue a subject's participation in the trial at any time if medically necessary. Unless the subject provides their written withdrawal of consent or there is other written documentation by the investigator confirming the subject's verbal intent to completely withdraw from the trial, subjects should be followed for all protocol-specified evaluations and assessments.

Complete withdrawal of consent requires a subject's refusal also of all of the methods of follow up.

Withdrawal of consent is a critical trial event and therefore should be approached with the same degree of importance and care as is used in initially obtaining informed consent. The reasons for a subject's intended withdrawal need to be completely understood, documented and managed to protect the rights of the subject and the integrity of the trial.

A subject may initially express his desire to interrupt or discontinue IMP administration, which is not equivalent to a complete withdrawal of consent for further participation.

A subject may, however, indicate that further trial participation is creating a burden on their work or social schedule. Therefore, the investigator should determine if the subject can continue participation in the trial if modifications to his/her treatment and/or schedule of assessments can be accommodated.

Only subjects who withdraw their permission for all of the above degrees of follow-up are considered to have completely withdrawn their consent to participate in the study.

In all cases of impending IMP discontinuation or consent withdrawal, investigators will be given instructions to meet and discuss with the subject their options of continuing in the trial, preferably on therapy. The investigator should ensure understanding and documentation of the reasons for the subject's desire to withdraw consent.

If a subject in randomized treatment period wishes to withdraw from the trial:

The investigator should first seek to understand the subject's motivation and wherever possible make accommodations to prevent treatment discontinuation or complete withdrawal of consent and maintain the fullest compliance with

1. Participation in a subset of protocol specified follow-up procedures (by a frequency schedule and method as agreed by subject and staff).
2. Participation in all regularly scheduled, study-related follow-up visits and EoTx visits.
3. Contact of the subject by trial personnel, even if only by telephone, to assess current medical condition and obtain necessary medical or laboratory reports relevant to the trial's objectives.

4. Contact of alternative person(s) who have been designated in source records as being available to discuss the subject's medical condition, even if only by telephone, mail or e-mail (e.g., family, spouse, partner, legal representative, friend, neighbor, physician).
5. Access to medical information from alternative sources (e.g., hospital/clinic medical records, referring doctor's notes, public records, dialysis, transplantation or vital registries, social media sources).

6. TREATMENT

6.1 Treatment dose and schedule

Subjects will be instructed to take the following doses for titration:

- 1 tablet 45 mg upon waking and 1 tablet 15 mg approximately 8 to 9 hours later for 1 week;
- 1 tablet 45 mg + 1 tablet 15 mg upon waking and 2 tablets 15 mg approximately 8 to 9 hours later for 1 week;
- 2 tablets 45 mg upon waking and 2 tablets 15 mg approximately 8 to 9 hours later for 1 week.

During run-in period, subjects will take the maximum dose of tolvaptan achieved during titration (or at least 45/15 mg).

For the Metformin titration period and subsequent run-in period, subjects will receive 2 packages at the first visit of metformin 500 mg.

Subjects will be instructed to take the following doses for titration:

- 1 tablet 500 mg upon waking for 1 week;
- 1 tablet 500 mg upon waking and 1 tablet 500 mg approximately 8 to 9 hours later for 1 week;

- 1 tablet 500 mg upon waking, 1 tablet 500 mg approximately 8 to 9 hours later and 1 tablet 500 mg approximately 8 to 9 hours later for 1 week.

During run-in period, subjects will take the maximum dose of metformin achieved during titration (or at least 500/500 mg).

If the subject cannot tolerate the lowest permitted doses, he/she will be deemed a titration failure or a run-in failure.

During the on-treatment period, the patient will take the maximum tolerated dose achieved during titration and run-in. The subject will stay on that dose for the entire trial, if not otherwise contraindicated by AEs or not tolerated.

Patients on metformin experiencing eGFR reduction below 45 ml/min, can not take more than 1000 mg a day of metformin (thus reducing from 1500 mg a day to 1000 mg a day).

Those patients on Jinarc will receive 1 package containing 4 blisters (irrespectively of the dosage).

Those patients on Zuglimet will receive 2 packages (all containing 3 blisters of 10 tablets) if on 500/500 mg a day, 3 packages if on 500/500/500 mg a day.

6.2 Investigational product

6.2.1 Investigational product description

Tolvaptan is provided as tablets. Metformin is provided as coated tables.

6.2.2 Packaging and labeling

All IMP will be provided to the investigator(s) by the promoter of the Coordinating Center or designated agent.

Tolvaptan (Jinarc®) will be provided as 3 types of packages:

1) package containing 4 blisters of tolvaptan. Each blister contains 14 tablets: 7 15 mg tablets (triangular, in a green background), and 7 45 mg tablets (squared, in a gray background);

2) package containing 4 blisters of tolvaptan. Each blister contains 14 tablets: 7 30 mg tablets (rounded, in a purple background), and 7 60 mg tablets (rectangular, in a golden background);

3) package containing 4 blisters of tolvaptan. Each blister contains 14 tablets: 7 60 mg tablets (rectangular, in a golden background); and 7 90 mg tablets (pentagonal, in an orange background).

Metformin (Zuglimet®) will be provided as a package containing 3 blisters. Each blister contains 10 500 mg tablets.

Each package used will be labeled to clearly disclose the subject identification number (ID), compound ID, trial number, Promoter's name and address, instructions for use, route of administration.

Each package of the designated IMP will be dispensed at the beginning of each specific period.

For the tolvaptan titration period and subsequent run-in, subjects will receive 2 cartons at the first visit of tolvaptan 45/15 mg.

6.2.3 Storage Conditions

All IMPs will be stored in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees. Neither investigators nor any designees may provide IMP to any subject not participating in this protocol.

The IMP should be stored according to the conditions specified in the IMP label. The clinical site staff will maintain a temperature log in the drug storage area recording the temperature at least once each working day.

6.2.4 Administration Method

IMP will be distributed to the patient during each visit it is expected drug dispensation. Patient will orally take IMP at home.

6.2.5 Treatment of Overdose

In case of overdose, the subject will be instructed to interrupt IMP assumption, skipping the same number and type of doses he has over-assumed.

If the patient has assumed twice the scheduled maximum daily dose, it also will be reported as IRE. An out of protocol visit has to be scheduled and the subject will undergo blood withdrawal in order to assess any AEs. IMP assumption can be restarted if no AE has arisen.

6.2.6 Retrieval and/or Destruction

The investigator or designee must maintain an inventory record of IMP received, dispensed, administered, and returned.

Upon completion or termination of the trial, all unused and/or partially used IMP must be returned to the sponsor or a designated agent.

All IMP returned to the sponsor must be accompanied by appropriate documentation and be identified by protocol number with trial site number on the outermost shipping container. Returned supplies should be in the original containers. The assigned trial monitor will facilitate the return of unused and/or partially used IMP.

6.3 Prohibited medications and dietary restrictions

Medications or surgical therapies used for the purpose or potential for modifying the progression of PKD cyst growth or development will be prohibited. These include, but are not restricted to, somatostatin agonists, rapamune (sirolimus), anti-sense ribonucleic acid (RNA) therapies, vasopressin antagonists, (mozavaptan), Vaprisol (conivaptan), or agonists (e.g., desmopressin) and cyst decompression surgery.

Continuous or short-term use of other medications, while not prohibited, may be restricted by the investigator because of their potential for interference with metabolism or efficacy endpoints. This includes the use of diuretics which may be used intermittently. Diuretics are not generally recommended in ADPKD due to their tendency to increase AVP levels through relative dehydration or volume depletion; thus, chronic use of diuretics (e.g., for hypertension) will be prohibited due to potential endpoint interference and is an exclusionary criterion for this trial. Subjects taking such agents must first sign an ICF and then agree to be switched to an alternate form of therapy in order to be eligible for the trial. Some drugs are known to alter creatinine concentrations so, while not prohibited, subjects should alert their trial doctor, and any other health-care providers, to take this into consideration when considering changes in their prescribed or over-the-counter medications. A brief list would include: cimetidine, NSAID medications like aspirin or ibuprofen, chemotherapy drugs, and cephalosporin, RAAS blockage, acarbose, guar gum, phenprocoumon, oral anticoagulants, thrombolytic drugs, ranolazin, cephalexin.

Since tolvaptan is a weak cytochrome P450 (CYP) 3A4 substrate, potent CYP3A4 inhibitors should be avoided during the trial, with the exception of amiodarone, which was found to have no effect on tolvaptan. A partial list of other CYP3A4 inhibitors include pomelo, grapefruit, or Seville orange and drugs listed in table 1.

Restriction of excess dietary sodium and cooked meat protein may prove beneficial to subjects with a history of, or predisposition for, hypertension or kidney disease in general

and should be applied to all subjects diagnosed with advanced ADPKD, particularly if there is evidence for a tendency towards rapid progression. In the absence of alternate regional practices, restrictions of dietary salt < 5g/day and dietary cooked meat protein < 1 g/kg/day and to limit caffeinated drinks/foods should also be given (no more than 2 coffee equivalents per day).

Additionally, fluid intake is generally encouraged in subjects with PKD. Given the potential for dehydration with tolvaptan treatment, all subjects should be instructed to ingest fluids in anticipation of, or at the first sign of thirst in order to avoid excessive thirst or dehydration. Upon consent, all subjects should receive the recommendation to ingestion of at least 2-3 liters of fluid (including in solid, semi-solid, and liquid foods) per day, unless otherwise directed by your study doctor. This recommendation should start during screening and continue through the end of the trial. Additionally, subjects should ingest 1 to 2 cups of water before bedtime regardless of perceived thirst and replenish fluids overnight with each episode of nocturia. Dehydration will be monitored by subject self-assessment of changes in body weight and reporting of symptoms. Acute changes of > 3% of body weight (increase or decrease) over any 7-day period should be noted.

7. STUDY DURATION

7.1 Study Duration

The trial has a 36 months overall duration, that include a 9 months recruitment period.

7.1.1 Screening Period:

Has a 2 weeks duration, it is included in the 9 months recruitment period.

7.1.3 Treatment Period:

It has a 25 months duration. Each month lasts 28 days. It includes the 3 weeks titration period and the 1 week run-in period.

7.1.4 Post-Treatment Follow-up Period:

It lasts 21 days.

7.1.4 Total Study Duration:

About 3 years.

8. STUDY ENDPOINTS

Trial endpoints were constructed based on advice obtained at scientific advisory boards and meetings with regulatory agencies from Japan, EU and the USA and were chosen to represent potentially clinically meaningful changes in levels of laboratory or physiological parameters and patient symptoms reasonably expected to develop or worsen with progressive ADPKD.

8.1 Effectiveness Endpoints

8.1.1 Primary Endpoint

Primary outcome of the study is to evaluate the *difference between Metformin and Tolvaptan in annualized slope of eGFR (CKD-EPI) for individual subjects*, that will be calculated using an appropriate baseline and post-randomization assessment. Traditionally, the primary endpoint in trials testing renoprotective effects of interventions has been the incidence of ESRD or doubling of serum creatinine, which correlates to a 57% reduction in eGFR. Of note, ADPKD is a relatively slowly progressive disease. In a population such as that of the TEMPO 3:4 trial, which was selected to have early-stage ADPKD (eGFR >60 mL/min), it cannot be expected that this endpoint will occur within the typical duration of a renal trial. Adopting this endpoint would therefore only pick up cases

of acute kidney injury and not be of help for studying the effect of interventions on progression of the disease itself. To stimulate progress in developing renoprotective agents, especially for studies in early-stage CKD and diseases that are relatively slow in progression, the nephrological community has pleaded for the use of alternative endpoints for renal trials, namely lesser declines in eGFR. Regulatory authorities have accepted this proposal. When studying the incidence of a 25% reduction in eGFR [a priori defined in the TEMPO 3:4 trial and accepted by the European Medicines Agency (EMA)], there was a significant 61% relative risk reduction with tolvaptan (number needed to treat to prevent one event was ∼11). One of the inclusion criteria for the pivotal TEMPO 3:4 trial was a creatinine clearance as estimated with the Cockroft-Gault equation ≥60 mL/min/1.73 m2. Due to tubular creatinine secretion, creatinine clearance overestimates GFR by ∼20%. Consequently, the TEMPO 3:4 trial included a considerable number of ADPKD patients (n = 247; 17%) with an eGFR, as determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, of <60 mL/min/1.73 m2. A post hoc analysis indicated that in these patients, treatment efficacy was similar or even slightly better than in those with higher eGFR. However, the number of patients with CKD stage 3b, i.e. an eGFR of 30–45 mL/min/1.73 m2, was small.

8.1.2 Secondary Endpoints

The key secondary endpoint is *the percent change from baseline in htTKV as measured by CT-scan at 24 months*. The use of htTKV as a surrogate biomarker for progression of ADPKD is supported by the Consortium for Radiologic Imaging Study of Polycystic Kidney Disease (CRISP) which showed that baseline TKV strongly predicts subsequent loss of GFR. TKV is now widely used in RCTs for ADPKD.

Other secondary efficacy end-points include:

- Difference in rate of albuminuria change;

- Difference in rate of urological events and of hypertension onset (for not hypertensive patients at baseline);
- Hypertension worsening or amelioration (for those who are hypertensive);
- Exploratory efficacy Outcomes (changes from baseline in urinary EGF and EGF/MCP-1 levels).

8.2 Safety Endpoints

They include:

- changes from baseline in creatinine;
- vital signs;
- laboratory values including liver function tests, rate of aquaretic AEs, thus including serum sodium, rate of Metformin Associated Lactic Acidosis, blood insulin and glucose levels, HOMA test in both treatment groups.

9. VISITS

9.1 Trial Procedures

9.1.1 Screening:

After written informed consent has been signed, assessments during the screening visits will include:

1) Confirm diagnosis and determine whether the subject meets inclusion and exclusion criteria and record demographic information;

2) Perform physical examination, including post-void body weight and height (first visit);

3) Record medical/PKD history (all visits);

4) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor (all visits);

5) Assess AEs, if reported (all visits);

6) Assess vital signs (include heart rate and blood pressure) (all visits);

7) Serum pregnancy test for WOCBP (1st visit);

8) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis (1st, 2nd visit);

9) Non contrast enhanced CT-scan of the kidneys for TKV measurement (3rd visit, once eligibility has been confirmed). In case a patient has undergone kidney CT- or MRI-scan in order to measure TKV within 6 months before randomization, basal CT scan for TKV measurement may be not undertaken).

9.1.2 Randomization

Assessments during the randomization visit will include:

1) Verification of diagnosis, inclusion and exclusion criteria and record demographic information;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported;

5) Assess vital signs (include heart rate and blood pressure);

6) IMP dispensation;

9.1.3 Titration period

Assessments during up-titration phone call will include:

1) Assess IMP tolerability;

2) Assess AEs, if reported.

9.1.3 Run-in Period

Assessment at the end of run-in period visit will include:

1) Perform physical examination, including post-void body weight and height;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported.

5) Assess vital signs (include heart rate and blood pressure);

6) Serum pregnancy test for WOCBP;

7) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis;

8) IMP dispensation;

9) IMP reconciliation (Subjects must return any unused IMP).

9.1.4 Treatment Period

Assessment at each on-treatment visit will include:

1) Perform physical examination, including post-void body weight and height;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported;

5) Assess vital signs (include heart rate and blood pressure);

6) Serum pregnancy test for WOCBP;

7) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis;

8) IMP dispensation;

9) IMP reconciliation (subjects must return any unused IMP);

10) Non contrast enhanced CT-scan of the kidneys for TKV measurement (at the end of month 24).

9.1.5 Follow-up Period

Assessments at each follow-up visit will include:

1) Perform physical examination, including post-void body weight and height;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported;

5) Assess vital signs (include heart rate and blood pressure);

6) Serum pregnancy test for WOCBP (second visit);

7) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis (second visit).

The above follow-up assessments will also be performed for a subject who interrupts IMP for more than 10 days, in order to collect their data in the event that they never restart IMP treatment.

9.1.6 Efficacy Assessments

9.1.6.1 Serum Creatinine for Estimated Glomerular Filtration Rate

The serum creatinine concentration is related to eGFR and is commonly used to estimate renal function in clinical practice. Alteration in metabolism of creatinine and methodological interference in its measurements may impact accuracy of the serum creatinine and renal function estimation. Below are suggested measures to decrease serum creatinine variability prior to the monthly blood draws required by this protocol:

- Maintain a stable dietary protein intake and avoid very different or high cooked meat protein meals the day before each scheduled serum creatinine assessment;
- Maintain a stable exercise routine and avoid very different or heavy physical activity/exercise the day before each scheduled serum creatinine assessment;

- Maintain a stable water intake, aimed at avoiding thirst consistently throughout the trial - recommended ingestion of at least 2-3 liters of fluid (including in solid, semi-solid, and liquid foods) per day, unless otherwise directed by your study doctor;
- Plan to arrive at the same time for each blood-draw and clinic visit to better standardize time of the sample collection throughout the trial;
- Avoid forbidden medications.

Serum creatinine is stable when stored frozen; therefore, blood sample will be analyzed by the laboratory as soon as it is received and accessioned.

Ongoing, analysis will be conducted for each subject upon his/her individual completion of all their assessments within the trial (not at the end of the trial).

The eGFR values will be calculated from the laboratory serum creatinine concentrations taken at screening and during every trial visit. In the screening period, the first two assessments must be used to determine the eGFR values that will be averaged for determination of meeting inclusion criteria.

9.1.6.2 TKV measurement

Abdomen-pelvis CT-scan will be performed by using a 320-row multi detector device and the following acquisition parameters will be used: slice thickness 0.5 mm, increment 0.5 mm, rotation time 0.5 s; 120/200 kVp/mAs.

An automatic dose modulation system will be used in all cases. Images will be acquired without intravenous injection of iodinate contrast material. Scans will be performed from the diaphragm to the pubic symphysis.

CT data will be centrally transferred to and analysed on a workstation (HPXW8600), present within the DETO equipped with software dedicated to image reconstruction (Vitrea FX 2.1, Vital Images, Minneapolis, MN, USA). For each patient, Multi-planar Reconstructions (MPR) on the axial, coronal and sagittal planes and Volume Rendering

(VR) images will be used in order to assess renal parenchyma. A radiologist with more than 10-year experience in the field of abdominal CT will manually trace both kidneys (renal parenchyma and all cysts including exophytic cysts), excluding the renal pelvis and other hilar vascular structures. The semi-automatic 3D Analysis software will be used in order to quantify the TKV for each CT examination. All changes in TKV (delta TKV) will be calculated by analyzing any variation of absolute TKV values deriving from the different CT examinations.

9.1.6.3 Polycystic Kidney Disease History and Outcomes Surveys

A short PKD history survey will be completed once during screening to capture information from the subject's recollection, and documented past medical history where available. The survey should be updated at each visit if new information regarding past history becomes available.

The PKD outcomes survey will collect information relevant to the medical, social and economic consequences of new and ongoing PKD-related morbidities. New clinically relevant information and specific questions about outcomes will be collected at the following visits: screening, end of run-in, and during the unblind randomized treatment period either monthly (over the phone or in person).

If a subject who has been randomized discontinues the use of IMP, PKD outcomes will be collected at the normally scheduled trial visits, or by telephone contact, to the date of the originally planned Month 12 visit, if the subject agrees.

9.1.6.4 Urinary Epidermal Growth Factor and Monocyte Chemotactic Peptide-1

A number of studies tried to identify molecular markers of rapid disease progression in ADPKD, but nowadays eGFR and TKV are the most important.

Epidermal Growth Factor (EGF) is a renal tubular-specific protein that exerts a key role in cellular differentiation and regeneration after kidney damage. For this reason, urinary EGF (uEGF) is considered a surrogate marker of renal tubular cells to regenerate after acute or chronic damage.

An Italian study showed lower uEGF levels in those ADPKD patients affected by renal insufficiency, than in those with normal renal function (77). Prognostic value of uEGF has been subsequently confirmed in another study conducted in CKD (78), that showed as uEGF is an independent risk factor for CKD progression characterized by higher sensitivity than serum creatinine.

Urinary Monocyte Chemotactic Peptide-1 (uMCP-1), in ADPKD seems to increase before than serum creatinine or than proteinuria and intracystic levels of uMCP-1 are higher than those found in urine or serum (79).

Moreover, as uEGF/uMCP-1 ratio was found to be a sensible prognostic marker of ESRD in IgA Nephropathy, likewise it could be a useful biomarker in ADPKD (80).

9.2 Definition of Source Data

The Investigator shall permit the authorized Investigator-Promoter, his representatives, and regulatory agencies to enter and inspect any site where the drug or records pertaining to the drug are held, and to inspect all records relating to an investigation, including subject records. Completed eCRFs must be available by the Investigator for review by the Investigator-Sponsor, his representatives, the monitor and the regulatory agencies. To ensure the accuracy of data submitted, it is mandatory that representatives of the Investigator-Promoter and of the regulatory agencies have direct access to source documents.

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents will include but are not limited to progress notes, electronic data, screening logs, and recorded data from automated instruments. All source documents pertaining to this trial will be maintained by the investigators.

Investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source data/documents by authorized persons as defined in the ICF.

9.2.1 Data Collection

During each subject's visit to the clinic, a clinician participating in the trial will record progress notes to document all significant observations. At a minimum, these notes will contain:

- Documentation of the informed consent process, including any revised consents;
- The date of the visit and the corresponding Visit or Day in the trial schedule;
- General subject status remarks, including any significant medical findings;
- The severity, frequency, duration, action taken, and outcome of any AEs and the investigator's assessment of relationship to IMP must also be recorded;
- Any changes in concomitant medications or dosages;
- A general reference to the procedures completed;
- The signature (or initials) and date of each clinician (or designee) who made an entry in the progress notes.

In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes as described above.

Any changes to information in the trial progress notes and other source documents will be initialed and dated on the day the change is made by a site staff member authorized to make the change. Changes will be made by striking a single line through erroneous data (so as not to obliterate the original data), and clearly entering the correct data (e.g., wrong data right data).

Information from the trial progress notes and other source documents will be entered by investigative site personnel directly onto eCRFs in the sponsor's electronic data capture system. Changes to the data will be captured by an automatic audit trail.

9.2.2 File Management at the Trial Site

The investigator will ensure that the trial site file is maintained in accordance with Section 8 of the ICH GCP Guideline and as required by applicable local regulations. The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

9.2.3 Records Retention at the Trial Site

Regulatory requirements for the archival of records for this trial necessitate that participating investigators maintain detailed clinical data for the longest of the following 3 periods:

- A period of at least 2 years following the date on which approval to market the drug is obtained (or if IMP development is discontinued, the date regulatory authorities were notified of discontinuation); OR
- A period of at least 3 years after the sponsor notifies the investigator that the final report has been filed with regulatory authorities.

The investigator must not dispose of any records relevant to this trial without written permission from the Promoter.

The investigator will be responsible to maintain adequate and accurate electronic or hard copy source documents of all observations and data generated during this trial. Such documentation is subject to inspection by the Promoter and relevant regulatory authorities. If the investigator withdraws from the trial (e.g., due to relocation or retirement), all trial-related records should be transferred to a mutually agreed-upon designee within a sponsor-specified timeframe. Notice of such transfer will be given to the Promoter in writing.

10. SAFETY MONITORING AND REPORTING

10.1 Adverse Event

This section describes the methods and timing for assessing, recording, and analyzing safety parameters, as well as the procedures for eliciting reports of and recording and reporting AEs and intercurrent illnesses and the type and duration of the follow-up of subjects after AEs.

ADPKD is a progressive disorder involving the kidney, liver and occasionally other organ systems. A number of AEs may be associated with this disorder and are endpoints in this trial, including urine concentration defects, hypertension, renal pain, renal infection, nephrolithiasis, hematuria, and ESRD. As such, these events are considered “expected” in this trial population and will not qualify for the purposes of regulatory expedited reporting.

These events will be evaluated on a regular basis by the trial’s Medical Monitor and the sponsor’s Safety group.

10.1 Adverse Event Definitions and Classifications

Subjects must be carefully monitored for adverse events. Adverse events should be assessed in terms of their seriousness, severity, and relationship to the study drug.

The intensity or severity of adverse events should be graded according to NCI CTCAE (v 5.0) criteria.

10.1.1 Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. (Definition per International Conference on Harmonization [ICH]).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

10.1.3 Serious Adverse Event

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death;
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires inpatient hospitalization or causes prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;

- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization);
- Transaminase levels raise above 5xUNL;
- Transaminase and total bilirubin 2xUNL;
- Clinical signs of Drug Induced Liver Injury (e.g., jaundice, right upper quadrant pain);
- Lactic Acidosis (as defined by pH<7,3 and Lactate> 4 mmol/l);
- Death;
- Pregnancy;
- Malignancies;
- Onset of permanent medical conditions contraindicating metformin or tolvaptan treatment (i.e.: myocardial infarction, severe chronic arrhythmias, chronic respiratory failure).

10.1.4 Serious Adverse Event Collection and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures.

If only limited information is initially available, follow-up reports are required.

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the Sponsor (or designee) using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

10.1.5 Unlisted (Unexpected) Adverse Event

An unlisted adverse event is an event the nature or severity of which is not consistent with the applicable product information. The expectedness of an adverse event will be determined by whether or not it is listed in the summary of product characteristics (SmPC).

10.1.6 Adverse Event Associated With the Use of the Drug

An adverse event is considered associated with the use of the drug if the attribution is possible, probable, or very likely by the definitions listed in Section 10.1.7.

10.1.7 Intensity (Severity) Reporting and Attribution

For both serious and non-serious adverse events, the Investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Intensity for each adverse event will be determined by using Version 4.0 of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) as a guideline, wherever possible; a copy of the NCI-CTCAE Version 4.0 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). In those cases where the NCI CTCAE does not apply, intensity should be defined according to the following criteria:

- **Mild**: Awareness of sign or symptom, but easily tolerated;
- **Moderate**: Discomfort enough to cause interference with normal daily activities;
- **Severe**: Inability to perform normal daily activities;
- **Life Threatening** Immediate risk of death from the reaction as it occurred.

Relationship to study drug administration will be determined as follows:

- **Not related**: An adverse event which is not related to the use of the drug;
- **Unlikely/Doubtful**:An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely;
- **Possible**: An adverse event which might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded;
- **Probable**: An adverse event which might be due to the use of the drug. The relationship in time is suggestive (e.g., confirmed by dechallenge). An alternative explanation is less likely, e.g., concomitant drug(s), concomitant disease(s);
- **Definite/Very Likely**: An adverse event which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g., it is confirmed by dechallenge and rechallenge).

10.1.8 Relationship of Adverse Event to Investigational Product

The assessment of the relationship of an adverse event to the administration of study drug is a clinical decision based on all available information at the time of the completion of the CRF.

An assessment of ‘No’ would include:

1. The existence of a clear alternative explanation (e.g., mechanical bleeding at surgical site) OR

2. Non-Plausibility (e.g., the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration).

An assessment of 'Yes' indicates that there is a reasonable suspicion that the adverse event is associated with the use of the investigational drug. Factors to be considered in assessing the relationship of the adverse event to study drug include:

1. The temporal sequence from drug administration: the event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
2. Recovery on discontinuation (de-challenge), recurrence on reintroduction (rechallenge):

Subject's response after drug discontinuation (de-challenge) or subjects response after drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question.

- Underlying, concomitant, intercurrent diseases: each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have;
- Concomitant medication or treatment: The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question;
- The pharmacology and pharmacokinetics (PK) of the test drug: The PK properties (absorption, distribution, metabolism and excretion) of the test drug(s), coupled with the individual subject's pharmacodynamics should be considered.

10.1.9 Reporting of Adverse Events

All adverse events that occur between the first study-related procedure and 100 days after the last dose of study drug will be reported. All events that meet the definition of a serious adverse event will be reported as serious adverse event, regardless of whether they are protocol-specific assessments.

All adverse events, regardless of seriousness, severity, or presumed relationship to study therapy, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as “upper respiratory infection”). Investigators must record in the CRF their opinion concerning the relationship of the adverse event to study therapy. All measures required for adverse event management must be recorded in the source document and reported according to sponsor instructions.

All grade 3 and 4 adverse events, considered related, must be followed until resolution of the event, or the event improves to a grade 2 or better.

10.1.10 Reporting of Serious Adverse Events/Pregnancy

Serious adverse events, including laboratory test abnormalities fulfilling the definition of serious, occurring during the study and follow-up period must immediately (within 24 hours of the investigator’s awareness) be reported to the Investigator-Sponsor or his delegate. Name and address for this purpose will be supplied on a Contact Information Form.

Information regarding serious adverse events will be transmitted to the Investigator-Sponsor or his delegate using the Serious Adverse Event Form, which must be signed by a member of the investigational staff. The initial report of a serious adverse event may be reported by fax or by email. It is preferable that serious adverse events be reported via fax. Subsequent to a report of a serious adverse event, a Serious Adverse Event Form must

be completed by the investigational staff and transmitted to the Investigator-Sponsor within 1 working day.

The cause of death of a subject in a clinical study, whether or not the event is expected or associated with the investigational agent, is considered a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a clinical study must be reported as a serious adverse event, except hospitalizations for:

- social reasons in absence of an adverse event;
- surgery or procedure planned before entry into the study (must be documented in the CRF);
- study drug administration;
- study related procedures defined in the protocol.

The Investigator-Sponsor should report serious unexpected adverse events to the appropriate Independent Ethics Committee (IEC) that approved the protocol unless otherwise required and documented by the IEC. The Investigator-Sponsor assumes responsibility for appropriate reporting of serious unexpected adverse events to Regulatory Authorities.

Each serious adverse event must be followed up until resolution or stabilization by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as a serious adverse event, unless the investigator assesses that the event meets standard International Conference on Harmonization (ICH) criteria for a serious adverse event (see definition Section 10.1.3).

CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as a serious adverse event, specifically when they are allowed or not

excluded by the protocol inclusion/exclusion criteria. If an investigator is in doubt about the applicable reporting obligations, he/she should consult with the chairman or the Investigator-Sponsor.

CTC grade 4 laboratory abnormalities will be recorded in the dedicated section of the electronic CRF and will be reviewed on a regular basis.

10.1.11 Pregnancy

Women of childbearing potential who are sexually active must use an effective method of birth control during the course of the trial and for 30 days after the last dose of IMP in a manner such that risk of failure is minimized. Unless the subject is sterile (i.e., women who have had a bilateral oophorectomy and/or hysterectomy or have been postmenopausal for at least 12 consecutive months) or remains abstinent, 2 of the following precautions must be used: vasectomy, tubal ligation, intrauterine device, birth control pills, birth control depot injection, birth control implant, condom or occlusive cap (diaphragm or cervical/vault caps) with spermicide. Any single method of birth control, including vasectomy and tubal ligation, may fail, leading to pregnancy.

Before enrolling WOCBP in this clinical trial, investigators must review guidelines about trial participation for WOCBP. The topics should generally include:

- General information;
- ICF;
- Pregnancy prevention information;
- Drug interactions with hormonal contraceptives;
- Contraceptives in current use;
- Guidelines for the follow-up of a reported pregnancy.

Prior to trial enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form stating that the above- mentioned risk factors and the consequences were discussed with her.

During the trial, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual cycle).

If a subject or investigator suspects that the subject may be pregnant prior to administration of the investigational product, administration must be withheld until the results of blood serum pregnancy tests are available. If the pregnancy is confirmed, the subject must not receive IMP or be enrolled in the trial. If pregnancy is suspected while the subject is receiving treatment, IMP must be withheld immediately (if reasonable, taking into consideration any potential withdrawal risks) until the result of a serum pregnancy test is known. If pregnancy is confirmed, IMP will be interrupted or withheld in an appropriate manner (e.g., dose tapering if necessary for subject safety) and the subject will continue to be monitored for the duration of the remainder of the trial or of their pregnancy. Subjects who permanently discontinue IMP due to pregnancy may continue to be monitored in the same manner as other subjects to their 24-month visit.

The investigator must immediately notify the Promoter of any pregnancy associated with IMP exposure during the trial and for 30 days after the last dose of IMP and record the event on the IRE form and forward it to the sponsor. The sponsor will forward Pregnancy Surveillance Form(s) for monitoring the outcome of the pregnancy.

Protocol required procedures for IMP discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report to the sponsor, on appropriate Pregnancy Surveillance form(s),

follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants will be followed for a minimum of 6 months.

10.2 Follow-up of Adverse Events

For this trial, AEs will be followed up for 7 days in subjects who discontinued prior to randomization and for 21 days after the last dose of IMP has been administered (follow- up period) in subjects who were randomized.

Subjects experiencing SAEs should be followed clinically until their health has returned to baseline status, or until all parameters have returned to normal, or have otherwise been explained.

For subjects who have discontinued IMP but have not withdrawn from the trial, vital status, AEs, concomitant medications, ESRD status, and scheduled laboratory data (including serum creatinine data) are planned to be collected regardless of IMP discontinuation until the scheduled end of the trial.

10.2.1 Follow-up of Non-serious Adverse Events

Non-serious AEs that are identified on the last scheduled contact must be recorded on the AE eCRF with the current status noted. All non-serious events that are ongoing at this time will be recorded as ongoing on the eCRF.

10.2.2 Follow-up of Post-Trial Serious Adverse Events

Serious AEs that are identified on the last scheduled contact must be recorded on the AE eCRF page and reported to the Promoter This may include unresolved previously reported SAEs, or new SAEs.

The investigator will follow SAEs until the events are resolved, or the subject is lost to follow-up. Resolution means the subject has returned to the baseline state of health, or the investigator does not expect any further improvement or worsening of the subject's condition. The investigator will continue to report any significant follow-up information to OPDC up to the point the event has been resolved.

10.2.3 Follow-up and Reporting of Serious Adverse Events Occurring after Last Scheduled Contact

Any new SAEs reported by the subject to the investigator that occur after the last scheduled contact, and are determined by the investigator to be reasonably associated with the use of the IMP, should be reported to OPDC. This may include SAEs that are captured on follow-up telephone contact or at any other time point after the defined trial period (i.e., up to last scheduled contact). The investigator should follow potentially IMP-related SAEs identified after the last scheduled contact until the events are resolved, or the subject is lost to follow-up. The investigator should continue to report any significant follow-up information to OPDC up to the point the event has been resolved.

10.3 Clinical Laboratory Assessments

Blood and/or urine samples will be collected as indicated in the schedule of assessments (Fig.1). It is preferable to collect the clinical laboratory samples from each subject at a consistent time of day throughout the trial, and to recommend that the subject have a similar diet, avoiding variation in protein intake (especially cooked meat protein), and exercise pattern during these periods, in order to reduce variability in the samples over time.

Liver panels will be analyzed monthly during the unblind, treatment period using both central and local labs, as needed, and per standard of care according to the subject's individual medical needs.

Serum pregnancy testing and Blood venous gas-analysis will be performed locally during screening period and then during every scheduled visit.

10.4 Physical Examination and Vital Signs

A full physical examination will be performed and documented during every visit and during End of Treatment visit. Any changes in medication or AEs will be recorded in the eCRF. Body weight will be taken post-void. It is preferable to use the same scale for each measurement.

The investigator or his/her appointed designee is primarily responsible to perform the physical examination. If the appointed designee is to perform the physical examination, he/she must be permitted to do so by local regulations and his/her name must be included on any globally and locally required documents.

Whenever possible, the same individual should perform all physical examinations. Any undesirable condition present at a post-treatment physical examination that was not present at the baseline examination should be documented as an AE and followed to a satisfactory conclusion.

Vital sign data, including seated blood pressure, heart rate, temperature, height, and weight, will be taken at the visits identified in the Schedule of Assessments.

10.5 Assessment of Liver Symptoms, Signs or Test Abnormalities

Testing for hepatic transaminase (ALT/AST), alkaline phosphatase, and BT will be performed during screening visits. Testing for hepatic transaminase (ALT/AST) will be performed during run-in and at each monthly visit in the tolvaptan arm, whereas it will be performed during three monthly visits in the metformin arm.

10.5.1 Requirements for Repeated Liver Testing

10.5.1.1 Repeated Liver Testing in Subjects with Normal Values at Screening

The appearance of any suspicious symptom or sign should trigger prompt testing of hepatic function (i.e., within 72 hours). Local laboratory testing is acceptable, ideally with a concurrent central laboratory sample for confirmation.

Any transaminase or bilirubin values which exceed 2 x ULN should also prompt immediate retesting within 72 hours. While values remain in an abnormal range, testing frequency should be increased to at least weekly for the first month, gradually returning to monthly/three monthly as indicated by the results.

Subjects exhibiting such an increase during the tolvaptan titration/run-in phases will be disqualified from randomization on safety grounds and should not be randomized.

Should the cause of the abnormality be determined to be unrelated to tolvaptan exposure (e.g., having identified a plausible alternative explanation) such a subject may be re-screened only with medical monitor approval.

10.5.1.2 Repeated Liver Testing in Subjects with Abnormal Values at Screening

Subjects found to have liver laboratory abnormalities at screening or who have a history of non-ADPKD-related liver disease will require further evaluation. These subjects will need to have the special liver eCRF completed and additional testing will be required during screening (to confirm the stability of the abnormality) and during the tolvaptan run-in phase at least 1 week prior to randomization (to confirm eligibility for randomization).

Management of such subjects should be closely coordinated with the trial's Medical Monitor. In these subjects, further changes in liver test levels of > 2 x upper limit of their highest screening value at any point post-screening should prompt re-testing within 72 hours. Should such increase occur in the tolvaptan titration/run-in phase, the subject will be disqualified from trial.

10.5.1.3 Liver Test Abnormalities and Interruption/Discontinuation of Investigational Medicinal Product

Liver transaminase or bilirubin levels reaching or exceeding 2 x ULN that have an uncertain or rapidly increasing trajectory should prompt at least temporary IMP interruption. IMP should not be resumed until monitoring indicates abnormalities have resolved, are stable or are not rapidly increasing, and then only with an increased frequency of monitoring.

Subjects would not typically be allowed to resume treatment with IMP if they have:

- transaminase levels rise above 8 x ULN;
- transaminase levels are > 5 x ULN for more than 2 weeks; or
- concurrent elevations of transaminase > 3 x ULN and BT > 2 x ULN.

Subjects with these levels of abnormality may be re-challenged with IMP if abnormalities were adjudicated as having a < 50% likelihood of being related to IMP (per DILI network [DILIN] probability criteria) by the investigator and medical monitor.

All elevations will be assessed by the medical monitoring team. The subject must also be willing to comply with these monitoring measures, be informed of the potential risks, and consent to IMP re-challenge.

10.6 Assessment of Metformin Associated Lactic Acidosis (MALA)

In order to assess acid-base status in treated patients and in order to limit the patient discomfort, peripheral venous blood gas analysis (pVBGA) will be performed during scheduled and unscheduled visits.

The appearance of any suspicious symptom or sign should trigger prompt testing (pVBGA) (i.e., within 24 hours). Local laboratory testing is always permitted.

The investigator must follow the following rules:

- lactate level is between 2-4 mmol/l and pH over 7.35, pVBGA has to be promptly repeated within 24 hours. If confirmed, patient has to reduce metformin dosage (from 1500 mg a day to 1000 mg a day or from 1000 mg a day to 500 mg a day). pVBGA has to be repeated every other day for 1 week till normalization. Once restored normal values, patient can up-titrate to the former dosage and pVBGA has to be retested after 7 days. If normal, patient can keep on treatment, otherwise meformin dosage has to be reduced as previously described till normalization. In this case higher dosage can not be restored.

  In both cases, if pVBGA fail to restore, metformin has to be permanently stopped. In this case, EoTx visit has to be collected and the patient has to be followed up for 21 days.
- lactate level is higher than 4 mmol/l with sVO_2 between 30-50% (irrespective of pH) or lactate between 2-4 mmol/l but associated to pH lower than 7.35. In these cases IMP must be suspended and patient hospitalized. If AEs fails to recover in 3 weeks, IMP must be permanently discontinued.

  Once AEs recovered the patient can undergo IMP re-challenge, by restarting since metformin titration. In this case pVBGA has to be performed every week prior to up-titration. If pVBGA is normal, the patient can uptitrate, otherwise meformin dosage has to be reduced till the minimum allowed dosage (that is 1000 mg a day) as previously described till normalization.

  If pVBGA fail to restore, metformin has to be permanently stopped. In this case, EoTx visit has to be collected and the patient has to be followed up for 21 days.

10.7 Assesment of Metformin Associated Megaloblastic Anemia (MAMA)

The pathogenic mechanisms of vitamin B_{12} deficiency in metformin treatment have not been fully elucidated. However, among the instances of bacterial overgrowth in the small intestine attributable to diabetes mellitus, changes in small bowel motility, alterations in the bacterial flora, competitive inhibition, the inactivation of vitamin B_{12} absorption, or the effect of calcium on cell membranes have been suggested to play a role.

Vitamin B_{12} deficiency is clinically important because it is a reversible cause of bone marrow failure and demyelinating nerve disease. Neurologic damage, a possible consequence of metformin-induced vitamin B_{12} deficiency, can present as peripheral neuropathy and may be mistaken for diabetic neuropathy in patients on metformin treatment.

Some studies (81–83) showed a clear relationship between the dosage or duration of metformin use and vitamin B_{12} deficiency in patients with type 2 diabetes, with an incidence of 19% after 4 years of treatment. Subjects with metformin use ≥10 years and daily dosage ≥2,000 mg show about a 4-fold higher risk of vitamin B_{12} deficiency compared to those with metformin use of <4 years and daily dosage of ≤1,000 mg. The presence of anemia shows a statistically positive association with vitamin B_{12} deficiency.

For these reasons Cianocobalamin blood dosage and Complete Blood Count will be regularly performed. In case of Cianocobalamin deficiency onset, oral supplementation will be allowed.

11. TREATMENT/STUDY DISCONTINUATION

Patients should be informed of circumstances under which their participation may be terminated by the Investigator without their consent.

A subject may temporarily or permanently discontinue IMP for a number of reasons listed below:

1. Reasons related to AE:
    a. Subject could not tolerate IMP due to an AE which is annoying or uncomfortable but not serious or hazardous,
    b. Physician determined that there are potential IMP related safety concern or SAE placing subject at undue hazard,
    c. Serious adverse event (SAE),
    d. Progression of disease leading to dialysis, transplantation or eGFR decline as determined by the investigator,
    e. Blood Venous Gas abnormalities meeting criteria for MALA,
    f. Clinical signs of MALA,
    g. Fever or diarrhea,
    h. Liver test abnormalities meeting criteria for permanent discontinuation,
    i. Clinical signs of DILI (e.g., jaundice, right upper quadrant pain).
2. Death;
3. Reasons unrelated to medical condition (e.g., pregnancy, trial too burdensome);
4. Withdrawal of informed consent (partial related to IMP or complete from the trial);
5. Lost to follow-up (detailed procedures to prevent subjects from becoming “lost to follow-up will be provided in the operations manual. These procedures must be followed by the investigator, their staff or other designated trial personnel);
6. Termination of all or part of the trial by the Promoter;
7. Fall of eGFR below 15 ml/min (in case of tolvaptan treatment), below 30 ml/min (in case of metformin treatment);

8. Peripheral BVG predicting lactic acidosis onset (sVO2 between 30-50% and Lactate levels greater than 3,5 mmol/l; in this case ABG must be performed). In these cases IMP must be suspended for maximum 3 weeks and resumed once AEs recovered. If AEs fails to recover in 3 weeks, IMP must be permanently discontinued.

Any administrative or other reasons for withdrawal must be documented and explained to the patient.

If the reason for removal of a patient from the study is an AE, the principal specific event will be recorded on the eCRF. The patient should be followed until the AE has resolved, if possible. All patients will be followed for 21 days following the last dose of study medication-

If the subject temporarily interrupts or discontinues IMP due to an AE, the investigator, or other trial personnel, will make every effort to follow the event until it has resolved or stabilized.

If lost to follow-up, the Investigator should make every effort to contact the patient by telephone or by sending a registered letter to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patient is withdrawing from the study.

If the Promoter terminates or suspends the trial for safety or unanticipated other reasons, prompt notification will be given to investigators, IRBs/IECs, and regulatory authorities in accordance with regulatory requirements.

The Investigator will notify the Promoter promptly if the trial is terminated by the investigator or the IRB/IEC at the site. If the investigator, IRB/IEC or sponsor decides to terminate or suspend the trial's conduct at a particular center for safety, non-enrollment of

subjects, non-compliance with the protocol, or unanticipated other reasons, the above and other parties, as required by the applicable regulatory requirements, will be promptly notified.

If a subject discontinues during the screening period (because of consent withdrawal or failure to meet all of the requirements to continue in the trial) that subject will be considered a “Screen failure”. Subjects who fail to meet trial requirements during the screening period may be rescreened at a later date.

Screen failure subjects will be recorded as such on the eCRF. Screening failure subjects do not require follow-up and can be considered for rescreening if the reason for the screen failure was not that the subject withdrew their consent. If rescreened, the subject will sign a new informed consent, will be assigned a new screening number, and will repeat all screening procedures.

If a subject discontinues during the IMP titration period or during the IMP Run-in period, that subject will be considered a “Titration failure” or a “Run-in failure”.

Discontinuation during these two periods may be due to any of the following reasons:

- Subject does not meet entry requirements (i.e., subject cannot tolerate IMP treatment) as specified for a particular pre-randomization period;
- Subject decides to formally withdraw consent and/or fails to return for subsequent appointments at the trial site;
- Investigator considers the subject unsuitable for further participation.

“Titration failure” or a “Run-in failure” subjects will be recorded as such on the eCRF, they will complete an EoTx visit upon withdrawal from the trial.

The EoTx visit assessments will include:

1) Perform physical examination, including post-void body weight and height;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported;

5) Assess vital signs (include heart rate and blood pressure);

6) Serum pregnancy test for WOCBP (second visit);

7) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis (second visit).

Run-in failure subjects will then be followed up after 7 days with a phone call to record any ongoing AEs; unless the subject fully withdraws consent to any further follow-up by written documentation

11.1 Temporary Treatment Discontinuation

In this trial, it is expected that subjects may have one or more treatment interruptions during the unblind, randomized treatment period.

If a subject’s IMP treatment must be interrupted for medical or surgical reasons; blood safety test abnormalities; use of a prohibited concomitant medication; or other reasons (e.g., hospital admission for an invasive procedure, a major medical condition, surgery; dental work, or a temporary situation that prevents subject compliance with the IMP administration schedule), the subject’s IMP should be resumed as early as the situation allows.

Any IMP interruption of <10 consecutive days will be recorded as missed doses rather than as a temporary interruption of IMP. The subject should immediately inform the investigator of any missed doses reaching or expected to be 2 days or more so that the investigator can continue to monitor the subject's treatments and prepare for a possible 10-day IMP interruption.

An IMP interruption that lasts >10 consecutive days will be recorded as a "10-day Treatment Interruption" on the eCRF and the subject will visit the clinic to collect vital signs and physical exam. Treatment may still be restarted during or after these assessments are completed. If treatment is restarted, and the subject continues to Month 24, the subject will complete the Month 24 visit and scheduled follow-up assessments.

If treatment does not restart, subjects will complete an EoTx visit then 2 follow-up visits (between +8 and +21) must be collected. Assessment at each follow-up visit will include:

1) Perform physical examination, including post-void body weight and height;

2) Record medical/PKD history;

3) Record concomitant medications and ensure subject's treatment meets current standard of care including lifestyle and dietary recommendations, especially for ingestion of at least 2-3 liters of fluid per day as appropriate, unless otherwise directed by study doctor;

4) Assess AEs, if reported;

5) Assess vital signs (include heart rate and blood pressure);

6) Serum pregnancy test for WOCBP (second visit);

7) Collect urine and blood samples for clinical laboratory analyses, including serum creatinine, liver function tests, glucose metabolism parameter, CBC and venous gas analysis (second visit).

11.2 Permanent Treatment Discontinuation

After randomization, a subject may stop treatment permanently before Month 24 for a variety of reasons. Treatment discontinuations may be initiated by a subject who is not satisfied with treatment or may become medically necessary due to AEs or other issues, as determined by the investigator. However, each investigator must comprehensively review the circumstances and offer the subject options for continued treatment to the degree possible.

A subject who permanently discontinues treatment will be recorded as an **IMP discontinuation** on the eCRF. The subject will then enter the follow-up period as though they had reached the Month 24 visit. During the first week of the follow-up period, no procedures will be done. During the last two weeks of the follow-up period, the subject will have a total of 2 samples collected.

After the follow-up period, the subject will continue with all assessments up to and including their scheduled Month 24 visit, but will not be required to complete follow- up beyond that visit.

11.3 Accountability

The investigator or designee must maintain an inventory record of IMP (including investigational, active control, or placebo) received, dispensed, administered, and returned.

12. STATISTICAL CONSIDERATIONS

12.1 Sample Size Determination and Rationale

In TEMPO 3:4, tolvaptan reduced the decline in eGFR from 3.70 to 2.72 ml/min per 1.73 m^2 per year (26.5%); P=0.001). A post hoc analysis showed that tolvaptan decreased the rate of eGFR decline by 0.40 ml/min per 1.73 m^2 per year (95% CI, 20.25 to 1.05; P=0.23) in patients with CKD1, by 1.13 (95% CI, 0.61–1.66; P=0.001) in those with CKD2, and by 1.66 (95% CI, 0.83–2.45; P=0.001) in those with CKD3, with a trend for a positive subgroup–treatment interaction (P=0.07). Results for patients in CKD3 were similar when split into CKD3a and CKD3b. Tolvaptan treatment effects on eGFR slope were confirmed by comparing the eGFR values before treatment at baseline and those after discontinuation of study drug, which favored tolvaptan across CKD1 through CKD3. Consistent with the beneficial effects of tolvaptan on the rates of eGFR decline, patients with CKD2 and CKD3 randomly assigned to placebo were more likely to progress to a higher CKD stage at the last follow-up visit than those treated with tolvaptan.

This study will be based upon recruitment of an opportunistic sample given the rarity of the condition and the lack of available studies in the setting.

We plan to recruit a total of 150 patients which are currently within reach of the network coordinated by the proponent and composed by 11 Nephrology Centres. This network treats a total of 1500 (already genetically studied) patients of which we expect (based on standard response rates recognized in the population) acceptance to participate in the study to a value of approximately 40% of patients. These will be then allocated to the experimental and control intervention.

The selected sample is adequate to evaluate a significant reduction in the slope of eGFR at 2 years by 10%, which is a clinically relevant piece of information at the current state of knowledge, as well as a complete assessment of the benefits-harms trade-off of the two interventions.

12.2 Randomization

Randomization process will be carried out by a WEB-based, internet-accessible, system (e-trial platform). Investigators willing to include a patient should log-in into the e-trial platform and complete the registration form.

12.3 Statistical Methods

A comparative analysis for the primary outcome will be carried out under the principle of '"intention-to-treat" and including data from all subjects who are randomized. The efficacy and safety of compared interventions will be assessed. Trial data will be summarized by the calculation of means and standard deviations for normally distributed variables, medians and interquartile ranges for non-normally distributed variables, and frequency and percentage for categorical variables. Missing data will be ignored. The primary and secondary outcomes, namely the slope of the annualized eGFR and TKV will be analyzed using standard statistics including linear mixed-effects models. A P value <0.05 will be deemed significant.There are no plans for interim analyses.

13. ETHICAL CONSIDERATIONS

13.1 Investigator Responsibilities

The trial will be conducted in accordance with the requirements of the International Conference on Harmonization (ICH), of the Good Clinical Practice (GCP) Guideline and of the local regulatory and must adhere to the ethical principles that have their origin in the Declaration of Helsinki.

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

The Investigator will report promptly to the IEC any new information that may adversely affect the safety of subjects or the conduct of the study. Similarly, the Investigator will submit written summaries of the study status to the IEC annually, or more frequently if requested by the IEC.

Upon completion of the study, the Investigator will provide the IEC with a brief report of the outcome of the study, if required.

13.2 Informed Consent

Each subject (or a legally acceptable representative) must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by reviewing IEC. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Investigator-Sponsor policy.

Before entry into the study, the Investigator or an authorized member of the investigational staff must explain to potential subjects or their legally acceptable representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort it may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his/her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment.

The subject or legally acceptable representative will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and

before entry to the study, consent should be appropriately recorded by means of either the subject's or his/her legally acceptable representative's dated signature. After having obtained the consent, a copy of the informed consent form must be given to the subject.

If the subject or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and personally date and sign the informed consent form after the oral consent of the subject or legally acceptable representative is obtained.

Investigators (or whoever required by local regulations) should determine whether or not a mentally impaired or incapacitated subject is capable of giving informed consent and should sign a statement to that effect. If the subject is deemed mentally competent to give informed consent, the investigator should follow standard procedures. If the subject is deemed not to be mentally competent to give informed consent, must not be enrolled in clinical study.

13.3 Independent Ethics Committee

Prior to initiation of the study at each site, the protocol, the informed consent form(s), the subject information sheet(s), details of the subject recruitment procedures and any other relevant study documentation will be submitted to local IEC. At the end of the study, the Investigator-Sponsor will notify the IEC about the study completion.

13.4 Amendments to the Protocol

Modifications of the signed protocol are only possible by approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The IEC must be

informed of all protocol amendments and should be asked for its opinion as to whether a full re-evaluation of the ethical aspects of the study is necessary by the IEC. This should be fully documented.

The Investigator must not implement any deviation from or change to the protocol without discussion with and agreement by the Investigator-Sponsor and prior review and documented approval/favorable opinion of the amendment from the relevant IEC, except where it is necessary to eliminate an immediate hazard to study subjects, or where the change(s) involves only logistical or administrative aspects.

13.5 Premature Termination of the Study

If the study is prematurely terminated or suspended, the Investigator-Sponsor will prompt inform the Investigator/Institution and the IEC of the termination or suspension and the reason(s) for the termination or suspension.

13.6 Data Protection

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

The Investigator-Sponsor ensures that the personal data will be:

- processed fairly and lawfully;

- collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes;
- adequate, relevant, and not excessive in relation to said purposes;
- accurate and, where necessary, kept current.

Explicit consent for the processing of personal data will be obtained from the participating subject (or his/her legally acceptable representative) before collection of data. Such consent should also address the transfer of the data to other entities and to other countries.

The subject has the right to request through the Investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

14. STUDY MONITORING

14.1 Monitoring

The Investigator-Sponsor, or designee, will perform all monitoring functions within this clinical study. Monitors will be responsible for establishing and maintaining regular contact between the Investigator and the Investigator-Sponsor.

14.2 Data Confidentiality

Information about study subjects will be kept confidential and managed under the applicable laws and regulations. The data collection system for this study uses built-in security features to encrypt all data for transmission in both directions, preventing

unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of individually assigned user identification codes and passwords, made available only to authorized personnel who have completed prerequisite training.

14.3 Audit and Inspection

Investigator site, the study database and the study documentation may be subject to quality assurance audits during the course of the study either by the Investigator-Sponsor or his appointed representatives. In addition, regulatory bodies at their discretion may conduct inspections.

14.4 Source Documents

The Investigator shall permit the authorized Investigator-Sponsor, his representatives, and regulatory agencies to enter and inspect any site where the drug or records pertaining to the drug are held, and to inspect all records relating to an investigation, including subject records. Completed eCRFs must be available by the Investigator for review by the Investigator-Sponsor, his representatives, the monitor and the regulatory agencies. To ensure the accuracy of data submitted, it is mandatory that representatives of the Investigator-Sponsor and of the regulatory agencies have direct access to source documents (e.g., subject medical records, charts, laboratory reports, etc.). Subject confidentiality will be protected at all times.

14.5 Case Report Form

This study will use an Electronic Data Capture (EDC) system. Investigator site staff will not be given access to the EDC system until they have been trained. Automatic validation

programs check for data discrepancies in the eCRFs allow modification or verification of the entered data by the investigator staff. The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner. The CRF and the protocol are both confidential. The CRF will remain the property of the Investigator-Sponsor at all times.

The Investigator-Sponsor will supply electronic CRFs. All e-CRFs are to be completed and reviewed by the Investigator.

The e-CRFs for any subject leaving the study should be completed at the time medication is terminated for whatever reason.

It is each Investigator's responsibility to ensure that e-CRFs accurately reflect data contained in subject's records (e.g., source documents).

14.6 Investigator Site File

At the beginning of the study, an Investigator's study file will be established at the Center. The Investigator/Institution is responsible for maintaining the study documents, as required by the applicable regulatory requirements. The Investigator/Institution must take measures to prevent accidental or premature destruction of these documents.

14.7 Patient Insurance and Indemnity

The Investigator-Sponsor will provide the insurance in accordance with local guidelines and requirements as a minimum for the patients participating in this study. The terms of insurance will be kept in the study files. Subject insurance and the publication policy for the trial will be provided and documented according to DM 14 Luglio 2009.

14.8 Clinical Study Report

A final integrated clinical/statistical report will be prepared at the end of the study.

14.9 Disclosure of Information and Results

In signing the protocol/protocol amendment(s), every participating Investigator agrees to keep all information and results concerning the study confidential. The confidentiality obligation applies to all personnel involved at each site.

14.10 Publication

The results of this study will be published and/or presented at scientific meetings. Any formal publication of study results will be a collaborative effort between the Investigator-Sponsor and the Investigators. All manuscripts or abstracts will be reviewed and approved in writing by the Investigator-Sponsor prior to submission.

14.11 Archiving and Data Retention

All study documents should be retained in compliance with the regulatory requirements.

The final database will be archived by the Investigator-Sponsor according to regulatory requirements.

15. FIGURES AND TABLES

FIGURE 1

	SV1	SV2	EoTI	EoRI	M3	M6	M9	M12	M15	M18	M21	M24	FU1	FU2
EXAMS A	X	X		X	X	X	X	X	X	X	X	X	X	X
EXAMS B	X													
EXAMS C	X			X	X	X	X	X	X	X	X	X	X	X
EXAMS D	X				X	X	X	X	X	X	X	X		
EXAMS E	X			X	X	X	X	X	X	X	X	X		X
EXAMS F	X				X	X		X		X		X		X
EXAMS G	X							X				X		
PHYSICAL EXAMINATION	X	X		X	X	X	X	X	X	X	X	X	X	X
ARTERIAL BLOOD PRESSURE	X	X		X	X	X	X	X	X	X	X	X	X	X
POST VOID BODY WEIGHT	X	X		X	X	X	X	X	X	X	X	X	X	X
HEIGHT	X													
KIDNEY CT SCAN (non contrast)		X										X		

SCREENING PERIOD (2 WEEKS)
SV 1 = SCREENING VISIT 1
SV 2 = SCREENING VISIT 1
KIDNEY CT SCAN
IMP TITRATION PERIOD (DAY -28, DAY -8)
EoTI = END OF TITRATION
IMP RUN-IN PERIOD (DAY -7, DAY -1)
EoRI = END OF RUN-IN
ON-TREATMENT PERIOD (24 MONTHS)
M = MONTH
FOLLOW-UP PERIOD (3 WEEKS)
FU1 = FOLLOW UP VISIT 1 (DAY +8)
FU2 = FOLLOW UP VISIT 2 (DAY +21)

FIGURE 1 (LEGEND EXAMS)

EXAMS A

CREATININEMIA

EXAMS B

PT INR

EXAMS C

AST

ALT

BILIRUBIN TOT+FRACTIONED

UREA

URATE

PHOSPHATE

GLYCEMIA

VENOUS BLOOD GAS ANALYSIS

EXAMS D

GLYCOSYLATED HAEMOGLOBIN

EXAMS E

SERUM B-HCG

EXAMS F

COMPLETE BLOOD COUNT

SERUM CYANOCOBALAMI

EXAMS G

uPCR*

uACR*

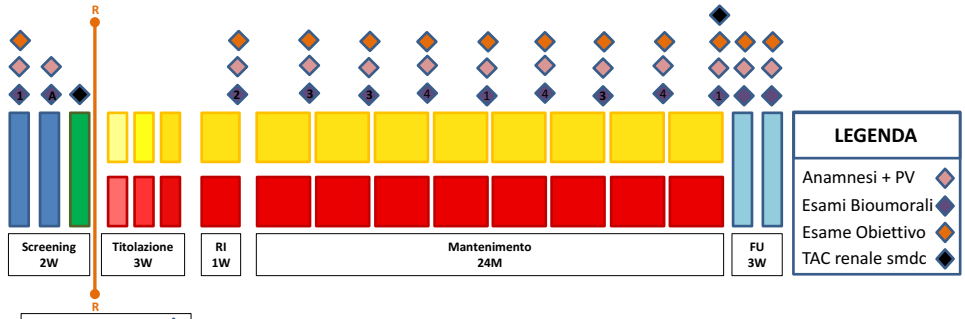
uMCP-1*

uEGF*

INSULINEMIA*

KIDNEY CT SCAN

FIGURE 2



Anamnesi

Storia clinica medica generale e PKD specifica, revisione terapia, aderenza alle norme dietetiche e comportamentali, parametri vitali.

Esami Bioumorali

A. sCreatinina, B. PT-INR, C. Ast, ALT, BLB tot e fraz, sUrea, sFosfato, sGlucsio, Emogasanalisi venosa D. Emoglobina Glicata E. βHCG sierica F. Emocromo completo, Cianocobalamina G. uACR, uPCR, uMCP-1, uEGF, Insulinemia	1. ESAMI A+B+C+D+E+F+G 2. ESAMI A+C+E 3. ESAMI A+C+D+E+F 4. ESAMI A+B+D+E 5. ESAMI A+C 6. ESAMI A+C+E+F

Esame Obiettivo

Esame fisico, peso corporeo – in Kg, altezza – in cm(questa solo alla prima visita).

TAC renale smdc

Spessore scansioni: 0.5mm, incremento 0.5mm, tempo di rotazione 0.5s, 120/200 kVp/mAs - da diaframma a sinfisi pubica (ogni centro).
Ricostruzione mediante software Vitrea FX 2.1, Vital Images, Minneapolis, MN USA (centro coordinatore).

FIGURE 3

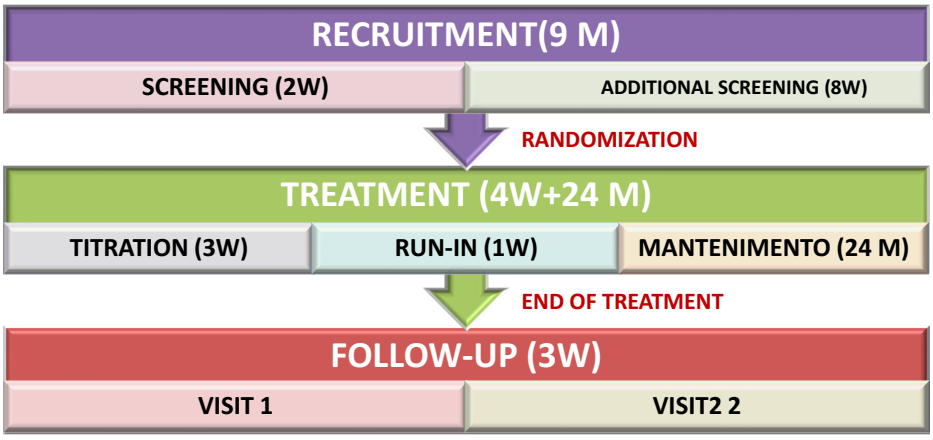


FIGURE 4

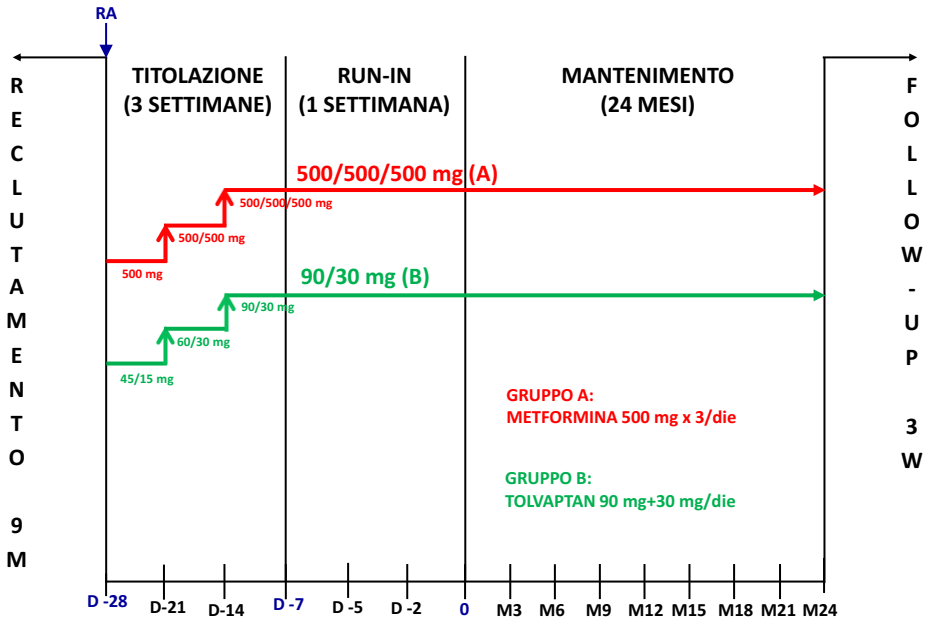


TABLE 1			
Boceprevir	Clrithromycin	Clotrimazole	Indinavir
Itraconazole	Ketoconazole	Lopinavir	Mibefradil
Nefazodone	Nelfinavir	Posaconazole	Ritonavir
Saquinavir	Telepravir	Telithromycin	Voriconazole

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