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Title: ADPKD current management and ongoing trials

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Abstract:	Autosomal dominant polycystic kidney disease (ADPKD) is the fourth diagnosis for both the incidence and prevalence of renal diseases that require replacement therapy. In Italy, there are at least 32,000 patients affected by ADPKD, of which about 2900 in dialysis. The pure costs of dialysis treatment for the Italian National Health Service can be conservatively estimated at 87 million euros per year. Even a modest slowdown in the evolution of the disease would obtain an important result in terms of reduction of health expenditure. In recent years, many new or repurposed drugs have been evaluated in clinical trials for ADPKD. In this review we will mainly focus on advanced stage clinical trials (phase 2 and 3). We have grouped these studies according to the molecular pathway addressed by the experimental drug or the therapeutic strategy. More than 10 years after the start of the first Phase III clinical trials in ADPKD, the first drug active in slowing disease progression is finally available. It cannot be considered a goal but only the beginning of a journey because of the significant side effects and the high cost of Tolvaptan. An exuberant basic research activity in the field, together with the large number of ongoing protocols, keep doctors' and their patients positive with regard to the discovery of new and better therapies in a not-too-distant future.
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Response to Reviewers:	Dear Editor, we thank you for reviewing our work which has allowed us to improve the final quality of our paper. We have carefully read the comments of the reviewers to whom we replied promptly as indicated in the following lines. Major Criticisms 1)The results from studies are reported without the support of numbers but only with a description and some comments. I believe this setting should be amended. The Authors should report the results in detail, especially for more clinically significative studies (Tolvaptan). Otherwise, the reader must look for the original manuscript to appreciate the data reported in the manuscript. The numeric details of the results are particularly crucial for Tolvapatan studies while it is comprehensible that in ongoing studies it is not worth summarising in details the results Support of numbers was added for the main clinical trials in particular for the Tolvaptan

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	and Somatostatin trials. These modifications can be checked in the main document by the ‘review tracking feature’ of Word. Minor Criticisms 1)The acronyms CFTR is used without an explanation. I suppose that it stands for fibrosis transmembrane conductance regulator The complete definition ‘cystic fibrosis transmembrane conductance regulator’ was reported at the first appearance of the CFTR acronym. 2)The formula of eGFR is written not correctly; indeed the 2 has to in superscript ml/min/1.73m2 ‘2 supercript’ was corrected for all the occurrences of the eGFR formula 3)In the Venglustat paragraph is reported "…increase in two central sphingolipids: glucosylceramide (GL-1) and ganglioside GM3 levels (34, 35)…" I suppose that levels refer to plasma levels. Please specify We specified plasma levels 4)In "Concluding remarks" the Authors say: "…only one molecule (Tolvaptan) has obtained authorization for treatment in ADPKD…". This point should be checked because I think that also the analogs of somatostatin have this authorization Octreotide-LAR is not EMA neither FDA authorized for ADPKD, but limited to the Italian Territory is reimbursed according to a special authorization of the Italian Agency of Drugs AIFA (‘DETERMINA 3 agosto 2018. Inserimento del medicinale octreotide a lunga durata d’azione nell’elenco dei medicinali erogabili a totale carico del Servizio sanitario nazionale,ai sensi della legge 23 dicembre 1996, n. 648’). This particular condition for the Italian patients and physician was reported in the ‘final remarks’ section with the following sentence : …. limited to the Italian territory Octreotide-LAR can be reimbursed by the Italian National Health System in adult ADPKD patients with stage 4 CKD and increased risk of rapid progression after the authorization of the Technical Scientific Advisory Board of the AIFA-CTS on the basis of the case-by-case assessment ….. Best Regards Riccardo Magistroni and Francesca Testa

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Title : ADPKD current management and ongoing trials

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the fourth diagnosis for both the incidence and prevalence of renal diseases that require replacement therapy. In Italy, there are at least 32,000 patients affected by ADPKD, of which about 2900 in dialysis. The pure costs of dialysis treatment for the Italian National Health Service can be conservatively estimated at 87 million euros per year. Even a modest slowdown in the evolution of the disease would obtain an important result in terms of reduction of health expenditure. In recent years, many new or repurposed drugs have been evaluated in clinical trials for ADPKD. In this review we will mainly focus on advanced stage clinical trials (phase 2 and 3). We have grouped these studies according to the molecular pathway addressed by the experimental drug or the therapeutic strategy. More than 10 years after the start of the first Phase III clinical trials in ADPKD, the first drug active in slowing disease progression is finally available. It cannot be considered a goal but only the beginning of a journey because of the significant side effects and the high cost of Tolvaptan. An exuberant basic research activity in the field, together with the large number of ongoing protocols, keep doctors' and their patients positive with regard to the discovery of new and better therapies in a not-too-distant future.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the fourth diagnosis for both the incidence and prevalence of renal diseases that require replacement therapy (1) and 1 in 10 patients needing renal replacement therapy has ADPKD (2). The predominant phenotype of ADPKD is the accumulation of cysts in renal parenchyma. The condition is genetically heterogeneous and is caused by the mutation of two polycystin genes, PKD1 and PKD2, and much more rarely, by other recently identified genes: GANAB (3), DNAJB11(4). The genetic defect of ADPKD subverts the normal differentiated phenotype of renal tubular epithelium. The final event of these alterations is end-stage renal disease requiring renal replacement therapy (dialysis or transplantation).

In Italy, there are at least 32,000 patients affected by ADPKD, of which about 2900 in dialysis and approximately as many are carriers of a renal transplant (5). Without considering the social cost of dialysis, which strongly reduces the quality of life of the patients and greatly increases their risk of death, the pure costs of dialysis treatment for the Italian National Health Service can be conservatively estimated at 87 million euros per year. In light of the clinical and economic considerations, the importance of interventions to reduce the progression to end-stage renal disease is clear. Even a modest slowdown in the evolution of the disease would obtain an important result in terms of reduction of health expenditure. In recent years, many new or repurposed drugs have been evaluated in clinical trials for ADPKD. Despite the extraordinary advances in therapeutic possibilities that are now available for ADPKD patients, to date, the therapeutic options for ADPKD cannot be considered satisfactory as they lack definitively curative therapies and consist of treatments aimed at controlling complications and of therapies aimed at slowing the progression of the disease.

In this review, we will not report the trials that considered the management of complications related to ADPKD, but rather, we will focus our discussion on therapies aimed at slowing down renal disease. Regarding the already-published clinical trials with results, we will mainly focus our discussion on clinical trials that have had a positive or at least suggestive outcome for therapeutic potential in the near future. With regard to clinical trials in progress without published data, we will mainly limit the discussion to molecules in advanced phase 3 clinical trials. The main features of the trials discussed in this review have been summarized in Table 1

VAPTANS AND INHIBITION OF VASOPRESSIN

Rationale for the use of Vaptans in ADPKD

The Vaptan drug family comprises agents that act by directly blocking the action of vasopressin at its receptors (V1AR, V1BR, and V2R). Before clinical validation in ADPKD, the V2R antagonist Tolvaptan has been developed for the treatment of hyponatremia in patients with congestive heart failure, liver cirrhosis, or syndrome of inappropriate antidiuretic hormone secretion (SIADH(6)). Studies on animal models have suggested that arginine vasopressin, through its second cAMP messenger, promotes cyst growth both through a proliferative stimulus and a secretion of fluids into the cyst lumen mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) chlorine channel. In fact, the cells of the collecting duct present the receptors of the arginine vasopressin of the V2R type. These receptors are coupled to adenylate cyclase 6, which, when activated, produces an increase in the cytosolic levels of cAMP. cAMP finally activates phosphokinase A (PKA), which is the central effector of this pathway. PKA activates the transcription of genes involved in cell proliferation. In parallel, CFTR is responsible for the chloride and bicarbonate permeability on the apical membrane of tubular epithelia. Its activity is modulated by the intracellular concentration of cAMP that is increased in cystic cells; the activation of the CFTR promotes the

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secretion of chloride in the cystic lumen, leading to intra-cystic fluid accumulation (7-9). Preclinical studies of selective blocking of the V2R receptor through an AVP receptor antagonist in ADPKD rodent models (8) have demonstrated a protective effect of this strategy, thus paving the way for clinical research of Vaptans in ADPKD.

Trials of Vaptans with published data

Tolvaptan

TEMPO 3:4 (10) is a large randomized double-blind controlled study that compared the efficacy of Tolvaptan compared to a placebo in two parallel arms of ADPKD patients. Patients were randomized into the two arms at a ratio of 2:1, respectively, Tolvaptan (961 patients) and placebo (484 patients). The inclusion criteria of the patients were defined with the aim of enrolling relatively young patients (aged 18-50 years) with a sufficiently preserved renal function (glomerular filtration calculated according to Cockroft and Gault higher than 60 ml / min) and with a rapidly progressive disease. The selection of progressive patients made use of the consolidated evidence that had defined the correlation between renal volume and evolution of the disease (11). Therefore, the authors defined a minimum cutoff of renal volume equal to 750 ml. Tolvaptan was administered orally in two daily administrations. The dose of Tolvaptan (or placebo) was titrated at the start of the study in the individual patient at weekly intervals for a period of 3 weeks, initially administered at a dose of 45 mg and 15 mg, in the morning and afternoon respectively, and titrated to 60 mg and 30 mg, and then at 90 mg and 30 mg, according to the tolerability reported by the patient. Throughout the study, the protocol favored the attempt to keep the drug at the maximum tolerated dosage. The study had a follow-up of 36 months with the main outcome being the evaluation of the effect of reducing the increase in kidney volume through MRI. At the beginning of the study, randomization on a large population obtained a balanced distribution of patients in terms of the clinical characteristics of the subjects enrolled between the two treatment arms. Subsequently, however, the balance between the two arms was reduced due to an increased drop-out of patients in the experimental arm due to the adverse effect of aquaresis, which reduced the compliance of the patients exposed to Tolvaptan.

Regarding the outcome of renal volume, the study demonstrates a significant reduction of approximately ~~49~~50 % in growth in patients treated with tolvaptan compared to placebo: over a 3-year period, the increase in total kidney volume in the tolvaptan group was 2.8% per year (95% confidence interval [CI], 2.5 to 3.1), versus 5.5% per year in the placebo group (95% CI, 5.1 to 6.0; P<0.001). The study also evaluated other secondary outcomes, among which, the outcome that generated the most clinical interest is certainly represented by the degree of preservation of renal function in patients under treatment: tolvaptan has shown a protective effect equal to a ~~26~~31.4 % reduction of functional loss compared to placebo (Tolvaptan was associated with a slower decline in kidney function (reciprocal of the serum creatinine level, −2.61 [mg per milliliter]−1 per year vs. −3.81 [mg per milliliter]−1 per year; P<0.001).
A subsequent post-hoc analysis (12) of the Tempo 3:4 study described the results as a function of the classes of renal failure (CKD) defined according to the KDIGO guidelines. This analysis suggested that Tolvaptan expresses its potential to reduce the volume increase in kidneys in all stages of renal failure in the Tempo 3:4 study (Tolvaptan reduced annualized TKV growth by 1.99%, 3.12%, and 2.61% per year across CKD1, CKD2 and CKD3, all P<0.001, ~~CKD1-2-3~~) and that this volume reduction is particularly pronounced in the first year of treatment but is also maintained in the remaining follow-up.

The same study showed that the advantage on renal function is evident in the CKD 2 stage ~~(1.13 ml / min / year)~~ and CKD 3 ~~(1.66 ml / min / year)~~. A statistically significant advantage is not, however, recordable in the CKD 1 stage (eGFR decline by 0.40 in CKD1 (P=0.23), 1.13 in CKD2 (P<0.001) and 1.66ml/min per 1.73m^2 per year inCKD3 (P<0.001)). This could be justified by the substantial stability in terms of renal function of the patients of this group for whom the recording of a glomerular filtration flexion would probably have required a longer follow-up than the 36 months foreseen in this study.

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Finally, another significant aspect to be taken into consideration in the clinical adoption of tolvaptan in ADPKD is the effect of rapid and reversible loss of renal function observed in the first weeks of treatment. This initial functional loss is limited (about 5% of the baseline value (10)) and reversible at the time of tolvaptan suspension. The cause of this phenomenon is not well understood: hemodynamic causes are invoked, and more recently, effects correlated to glomerular tubular feedback have been called into question(13, 14). However, the continuous and prolonged use of the drug produces a preservation of renal function, which amply compensates for the initial glomerular filtration flexion. For obvious reasons, this advantage appears more evident at the suspension of the treatment, as demonstrated in the TEMPO 3:4 study and confirmed by the extension study Tempo 4:4 (15).

The TEMPO 3:4 study, showed several additional clinical elements related to the phenomena of compensatory hyperfiltration and proteinuria. Albuminuria was a parameter measured during the trial and its analysis was the subject of a detailed post-hoc analysis report (16); albuminuria levels were normal in 47.9% of patients at the time of enrollment, moderately increased in 48.7% of cases, and severely increased in 3.4% of cases; in the study, albuminuria represented a predictive parameter of the future loss of eGFR, regardless of the remaining clinical features of the patient, except the TKV to which it is strongly correlated. The tolvaptan-treated arm achieved a decrease in albuminuria compared to placebo, independent of blood pressure. The efficacy of tolvaptan treatment against slowing TKV growth and eGFR loss was more easily detected in patients with high albuminuria values.

During the TEMPO 3:4 study and its extension study, TEMPO 4:4, a signal of liver toxicity risk emerged (17). A concentration of Alanine aminotransferase (ALT) that was three times the upper limit of normal was observed more frequently for subjects receiving Tolvaptan (4.4 %) compared to placebo (1.0 %). Two subjects (0.2%) during the trial TEMPO 3:4 and a further one during the TEMPO 4:4 study met the definition of the cases stated in Hy's Law (ALT greater than three times the upper limit of normality and total bilirubin greater than nine times the upper limit of normality); this is a condition of high risk for developing acute liver failure. The hepatic toxicity is dose independent and it was not possible to identify any possible risk factors related to the chance of developing this severe adverse effect. However, the condition was always reversible and it occured within the first 18 months of treatment. Since closer monitoring of transaminases has been included during the TEMPO 3:4 study by a protocol amendment and in subsequent studies, including the REPRISE study, no further cases have been recorded that comply with Hy's law. In the post-registration phase, the drug is only distributed by highly qualified centers that are required to carry out close monitoring of liver toxicity. At the moment, this strategy has been effective and there have been no reported cases of liver failure.

REPRISE (Replicating Evidence of Preserved Renal Function: an Investigation of Tolvaptan Safety and Efficacy in ADPKD) (18) is a clinical study of Tolvaptan that tested the drug's efficacy in a more advanced stage of renal failure compared to the TEMPO 3:4 study. Inclusion criteria included the recruitment of patients aged 18-55 years old with eGFR between 65 and 25 ml / min / 1.73m^2 (regardless of renal volume). In addition, patients aged 56-65 years with eGFR between 45 and 25 ml / min / 1.73m^2 were recruited, which thus showed a significant historical decline in renal function (loss greater than 2.0 ml / min / 1.73m^2 in the last year). This one-year study recruited 1370 patients who were randomized 1: 1 placebo: Tolvaptan. The study had a run-in period in which all patients were exposed to increasing doses of Tolvaptan before randomization. In this way, patients who were not compliant with Tolvaptan therapy were excluded from the study before randomization, avoiding the unbalance problems of the two arms of treatment that occurred in the TEMPO 3:4 study (10). Tolvaptan resulted in a slower decline than placebo in the estimated GFR over a one-year period: the change from baseline in the estimated GFR was −2.34 ml per minute per 1.73 m2 (95% confidence interval [CI], −2.81 to −1.87) in the tolvaptan group, as compared

with −3.61 ml per minute per 1.73 m2 (95% CI, −4.08 to −3.14) in the placebo group (difference, 1.27 ml per minute per 1.73 m2; 95% CI, 0.86 to 1.68; P<0.001).

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The analysis of the sub-populations enrolled in the study showed a very positive result in the subjects of class 2 and 3a of renal failure, with deflection of therapeutic efficacy in the subjects of the upper classes (3b and 4), though still within the range of clinically relevant estimates. The study did not show any therapeutic efficacy in subjects older than 55 years (the change from baseline in the estimated GFR was −2.54 ml per minute per 1.73 m2 in the tolvaptan group, as compared with −2.34 ml per minute per 1.73 m2 in the placebo group;difference, -0.20 ml per minute per 1.73 m2, P=0.65). It is difficult to explain this reduced efficacy of the drug in the older age group: this may be due to a mix of causes, among which, one of the most relevant may be enrichment in the population over 55 years of patients suffering from mild forms of ADPKD. In this sense, the criterion of historical decline larger than 2 ml / min / 1.73m^2 was probably not sufficiently selective to exclude slowly progressive patients.

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Ongoing Trial on Vaptans

Lixivaptan

Lixivaptan is a newer, nonpeptide, oral V2-receptor-specific antagonist. Like other vaptans, the molecule was previously tested for its possible use for hyponatremic conditions (SIADH, heart failure, liver failure) without being approved for marketing by the FDA. The molecule was then acquired by a new company that started testing it for the treatment of ADPKD. "ELISA (Evaluation of Lixivaptan in Subjects With Autosomal Dominant Polycystic Kidney Disease)" is a Phase 2 clinical trial that will evaluate the safety, pharmacokinetics, and pharmacodynamics of multiple doses of lixivaptan in patients with ADPKD with relatively preserved kidney function (chronic kidney disease stages 1 and 2) and moderately impaired renal function (stage 3). The study is currently enrolling and is expected to include up to 32 patients at approximately 15 sites in the United States. Although the primary objectives of the trial consist of the study of toxicity and pharmacokinetics of the molecule, some pharmacodynamic data will also be collected as secondary objectives, including total kidney volume and serum creatinine. The study should be completed by September 2019. (Source www.clinicaltrials.gov : NCT03487913).

Water as a therapeutic prescription in ADPKD

The administration of Tolvaptan has a therapeutic action in patients with ADPKD, which is mediated by the arginine vasopressin block (AVP) and by the related cAMP dependent water reabsorption, which gives rise to the important aquaretic side effect of the drug. We can assume that the intake of a significant amount of water can mimic the AVP antagonistic effect produced by Tolvaptan by reducing the circulating levels of AVP. An experiment carried out on rats with a recessive form of polycystic kidney (PCK rats) confirmed this. In that experiment, the hypothesis was that an addition of 5% glucose in the drinking water increased fluid intake approximately 3.5-fold compared with rats that received tap water. High water intake reduced the kidney/body weight ratio by 28.0% and improved renal function (19). On the basis of these preclinical data, a human pilot study (20) was developed to obtain a target urinary osmolality in the recruited patients. In eight patients, the amount of water needed to obtain urine with a target osmolality of 285 mOsm was calculated based on the urinary osmolar excretion. The pilot study conducted on only eight patients did not allow for any clinically appreciable results, however, it did have the role of elaborating a water dosing strategy to be administered to patients and of concluding that this strategy is potentially prosecutable. Another pilot study evaluated the effect of water prescription (about 3 liters of water per day) on urine osmolarity and cAMP urinary excretion, either in acute or chronic condition (21). This small study suggested that acute water load obtained a reduction of cAMP excretion, however, this effect was not replicated during chronic water prescription. Paradoxically, another small, non-randomized study reported that high water intake worsened renal function compared to the control group (22). Other feasibility studies (23, 24) were published or are planned to be concluded in the months following the publication of this paper (25)

(Source www.clinicaltrials.gov : NCT00759369 trial). At the Rogosin Institute in New York, a clinical trial based on the prescription of water in 32 polycystic patients began in 2017. This non-randomized sequential study involves 32 participants with a follow-up of 18 months. The primary outcome will be renal volume. The kidney volumes recorded at the end of a period of six months with a usual water intake will be compared with the renal volumes recorded at the end of the following 12-month period in which an increased water intake, as prescribed by the investigator, will be implemented on the basis of an urine test of the participant. This study is ongoing and should be concluded in December 2019 (source www.clinicaltrials.gov, NCT03102632). An even larger randomized study (180 patients) has been announced by the Australasian Kidney Trials Network with the acronym of "PREVENT-ADPKD"(26)

ANALOGS OF SOMATOSTATIN

Rationale for the use of analogs of somatostatin in ADPKD

The authors of the first clinical report (27) on the potential use of somatostatin analogs in ADPKD report the origin of this idea to a clinical case concerning a patient with ADPKD being treated with Octreotide due to an adenoma of the secreting growth pituitary hormone. Assessment by abdominal CT series had indicated a stability of renal volumes; likewise, the patient's renal function had not deteriorated in a two-year follow-up. Details deriving from the experiences on elasmobranches fish, and in particular, on the rectal gland of sharks (28), suggested the possible role of somatostatin in inhibiting the chlorine channel encoded by the CFTR gene by stimulation of the somatostatin receptors present in renal tubular cells (29, 30).

Completed Clinical Trials: Octreotide and Lanreotide

The first experiences with somatostatin analogs have involved small cohorts of pilot studies that have suggested promising preliminary results concerning the reduction of the progression of renal (27) and hepatic volumetric increase (31). Subsequently, in larger studies, researchers have evaluated the effect of the treatment on renal function. The most significant studies are the ALADIN studies (which involved early disease phase in ALADIN 1 (32) and late stage of disease in the ALADIN 2 study (33)) and the DIPAK-1 study (34).

Overall, these studies have recruited populations that are much lower than the experience gained with Tolvaptan. The most numerically representative trial is the DIPAK-1 study, which recruited 309 patients, while the ALADIN studies reported more limited experiences (79 patients in ALADIN 1 and 100 patients in ALADIN 2). The DIPAK 1 study involved patients aged between 18 and 60 years and stage 3a and 3b of chronic renal failure, and the follow up had a duration of 2.5 years. Although the study confirmed the ability of Lanreotide to determine a reduction in the progression of increase in renal size, the primary outcome of the study, the slowing of the worsening of renal function, was not successful: there were no significant differences for incidence of worsening kidney function (hazard ratio, 0.87 [95%CI, 0.49 to 1.52]; P = .87) and change in eGFR (−3.58 vs −3.45; difference, −0.13 mL/min/1.73m^2 per year [95%CI, −1.76 to 1.50]; P = .88). For this reason, the authors concluded that Lanreotide was not indicated in the treatment of advanced stages of ADPKD.

The ALADIN studies focused on the analog of somatostatin Octreotide. ALADIN 1 recruited 79 patients at a relatively early stage of disease (GFR greater than 40 ml / min / 1.73m^2 -MDRD formula- and age above 18 years). The primary outcome was the evaluation of the effect on the renal volume, which was positive at the end of the first year of follow-up but statistically not significant by the third year. The most clinically significant outcome~~s~~ of glomerular filtration variation ~~were~~ was assessed as secondary outcome~~s~~ and

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~~showed discordant~~ resulted not significant ~~trends~~ in measured GFR (based on iohexol) ~~and calculated GFR (based on plasma creatinine) assessments, thus making a definitive evaluation at least problematic~~(annual slope of GFR in Octreotide-LAR group –3·85 mL/min per 1·73m^2 per year (–6·20 to –1·92) vs –4·95 mL/min per 1·73m^2 per year (–7·49 to –1·97) in placebo group; p=0.13). The ALADIN 2 study recruited patients in a more advanced disease phase (estimated GFR between 15 and 40 ml / min / 1.73m2). The study showed a reduction effect on renal volume growth in the first and third years. However, the authors were not able to demonstrate efficacy based on the co-primary outcome of the reduction of renal function (GFR was measured ~~GFR~~ by the iohexol method, reduction in the median (95% CI) rate of GFR decline (0.56 [−0.63 to 1.75] ml/min/1.73 m^2 per year) was not significant (p = 0.295)). In this study, an exploratory analysis based on a composite clinical outcome (initiation of replacement treatment and/or doubling of creatinine) would have suggested a protective effect of lanreotide as compared to placebo. However, in the study the two groups do not appear perfectly balanced as the treatment group with Octreotide shows lower renal volumes and more preserved renal function than the placebo group. Furthermore, as in Aladin 1, also in Aladin 2 do the outcomes of renal function appear to be discrepant between what is recorded with the iohexol method and what was observed on creatinine (~~and the outcomes are the opposite in the Aladin 2 study compared to Aladin 1. I~~in the Aladin 2 study, the mGFR is not significant while the composite clinical outcome based on the doubling of plasma creatinine is significant thus making a definitive evaluation at least problematic).

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Ongoing Trial

The "Lanreotide In Polycystic kidney disease Study" (LIPS) is an ongoing trial that was recorded for the first time in ClinicalTrials.gov in April 2014. It will recruit 156 patients that will be randomized to either placebo or to the experimental arm. Patients of both sexes older than 18 years of CKD class 2 and 3 will be recruited for a 36-month follow-up period. The main outcome consists of evaluating the variations of GFR between the two groups. The study should be completed in September 2019. (source www.clinicaltrials.gov, NCT02127437)

SUBSTRATE REDUCTION THERAPY AGAINST SPHINGOLIPIDS

Rationale for the use of Venglustat in ADPKD

Sphingolipids, despite constituting a very modest proportion of all cell lipids, play a central role in the control of mechanisms that regulate critical cellular functions, including proliferation and apoptosis. Historically, attention to these molecules has originated from lysosomal storage diseases, such as Fabry's disease. More recently, attention to this class of molecules has involved research fields ~~in~~ of diseases that are even more common, such as diabetic nephropathy and polycystic kidney disease (35). Sphingolipid synthesis is closely coupled with the availability of glucose metabolites from aerobic glycolysis (see the following paragraph "METABOLIC AND DIETETIC APPROACH") which is activated in conditions of stimulation of cell proliferation and growth. Polycystic kidney animal models have shown a significant increase in two central sphingolipids: glucosylceramide (GL-1) and ganglioside GM3 plasma levels (36, 37). In the treatment of animal models with glucosylceramide synthase (GCS) inhibitors, a key enzyme in the synthesis of sphingolipids of the globosid class, there have been important reductions in the progression of cystic disease (36, 37). Venglustat is a potent oral inhibitor of GCS, the enzyme that transforms ceramide into glucosylceramide (GL-1). GL-1 is the precursor of many important pathogenetic sphingolipids in a wide range of diseases (Gaucher disease Type 3, Parkinson's disease, acid β-glucosidase mutation, polycystic kidney diseases). The treatment with Venglustat is based on the strategy of 'substrate reduction therapy', which reduces the availability of an intermediate necessary for the biochemical synthesis of subsequent molecules directly involved in the disease of interest. According to the preclinical data of efficacy of

Venglustat, the pharmaceutical pipeline of the molecule has been extended to several conditions, with ADPKD among them.

Ongoing Trial: Venglustat

"A Medical Research Study Designed to Determine if Venglustat Can be a Future Treatment for ADPKD Patients (STAGED-PKD)" is a worldwide Phase 3 clinical trial that will recruit 560 patients. The subjects will be randomized to the experimental product (at two different dosages) or to placebo and will have a follow-up of 24 months. Patients will be adults aged between 18 and 50 years of both sexes. Patients will be selected to have a CKD stage 2 and 3a. Patients will also be selected for having a rapidly progressive condition based on the Mayo Imaging Classification of ADPKD (38) (Class 1C, 1D, or 1E). The primary outcomes of the study concern the rate of kidney growth and the rate of glomerular filtration change. The study is currently recruiting and should be completed by January 2023 (source www.clinicaltrials.gov: *NCT03523728).*

METABOLIC AND DIETETIC APPROACH

Rationale of the interventions oriented to the correction of the metabolic derangement of ADPKD

Although many cellular pathways that are dysregulated in ADPKD have been identified, new pathways are still emerging. In recent years, convincing data have accumulated regarding the presence of profound alterations of cellular metabolism in ADPKD. In particular, these data suggest that cystic cells shift their energy metabolism from oxidative phosphorylation to aerobic glycolysis (39), an alteration of the energy metabolism previously described in neoplastic cells (Warburg effect) (40, 41). The role of aerobic glycolysis and its therapeutic potential in ADPKD has been extensively studied in pre-clinical models. In particular, at least three independent research groups have replicated the positive effect of the metabolic interference produced by the administration of 2-deoxy glucose in orthologous and non-orthologous rodent and rat models of ADPKD (42-45). The hypothesis is that, in ADPKD, the energetic metabolic derangement is related to the alteration of the activity of the metabolic sensors, such as the mTOR complex (46), AMPK (39, 43, 47), and Sirtuins (47-51). All these pathways are theoretically amenable for pharmacologic modulation: mTOR complex can be inhibited by the class of the mTOR inhibitors (everolimus and sirolimus); metformin, a common hypoglycemic drug, is an activator of AMPK; and finally, several natural polyphenols, including resveratrol, can modulate the sirtuin family. In addition to potential pharmacological interventions, recent preclinical experiences have suggested the possible role of dietary manipulations targeting the same metabolic sensors. Warner et al. applied a caloric restriction of 40% compared to an ad libitum feeding in a mouse model of ADPKD, and obtained an extraordinary reduction of the cystic growth (52). Kipp et al., in their preclinical mouse model, showed that a substantial benefit can be maintained, even with a small reduction of food intake (23% reduction of food intake) (53). Furthermore, the same group tested the hypothesis that the beneficial effects obtained by the diet are due to ketosis caused by intermittent starvation rather than caloric restriction per se (54).

Clinical trials with strategy active against the metabolic derangement of ADPKD

mTOR inhibitors

Despite a number of promising preclinical studies, the results of clinical trials on mTOR inhibitors in ADPKD have been extremely frustrating. Both the everolimus study (51) on a cohort of 433 patients characterized by a relatively advanced phase of the disease, as well as the study on sirolimus (50) of a lower number (100 subjects) of patients at an earlier stage had negative results. This discrepancy between the excellent preclinical results and the demoralizing clinical failure is probably due to a number of contributing factors that are not easy to identify. One of the hypotheses put forward regarding the Everolimus study concerns the possibility that the recruited population was in an advanced stage of disease, whereby the fibrotic processes were relatively irreversible. This hypothesis appears weaker in light of subsequent studies on Tolvaptan, and in particular, the REPRISE study (16), which showed therapeutic potential, even in the

advanced stage of the disease. Another hypothesis is that, in the study by Walz et al., Everolimus-inhibited phenomena of compensatory hypertrophy and glomerular hyperfiltration would lead to a worsening of renal function. This hypothesis would leave room for hope that, in the long term, the therapy could have shown beneficial effects, precisely as a function of the long-term protective potential of the inhibition of glomerular hyperfiltration, but this remains completely speculative. However, despite these considerations, the long-term tolerability profile of Everolimus appears low. In fact, in the study, the dropout of the experimental group was about 25%.

The evaluation of the Sirolimus study is more problematic, although, in fact, the study by Serra et al. had a smaller numerosity, this work was not even able to demonstrate an effect on renal volume reduction. It was hypothesized that the exposure concentrations of Sirolimus in mice in preclinical studies (55) were extremely superior to what was tolerable, and therefore, applied in clinical studies. Furthermore, it is possible that the Sirolimus blood dosages used for the inhibition of circulating leukocytes are not effective at the level of the renal tubule, as suggested by a case report (56) in which the researchers reported the outcome of the accidental renal transplantation in two recipients of the kidneys of a donor with a mild form of ADPKD. One recipient was treated with Sirolimus, while the other was treated with immunosuppressive therapy without Sirolimus: both subjects developed a similar progressive cystic disease despite the presence or absence of Sirolimus in the immunosuppressive regimen.

An important fact that emerged from the experience on Everolimus was the decoupling between the renal and kidney function data. In fact, until the study on Everolimus, renal volume and renal function were considered closely linked based on the seminal work of the CRISP group (57). Violation of this principle with uncoupling between renal volume and renal function has been clearly replicated in other subsequent experiences, and in particular, in the studies on somatostatin analogs (32-34, 58). Consequently, according to these data, the renal volumetric assessment alone is no longer accepted as the primary outcome by the drug regulatory agencies in the ADPKD registration studies.

METFORMIN

As described in the previous section, Metformin is a molecule capable of stimulating the 5' AMP-activated protein kinase (AMPK), a metabolic sensor that appears to be inhibited in ADPKD. AMPK, in turn, inhibits the CFTR channel, which is involved in intracystic fluid flows, and mTOR, another metabolic sensor implicated in the activation of cell proliferation. Preclinical studies based on metformin have shown a decrease in the cystic index in two mouse models of ADPKD (47). There are currently three clinical trials in progress to evaluate the role of metformin in ADPKD.

“Metformin as a Novel Therapy for Autosomal Dominant Polycystic Kidney Disease (TAME)” is a phase 2 controlled against placebo randomized study that will recruit 97 participants. In the study, the experimental drug (and the placebo in the control arm) will be uptitrated from 1 g to 2 g per day according to the patient’s tolerability. The inclusion criteria will select subjects of age 18-60 years, non-diabetic, of both sexes, with a GFR larger than 50 ml/min/1.73m^2. Because of the phase of the study, the primary outcomes regard compliance, tolerability, and toxicity of the drug; as secondary outcomes, renal volume and variation of GFR will be compared between the two arms. The study is expected to end in December 2020 (source ClinicalTrials.gov NCT02656017*).*

“Feasibility Study of Metformin Therapy in ADPKD” is a phase 2 controlled against placebo randomized study that will recruit 50 participants. The titration of the drug is closely related to the TAME study, starting from 1 g to a maximum of 2 g per day according to patient tolerance. The inclusion criteria are slightly different as they recruit an older population (30-60 years old non-diabetic patients) of both sexes and restricting the GFR between 50 and 80 ml/min/1.73m2. The study is expected to end in March 2020. (Source ClinicalTrials.gov , NCT02903511)

Finally, the “Metformin vs Tolvaptan for Treatment of Autosomal Dominant Polycystic Kidney Disease (METROPOLIS)” is an Italian study that, in contrast with the other metformin trials, will compare metformin against an active comparator: Tolvaptan. This is a phase 3 controlled study that will recruit 150 subjects. The inclusion criteria are also different from those of the previous studies as a principle of genetic selection will be adopted in this work. The study will recruit non-diabetic patients with truncating mutations of the PKD1 gene, aged between 18 and 50 years and a GFR equal or larger than 45 ml/min/1.73m2. The study is expected to end in September 2021. (Source ClinicalTrials.gov , NCT03764605).

2 DEOXY GLUCOSE

2-deoxy glucose(2DG) is a glucose analog that can be internalized into the cells by the same plasma membrane carrier of the glucose. Inside the cell, like glucose, it is phosphorylated, but it cannot be further metabolized. It accumulates in the cell, causing the energetic metabolic paralysis of the glycolytic pathway. The use of 2-deoxy glucose in ADPKD is based on the strategy of directly targeting the energy demand of the cystic epithelia. This strategy takes advantage of the evidence that cystic cells are completely dependent on glucose metabolism for their energy needs because they do not have the possibility of switching to other metabolic fuels (amino acids or lipids) due to mitochondrial inhibition (39, 43). The dependence of cystic tubular cells from glucose and the inability to metabolize other energetic sources as fatty acids or amino acids makes them highly sensitive to the toxicity of 2DG. In this regard, not-cystic cells are protected from 2DG toxicity by their ability of switching to mitochondrial oxidative phosphorylation. This glycidic dependency is in accordance to the neoplastic paradigm of the aerobic glycolysis or Warburg Effect (40, 59-61). This approach effectively slowed down the disease progression in several distinct orthologous models of the disease (42, 43) and obtained similar results in the Han:SPRD rat model (44). The 2DG is not currently registered for any therapeutic indication, although it has been tested in oncology clinical trials (62-66). A phase 1 clinical trial coordinated by an Italian collaborative group has the aim of assessing the drug's toxicity, tolerability, and pharmacokinetics in an ADPKD cohort (67). The study will recruit 18 patients with a 3-month follow-up, with the study scheduled to end in July 2020 (Source: personal communication).

PIOGLITAZONE

As already anticipated, fatty acid oxidation (FAO) is inhibited in ADPKD in the process of the metabolic rewiring of cystic cells that preserve molecules, such as lipids from energy consumption, because useful in anabolic processes (68). The peroxisome proliferator-activated receptor family (PPARα and PPARγ) are nuclear hormone receptors that are activated by fatty acids or their prostaglandin derivatives. At the nuclear level, they promote the gene expression of several factors involved in metabolism, including elements of activation of lipid peroxidation. PPARα is mainly expressed in the organs in which FAO is most active and its downregulation has been identified in cystic tubular cells (69). The fibrates, a known class of molecules with lipid-lowering activity, have a PPARα-activating capacity and have shown a protective role in animal models of ADPKD (69). Also, altered levels of PPARγ have been identified in ADPKD and in analogy to what has been shown for PPARα, stimulation of PPARγ has demonstrated a protective effect in animal models (70-73). Thiazolidinediones are a family of molecules that have the ability to activate PPARγ. Some molecules of this family are in clinical use as hypoglycemic agents, such as Pioglitazone and Rosiglitazone (Rosiglitazone’s authorization has been suspended in Europe because of cardiovascular safety concerns).

Pioglitazone is the subject of the PIOPKD clinical trial (Use of Low Dose Pioglitazone to Treat Autosomal Dominant Polycystic KidneyDisease). PIOPKD is a phase 2 clinical trial that will evaluate the safety of 18 participants during one year of treatment. The study will be followed up for 2 years. The non-diabetic patients of 18-55 years of age and with a GFR above ≥ 50 ml/min/1.73 m2 will be enrolled. The patients will

be randomized to PIOGLITAZONE at a 15 mg per day regimen or placebo. The primary outcome of the study is to evaluate the safety of this treatment, while secondary outcomes comprise the evaluation of renal volume by MRI and bone marrow fat content through the use of MRI spectrometry. The study should be completed by October 2019. (Source: ClinicalTrials.gov: NCT02697617)

Caloric Restriction and Ketogenic Diet

In consideration of the important metabolic imprint highlighted in this disease, a dietary approach has been purposed (52, 54). In theory, although clinical efficacy is demonstrated, this approach has some indisputable advantages: a diet therapy has low toxicity and low costs. On the other hand, diet therapy clashes with a problematic compliance that is particularly exacerbated in the case of diets that implement important caloric restrictions. Finally, since these approaches go beyond specific industrial interests, it is difficult to obtain substantial funding in the development of these programs.

Our group recently published a small pilot trial (the GREASE 1 trial) to explore the feasibility of a ketogenic diet in ADPKD (74). The central idea of any ketogenic diet essentially consists of a high fat and restricted carbohydrate content; this dietetic regimen produces a metabolic response that mimics starvation, whereby ketone bodies become the main fuel for the energetic need of cellular metabolism. According to the glucose dependency of cystic cells, ketogenic promises to be another non-toxic approach for disease management. The pilot study involved three patients for three months. The patients showed a positive compliance to the dietetic regimen and glycemia was significantly reduced during the follow up. The largest side effect was the increase in cholesterol levels. A larger randomized trial will be organized in the months following the publication of this paper (GREASE 2 trial).

A Caloric Restriction strategy has been adopted in the study "Daily Caloric Restriction and Intermittent Fasting in Overweight and Obese Adults With Autosomal Dominant Polycystic Kidney Disease" which is currently in the active recruitment phase. This is a randomized trial that will be conducted on 40 overweight or obese patients (BMI 25-45 kg/m^2) suffering from ADPKD, 18-65 years old, and with a GFR equal or larger than 30 ml/min/1.73m2. Patients will be randomized to intermittent or continuous energy intake reduction (in both cases by a 34% weekly energy deficit). The primary outcomes of the study mainly regard weight loss, tolerability, and compliance. Renal volumes by MRI will be analyzed in a secondary outcome. The study should be concluded by September 2020 (Source ClinicalTrials.gov: NCT03342742)

TYROSIN KINASE INHIBITION

Rationale for the use of Tyrosin Kinase Inhibitors

Tyrosin kinases are enzymes capable of transferring a phosphate unit derived from a donor, often an ATP molecule, to an acceptor protein whose activity is modified by this covalent modification. Many cellular pathways are controlled through these enzymatic cascades, and in ADPKD, the role of tyrosin kinases is also central; for example, in the EGFR pathway that constitutes one of the central stimuli of cystic cell proliferation (75). C-src is a tyrosin kinase closely implicated in the EGFR cascade, although the molecular details have not yet been fully elucidated (76). Pre-clinical studies have supported the role of tyrosin kinase inhibitors in cystic diseases (75, 77, 78).

Trials of Tyrosin Kinase Inhbitors with results

Bosutinib (SKI-606) is an oral dual Src/Bcr-Abl tyrosine kinase inhibitor (TKI) approved for the treatment of Philadelphia chromosome–positive chronic myeloid leukemia. A phase two trial that enrolled 172 patients has been published (79). The treatment obtained a 66% reduction of renal volume for Bosutinib versus placebo. However, the eGFR decline was not statistically significant in the comparison of the placebo group versus the treatment groups in the two groups during the 3 years follow up of the trial (however, the

decline was numerically higher in the treatment group). Furthermore, the treatment showed the known adverse effects for this class of oncologic drugs (diarrhea and nausea) in a large proportion of patients, thereby raising doubts about the real potential of chronic treatment prolonged for many years, as needed in ADPKD.

Ongoing Trials of Tyrosin Kinase Inhbitors

Tesevatinib is a multi-kinase inhibitor that promotes the inhibition of c-Src, thereby decreasing the activity of the EGFR axis (80). The Trial "A Safety, Pharmacokinetic and Dose-Escalation Study of KD019 (Tesevatinib) in Subjects With ADPKD" is active but not yet recruiting. This is a non-randomized Phase 1/2 trial that will enroll 74 participants, with the age range between 22 and 62 years, a GFR higher or equal to 35 ml/min/1.73m^2, and a htTKV ≥ 1000 mL (Total Kidney Volume corrected for height). The primary outcomes of the study will be evaluation of safety, pharmacokinetics, maximum tolerated dose, and Glomerular Filtration Rate (Source: ClinicalTrials.gov: NCT01559363).

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Concluding remarks

In recent years, a high number of molecules have been experimentally evaluated in ADPKD, with a number of these strategies promoted to advanced phase clinical trials. Figure 1 shows the temporal projection of the concluded trials and those that are on-going or in activation. Also documented in other fields, the attrition rate of these molecules is generally high, and to date, only one molecule (Tolvaptan) has obtained authorization for treatment in ADPKD (limited to the Italian territory Octreotide-LAR can be reimbursed by the Italian National Health System in adult ADPKD patients with stage 4 CKD and increased risk of rapid progression after the authorization of the Technical Scientific Advisory Board of the AIFA-CTS on the basis of the case-by-case assessment). The high failure rate in the clinical development of pharmaceutical molecules is well known and has been discussed in many previous reviews (81, 82), with ADPKD no exception. Many very promising approaches in preclinical models were not successful at the time of clinical validation. The reasons for these failures are heterogeneous and complex and should be taken into account in each individual case. Overall, we can draw a hypothesis that, in some cases, clinical studies have been undertaken after an excessively hasty preclinical evaluation. Further, pharmacokinetic and toxicity assessments have not been analyzed with sufficient depth in the preclinical phase and have, therefore, failed in the translational phase from animal to human. In this sense, the failure of Everolimus and Sirolimus in particular could be paradigmatic (83). A large number of clinical validation failures have predominantly matured in academic studies, with these studies only marginally supported by the pharmaceutical industry. Although academic research has the great advantage of complete intellectual freedom, on the other hand, it often does not have the sufficient resources to completely carry out expensive clinical protocols. This limitation of funds often results in studies with reduced sample sizes and/or reduced follow up, and ultimately, with low statistical power. Figure 2 shows the relationship between the length of the follow-up and the sample size of the phase III clinical trials in ADPKD in the concluded trials and in the on-going or in activation trials. The graph concerning the completed studies clearly shows how the studies on Tolvaptan are distinguished from the studies conducted on other molecules. Considering the trials conducted until now, the graph suggests that academic studies (all except those of Tolvaptan) have, most likely, been able to develop less statistical power due to a reduced sample size, among other factors. Studies on Tolvaptan have enrolled more than 2500 patients (points 6,7 and 8 of Fig.2). Even when considering the family of molecules most studied after Tolvaptan (i.e., the somatostatin analogs), the sample size did not exceed 600 patients (points 1,2 and 4 of Fig.2): one-fourth compared to what was expressed by Tolvaptan. It is possible that this reduced recruitment capacity has partly contributed to determining some of the failures in clinical trials in ADPKD. This trend does not seem to be different in either on-going or in-activation studies. Indeed, even an industrial study such as that on Venglustat does not express a sample size comparable to that of the Tolvaptan studies, a hint that suggests

the expectation for a high efficacy compared to the previous experiences, an expectation that only the outcome of this trial can decree if well placed.

More than 10 years after the start of the first Phase III clinical trials in ADPKD, a drug active in slowing disease progression is finally available. It cannot be considered a goal but only the beginning of a journey: the significant side effects, especially those of the aquaretic type, as well as the high cost of the drug, make this therapeutic option applicable only in a modest fraction of the affected patients. Even in patients undergoing treatment, it is not possible to obtain a cure but only a slowing of the progression of the disease. All the other molecules considered in this review had a negative clinical outcome or at best they require the confirmation of new or ongoing clinical studies before a possible clinical adoption. An exuberant basic research activity in the field, together with the large number of ongoing protocols, keep doctors' and their patients positive with regard to the discovery of new and better therapies in a not-too-distant future.

Agent/	Type of Trial / status	Inclusion Criteria	Results/ Outcomes
Vaptans:			
Tolvaptan	TEMPO 3: 4 phase 3, double blinded placebo-controlled RCT 1445 patients , 36 months Published (10)	Age 18-50years eCrCL≥60ml/min TKV≥750ml (MRI)	TKV growth reduction by 49% eGFR loss protected by 26%
	TEMPO 4:4 phase 4, extension trials of TEMPO 3:4 871 patients, 24 months Published (15)	Patients from TEMPO 3:4	sustained disease-modifying effect of tolvaptan on eGFR
	REPRISE phase 3, double blinded placebo-controlled randomized withdrawal trial 1370 patients, 12 months Published (18)	Age 18-55 years with eGFR 25-65 ml/min/1.73m^2 Or Age 56-65 years with eGFR 25-45ml/min/1.73m^2 and progressive disease	eGFR loss significantly reduced , but not in patients >55y
Lixivaptan	ELISA phase 2, open-label 32 patients, 12 months Recruiting NCT03487913	Age 18-65 years and eGFR > 30 mL/min/1.73 m^2	Primary : toxicity and pharmacokinetic studies of the molecule, Secondary: TKV and creatinine
mTOR inhibitors:			
Everolimus	Phase 3, double blinded placebo-controlled RCT 431 patients, 24 months Published (51)	eGFR≥ 30 ml/min1.73 m^2 and TKV>1000ml	Significant reduction in TKV growth, no effect on eGFR
Sirolimus	Phase 3, open-label placebo controlled RCT 100 patients, 24 months Published (50)	Age 18-40years and eCrCl≥ 70 ml/min/1.73 m^2	No effect on TKV and eGFR
Somatostatin analogues :			
Octreotide	ALADIN 1 Phase 3, single-blind placebo-controlled RCT, 79 patients, 36 months Published (32)	Age >18 years and mGFR ≥ 40ml/min/1.73m^2	Positive effect on TKV at the end of the 1° year, not significant at the 3° year
Octreotide	ALADIN 2 Phase 3, double-blind placebo-controlled RCT, 100 patients, 36 months Published (33)	Age >18years and mGFR 15-40ml/min/1.73 m^2	Positive effect on TKV at 1° and 3° year, not significant on mGFR
Lanreotide	DIPAK 1 phase 3, open-label RCT, 309 patients , 30 months Published (34)	Age 18-60 years and eGFR 30-60ml/min/1.73m^2	No positive effect on eGFR, significant reduction in TKV growth
Lanreotide	LIPS Phase 3, double-blind RCT, 156 patients Active, not recruiting NCT02127437	Age > 18 years and eGFR 30-89ml/min/1.73m^2	Variations of GFR between the two groups

Substrate reduction therapy against Sphingolipids:			
Venglustat	Phase 3, double-blind placebo-controlled RCT 560 patients, 24 months, Recruiting NCT03523728	Age 18-50 years and eGFR 45-90ml/Min/1.73 m^2 TKV Mayo 1C-1E	Variation of kidney growth and change of glomerular filtration
Metabolic and Dietetic approach:			
Metformin	TAME Phase 2, double-blind placebo-controlled RCT, 97 patients, 26 months Active, not recruiting NCT02656017	Age 18 -60 years, non-diabetic and eGFR > 50 ml/min/1.73m^2	Compliance, tolerability and toxicity of the drug; secondary outcomes TKV and changes in eGFR
Metformin	phase 2, double-blind placebo-controlled RCT, 50 patients ,12 months Recruiting NCT02903511	Age 30- 60 years, non-diabetic with eGFR between 50 and 80 ml/min/1.73m^2	changes of TKV and eGFR
Metformin	METROPOLIS Phase 3, RCT Tolvaptan - controlled, 150 patients, 25 months Not yet recruiting NCT03764605	Age 18-50 with eGFR ≥45 ml/min/1.73m^2 non-diabetic with truncating mutations of the PKD1 gene.	changes of TKV and eGFR
2-Deoxy Glucose (2DG)	Phase 1, 18 patients, 3 months	Age 18-55 with eGFR > 45-ml/min/1.73m^2, TKV Mayo 1C-1E	tolerability and toxicity
Pioglitazone	PIOPKD Phase 2, double-blind placebo-controlled RCT, 18 patients, 24 months Active, not recruiting NCT02697617	Age 18-55 years with eGFR ≥ 50 ml/min/1.73 m^2 non-diabetic	safety; secondary: TKV by MRI and bone marrow fat content by MRI spectrometry
Caloric Restriction Diet	Randomized to intermittent or continuous energy intake reduction , 40 patients , 18 months, recruting NCT03342742	18-65 years, BMI 25-45 kg/m^2 with eGFR≥ 30 ml/min/1.73m^2	Primary: weight loss, tolerability and compliance. Secondary: TKV by MRI
Tyrosin Kinase Inhibition:			
Bosutinib (SKI-606)	Phase 2, RCT, 172 patients, 36 months Published (79)	Age 18-50 with eGFR ≥ 60 ml/min/1.73 m^2 TKV ≥ 750mL	66% reduction of TKV for bosutinib versus placebo the eGFR decline was not statistically significant
Tesevatinib	Non-randomized Phase 1/2 trial, 74 patients, 24months Active , not yet recruiting NCT01559363	Age 22-62 years with eGFR ≥35 ml/min/1.73m^2 and a htTKV ≥ 1000 mL	safety, pharmacokinetics, maximum tolerated dose and eGFR

Table 1: summary data of the main clinical trials completed, ongoing or activated in ADPKD. RCT = Randomized Clinical Trial, eGFR = estimated glomerular filtration rate, TKV = Total Kidney Volume, BMI = Body Mass Index

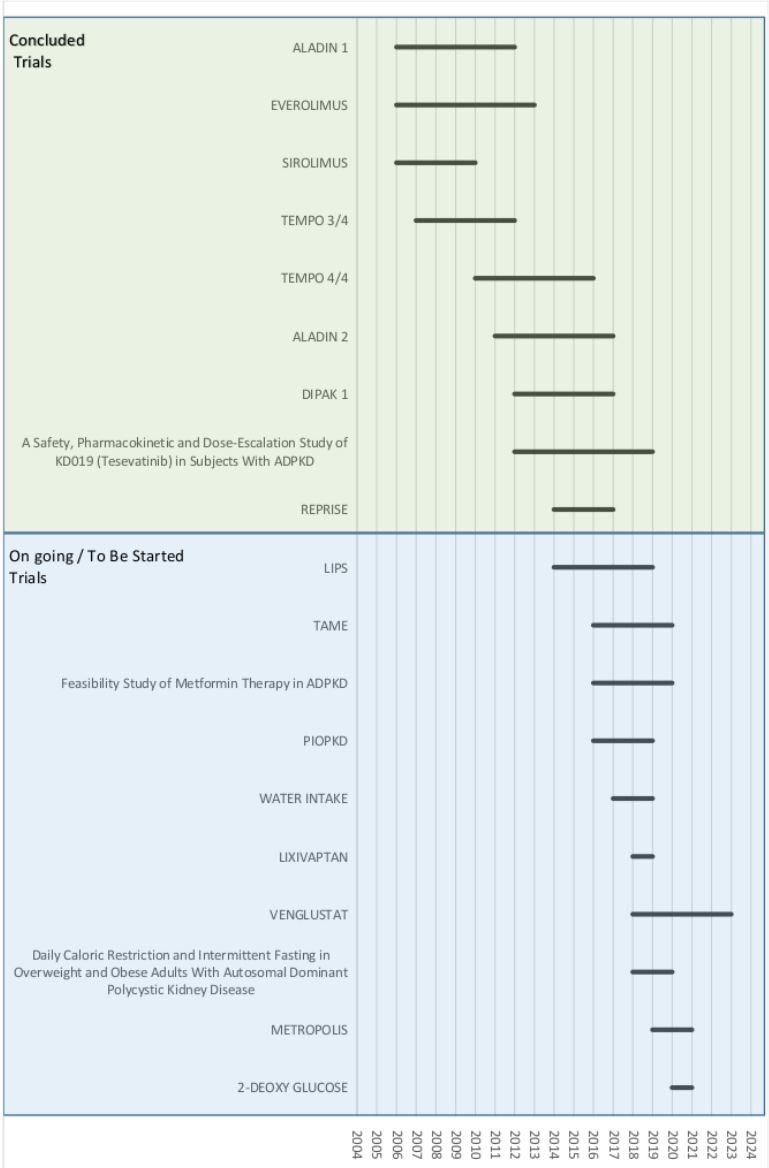

Fig. 1: representation of the temporal distribution of completed, on-going or in activation clinical trials

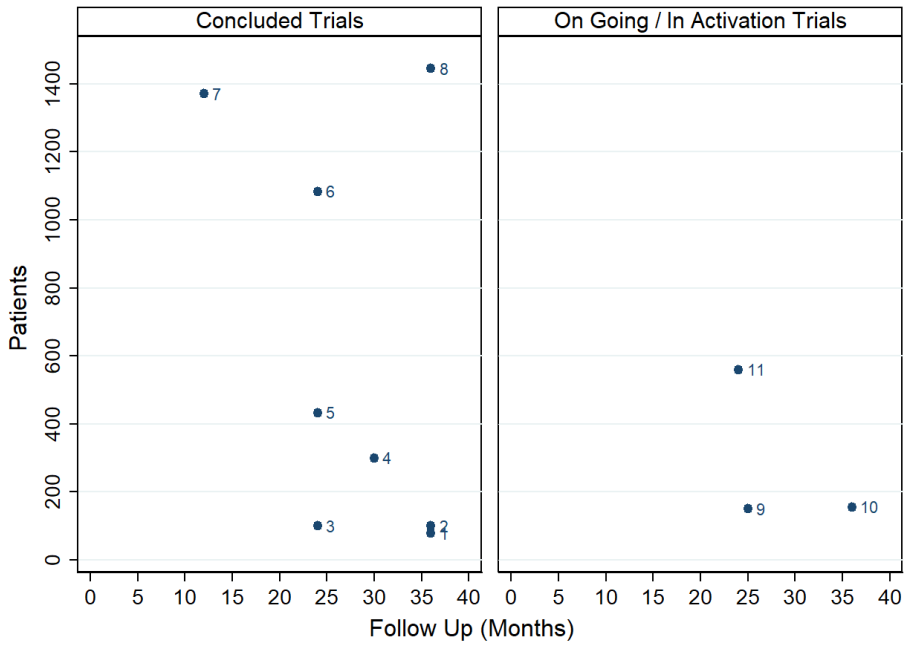

Fig. 2: graph representing the ratio between sample size and duration of the FUP of the main phase 3 clinical trials. The left panel shows thecompleted studies, while the right panel shows on-going and in activation clinical trials. 1 = Aladin 1 (32); 2 = Aladin 2 (33); 3 = Sirolimus (50); 4 = DIPAK 1 (34); 5 = Everolimus (51); 6 = Tempo 4:4 (15); 7 = REPRISE (18); 8 TEMPO 3:4 (10); 9 = METROPOLIS (NCT03764605); 10 = LIPS (NCT02127437); 11 = VENGLUSTAT (NCT03523728)

Disclosure of Financial Interests and Conflict of Interest

R.M. was involved in the trials of Tolvaptan sponsored by Otsuka Pharmaceutical as Principal Investigator. R.M. is scientific advisor of Otsuka Italia. F.T. does not declare any conflict of interest.

Compliance with Ethical Standards”

This paper did not involve Human Participants and/or Animals

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Title : ADPKD current management and ongoing trials

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the fourth diagnosis for both the incidence and prevalence of renal diseases that require replacement therapy. In Italy, there are at least 32,000 patients affected by ADPKD, of which about 2900 in dialysis. The pure costs of dialysis treatment for the Italian National Health Service can be conservatively estimated at 87 million euros per year. Even a modest slowdown in the evolution of the disease would obtain an important result in terms of reduction of health expenditure. In recent years, many new or repurposed drugs have been evaluated in clinical trials for ADPKD. In this review we will mainly focus on advanced stage clinical trials (phase 2 and 3). We have grouped these studies according to the molecular pathway addressed by the experimental drug or the therapeutic strategy. More than 10 years after the start of the first Phase III clinical trials in ADPKD, the first drug active in slowing disease progression is finally available. It cannot be considered a goal but only the beginning of a journey because of the significant side effects and the high cost of Tolvaptan. An exuberant basic research activity in the field, together with the large number of ongoing protocols, keep doctors' and their patients positive with regard to the discovery of new and better therapies in a not-too-distant future.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the fourth diagnosis for both the incidence and prevalence of renal diseases that require replacement therapy (1) and 1 in 10 patients needing renal replacement therapy has ADPKD (2). The predominant phenotype of ADPKD is the accumulation of cysts in renal parenchyma. The condition is genetically heterogeneous and is caused by the mutation of two polycystin genes, PKD1 and PKD2, and much more rarely, by other recently identified genes: GANAB (3), DNAJB11(4). The genetic defect of ADPKD subverts the normal differentiated phenotype of renal tubular epithelium. The final event of these alterations is end-stage renal disease requiring renal replacement therapy (dialysis or transplantation).

In Italy, there are at least 32,000 patients affected by ADPKD, of which about 2900 in dialysis and approximately as many are carriers of a renal transplant (5). Without considering the social cost of dialysis, which strongly reduces the quality of life of the patients and greatly increases their risk of death, the pure costs of dialysis treatment for the Italian National Health Service can be conservatively estimated at 87 million euros per year. In light of the clinical and economic considerations, the importance of interventions to reduce the progression to end-stage renal disease is clear. Even a modest slowdown in the evolution of the disease would obtain an important result in terms of reduction of health expenditure. In recent years, many new or repurposed drugs have been evaluated in clinical trials for ADPKD. Despite the extraordinary advances in therapeutic possibilities that are now available for ADPKD patients, to date, the therapeutic options for ADPKD cannot be considered satisfactory as they lack definitively curative therapies and consist of treatments aimed at controlling complications and of therapies aimed at slowing the progression of the disease.

In this review, we will not report the trials that considered the management of complications related to ADPKD, but rather, we will focus our discussion on therapies aimed at slowing down renal disease. Regarding the already-published clinical trials with results, we will mainly focus our discussion on clinical trials that have had a positive or at least suggestive outcome for therapeutic potential in the near future. With regard to clinical trials in progress without published data, we will mainly limit the discussion to molecules in advanced phase 3 clinical trials. The main features of the trials discussed in this review have been summarized in Table 1

VAPTANS AND INHIBITION OF VASOPRESSIN

Rationale for the use of Vaptans in ADPKD

The Vaptan drug family comprises agents that act by directly blocking the action of vasopressin at its receptors (V1AR, V1BR, and V2R). Before clinical validation in ADPKD, the V2R antagonist Tolvaptan has been developed for the treatment of hyponatremia in patients with congestive heart failure, liver cirrhosis, or syndrome of inappropriate antidiuretic hormone secretion (SIADH(6)). Studies on animal models have suggested that arginine vasopressin, through its second cAMP messenger, promotes cyst growth both through a proliferative stimulus and a secretion of fluids into the cyst lumen mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) chlorine channel. In fact, the cells of the collecting duct present the receptors of the arginine vasopressin of the V2R type. These receptors are coupled to adenylate cyclase 6, which, when activated, produces an increase in the cytosolic levels of cAMP. cAMP finally activates phosphokinase A (PKA), which is the central effector of this pathway. PKA activates the transcription of genes involved in cell proliferation. In parallel, CFTR is responsible for the chloride and bicarbonate permeability on the apical membrane of tubular epithelia. Its activity is modulated by the intracellular concentration of cAMP that is increased in cystic cells; the activation of the CFTR promotes the

secretion of chloride in the cystic lumen, leading to intra-cystic fluid accumulation (7-9). Preclinical studies of selective blocking of the V2R receptor through an AVP receptor antagonist in ADPKD rodent models (8) have demonstrated a protective effect of this strategy, thus paving the way for clinical research of Vaptans in ADPKD.

Trials of Vaptans with published data

Tolvaptan

TEMPO 3:4 (10) is a large randomized double-blind controlled study that compared the efficacy of Tolvaptan compared to a placebo in two parallel arms of ADPKD patients. Patients were randomized into the two arms at a ratio of 2:1, respectively, Tolvaptan (961 patients) and placebo (484 patients). The inclusion criteria of the patients were defined with the aim of enrolling relatively young patients (aged 18-50 years) with a sufficiently preserved renal function (glomerular filtration calculated according to Cockroft and Gault higher than 60 ml / min) and with a rapidly progressive disease. The selection of progressive patients made use of the consolidated evidence that had defined the correlation between renal volume and evolution of the disease (11). Therefore, the authors defined a minimum cutoff of renal volume equal to 750 ml. Tolvaptan was administered orally in two daily administrations. The dose of Tolvaptan (or placebo) was titrated at the start of the study in the individual patient at weekly intervals for a period of 3 weeks, initially administered at a dose of 45 mg and 15 mg, in the morning and afternoon respectively, and titrated to 60 mg and 30 mg, and then at 90 mg and 30 mg, according to the tolerability reported by the patient. Throughout the study, the protocol favored the attempt to keep the drug at the maximum tolerated dosage. The study had a follow-up of 36 months with the main outcome being the evaluation of the effect of reducing the increase in kidney volume through MRI. At the beginning of the study, randomization on a large population obtained a balanced distribution of patients in terms of the clinical characteristics of the subjects enrolled between the two treatment arms. Subsequently, however, the balance between the two arms was reduced due to an increased drop-out of patients in the experimental arm due to the adverse effect of aquaresis, which reduced the compliance of the patients exposed to Tolvaptan.

Regarding the outcome of renal volume, the study demonstrates a significant reduction of approximately 50 % in growth in patients treated with tolvaptan compared to placebo: over a 3-year period, the increase in total kidney volume in the tolvaptan group was 2.8% per year (95% confidence interval [CI], 2.5 to 3.1), versus 5.5% per year in the placebo group (95% CI, 5.1 to 6.0; P<0.001). The study also evaluated other secondary outcomes, among which, the outcome that generated the most clinical interest is certainly represented by the degree of preservation of renal function in patients under treatment: tolvaptan has shown a protective effect equal to a 31.4 % reduction of functional loss compared to placebo (Tolvaptan was associated with a slower decline in kidney function (reciprocal of the serum creatinine level, −2.61 [mg per milliliter]−1 per year vs. −3.81 [mg per milliliter]−1 per year; P<0.001).
A subsequent post-hoc analysis (12) of the Tempo 3:4 study described the results as a function of the classes of renal failure (CKD) defined according to the KDIGO guidelines. This analysis suggested that Tolvaptan expresses its potential to reduce the volume increase in kidneys in all stages of renal failure in the Tempo 3:4 study (Tolvaptan reduced annualized TKV growth by 1.99%, 3.12%, and 2.61% per year across CKD1, CKD2 and CKD3, all P<0.001,) and that this volume reduction is particularly pronounced in the first year of treatment but is also maintained in the remaining follow-up.

The same study showed that the advantage on renal function is evident in the CKD 2 stage and CKD 3. A statistically significant advantage is not, however, recordable in the CKD 1 stage (eGFR decline by 0.40 in CKD1 (P=0.23), 1.13 in CKD2 (P<0.001) and 1.66ml/min per 1.73m^2 per year inCKD3 (P<0.001)). This could be justified by the substantial stability in terms of renal function of the patients of this group for whom the recording of a glomerular filtration flexion would probably have required a longer follow-up than the 36 months foreseen in this study.

Finally, another significant aspect to be taken into consideration in the clinical adoption of tolvaptan in ADPKD is the effect of rapid and reversible loss of renal function observed in the first weeks of treatment. This initial functional loss is limited (about 5% of the baseline value (10)) and reversible at the time of tolvaptan suspension. The cause of this phenomenon is not well understood: hemodynamic causes are invoked, and more recently, effects correlated to glomerular tubular feedback have been called into question(13, 14). However, the continuous and prolonged use of the drug produces a preservation of renal function, which amply compensates for the initial glomerular filtration flexion. For obvious reasons, this advantage appears more evident at the suspension of the treatment, as demonstrated in the TEMPO 3:4 study and confirmed by the extension study Tempo 4:4 (15).

The TEMPO 3:4 study, showed several additional clinical elements related to the phenomena of compensatory hyperfiltration and proteinuria. Albuminuria was a parameter measured during the trial and its analysis was the subject of a detailed post-hoc analysis report (16); albuminuria levels were normal in 47.9% of patients at the time of enrollment, moderately increased in 48.7% of cases, and severely increased in 3.4% of cases; in the study, albuminuria represented a predictive parameter of the future loss of eGFR, regardless of the remaining clinical features of the patient, except the TKV to which it is strongly correlated. The tolvaptan-treated arm achieved a decrease in albuminuria compared to placebo, independent of blood pressure. The efficacy of tolvaptan treatment against slowing TKV growth and eGFR loss was more easily detected in patients with high albuminuria values.

During the TEMPO 3:4 study and its extension study, TEMPO 4:4, a signal of liver toxicity risk emerged (17). A concentration of Alanine aminotransferase (ALT) that was three times the upper limit of normal was observed more frequently for subjects receiving Tolvaptan (4.4 %) compared to placebo (1.0 %). Two subjects (0.2%) during the trial TEMPO 3:4 and a further one during the TEMPO 4:4 study met the definition of the cases stated in Hy's Law (ALT greater than three times the upper limit of normality and total bilirubin greater than nine times the upper limit of normality); this is a condition of high risk for developing acute liver failure. The hepatic toxicity is dose independent and it was not possible to identify any possible risk factors related to the chance of developing this severe adverse effect. However, the condition was always reversible and it occured within the first 18 months of treatment. Since closer monitoring of transaminases has been included during the TEMPO 3:4 study by a protocol amendment and in subsequent studies, including the REPRISE study, no further cases have been recorded that comply with Hy's law. In the post-registration phase, the drug is only distributed by highly qualified centers that are required to carry out close monitoring of liver toxicity. At the moment, this strategy has been effective and there have been no reported cases of liver failure.

REPRISE (Replicating Evidence of Preserved Renal Function: an Investigation of Tolvaptan Safety and Efficacy in ADPKD) (18) is a clinical study of Tolvaptan that tested the drug's efficacy in a more advanced stage of renal failure compared to the TEMPO 3:4 study. Inclusion criteria included the recruitment of patients aged 18-55 years old with eGFR between 65 and 25 ml / min / 1.73m^2 (regardless of renal volume). In addition, patients aged 56-65 years with eGFR between 45 and 25 ml / min / 1.73m^2 were recruited, which thus showed a significant historical decline in renal function (loss greater than 2.0 ml / min / 1.73m^2 in the last year). This one-year study recruited 1370 patients who were randomized 1: 1 placebo: Tolvaptan. The study had a run-in period in which all patients were exposed to increasing doses of Tolvaptan before randomization. In this way, patients who were not compliant with Tolvaptan therapy were excluded from the study before randomization, avoiding the unbalance problems of the two arms of treatment that occurred in the TEMPO 3:4 study (10). Tolvaptan resulted in a slower decline than placebo in the estimated GFR over a one-year period: the change from baseline in the estimated GFR was −2.34 ml per minute per 1.73 m2 (95% confidence interval [CI], −2.81 to −1.87) in the tolvaptan group, as compared

with −3.61 ml per minute per 1.73 m2 (95% CI, −4.08 to −3.14) in the placebo group (difference, 1.27 ml per minute per 1.73 m2; 95% CI, 0.86 to 1.68; P<0.001).

The analysis of the sub-populations enrolled in the study showed a very positive result in the subjects of class 2 and 3a of renal failure, with deflection of therapeutic efficacy in the subjects of the upper classes (3b and 4), though still within the range of clinically relevant estimates. The study did not show any therapeutic efficacy in subjects older than 55 years (the change from baseline in the estimated GFR was −2.54 ml per minute per 1.73 m2 in the tolvaptan group, as compared with −2.34 ml per minute per 1.73 m2 in the placebo group;difference, -0.20 ml per minute per 1.73 m2, P=0.65). It is difficult to explain this reduced efficacy of the drug in the older age group: this may be due to a mix of causes, among which, one of the most relevant may be enrichment in the population over 55 years of patients suffering from mild forms of ADPKD. In this sense, the criterion of historical decline larger than 2 ml / min / 1.73m^2 was probably not sufficiently selective to exclude slowly progressive patients.

Ongoing Trial on Vaptans

Lixivaptan

Lixivaptan is a newer, nonpeptide, oral V2-receptor-specific antagonist. Like other vaptans, the molecule was previously tested for its possible use for hyponatremic conditions (SIADH, heart failure, liver failure) without being approved for marketing by the FDA. The molecule was then acquired by a new company that started testing it for the treatment of ADPKD. "ELISA (Evaluation of Lixivaptan in Subjects With Autosomal Dominant Polycystic Kidney Disease)" is a Phase 2 clinical trial that will evaluate the safety, pharmacokinetics, and pharmacodynamics of multiple doses of lixivaptan in patients with ADPKD with relatively preserved kidney function (chronic kidney disease stages 1 and 2) and moderately impaired renal function (stage 3). The study is currently enrolling and is expected to include up to 32 patients at approximately 15 sites in the United States. Although the primary objectives of the trial consist of the study of toxicity and pharmacokinetics of the molecule, some pharmacodynamic data will also be collected as secondary objectives, including total kidney volume and serum creatinine. The study should be completed by September 2019. (Source www.clinicaltrials.gov : NCT03487913).

Water as a therapeutic prescription in ADPKD

The administration of Tolvaptan has a therapeutic action in patients with ADPKD, which is mediated by the arginine vasopressin block (AVP) and by the related cAMP dependent water reabsorption, which gives rise to the important aquaretic side effect of the drug. We can assume that the intake of a significant amount of water can mimic the AVP antagonistic effect produced by Tolvaptan by reducing the circulating levels of AVP. An experiment carried out on rats with a recessive form of polycystic kidney (PCK rats) confirmed this. In that experiment, the hypothesis was that an addition of 5% glucose in the drinking water increased fluid intake approximately 3.5-fold compared with rats that received tap water. High water intake reduced the kidney/body weight ratio by 28.0% and improved renal function (19). On the basis of these preclinical data, a human pilot study (20) was developed to obtain a target urinary osmolality in the recruited patients. In eight patients, the amount of water needed to obtain urine with a target osmolality of 285 mOsm was calculated based on the urinary osmolar excretion. The pilot study conducted on only eight patients did not allow for any clinically appreciable results, however, it did have the role of elaborating a water dosing strategy to be administered to patients and of concluding that this strategy is potentially prosecutable. Another pilot study evaluated the effect of water prescription (about 3 liters of water per day) on urine osmolarity and cAMP urinary excretion, either in acute or chronic condition (21). This small study suggested that acute water load obtained a reduction of cAMP excretion, however, this effect was not replicated during chronic water prescription. Paradoxically, another small, non-randomized study reported that high water intake worsened renal function compared to the control group (22). Other feasibility studies (23, 24) were published or are planned to be concluded in the months following the publication of this paper (25)

(Source www.clinicaltrials.gov : NCT00759369 trial). At the Rogosin Institute in New York, a clinical trial based on the prescription of water in 32 polycystic patients began in 2017. This non-randomized sequential study involves 32 participants with a follow-up of 18 months. The primary outcome will be renal volume. The kidney volumes recorded at the end of a period of six months with a usual water intake will be compared with the renal volumes recorded at the end of the following 12-month period in which an increased water intake, as prescribed by the investigator, will be implemented on the basis of an urine test of the participant. This study is ongoing and should be concluded in December 2019 (source www.clinicaltrials.gov, NCT03102632). An even larger randomized study (180 patients) has been announced by the Australasian Kidney Trials Network with the acronym of "PREVENT-ADPKD"(26)

ANALOGS OF SOMATOSTATIN

Rationale for the use of analogs of somatostatin in ADPKD

The authors of the first clinical report (27) on the potential use of somatostatin analogs in ADPKD report the origin of this idea to a clinical case concerning a patient with ADPKD being treated with Octreotide due to an adenoma of the secreting growth pituitary hormone. Assessment by abdominal CT series had indicated a stability of renal volumes; likewise, the patient's renal function had not deteriorated in a two-year follow-up. Details deriving from the experiences on elasmobranches fish, and in particular, on the rectal gland of sharks (28), suggested the possible role of somatostatin in inhibiting the chlorine channel encoded by the CFTR gene by stimulation of the somatostatin receptors present in renal tubular cells (29, 30).

Completed Clinical Trials: Octreotide and Lanreotide

The first experiences with somatostatin analogs have involved small cohorts of pilot studies that have suggested promising preliminary results concerning the reduction of the progression of renal (27) and hepatic volumetric increase (31). Subsequently, in larger studies, researchers have evaluated the effect of the treatment on renal function. The most significant studies are the ALADIN studies (which involved early disease phase in ALADIN 1 (32) and late stage of disease in the ALADIN 2 study (33)) and the DIPAK-1 study (34).

Overall, these studies have recruited populations that are much lower than the experience gained with Tolvaptan. The most numerically representative trial is the DIPAK-1 study, which recruited 309 patients, while the ALADIN studies reported more limited experiences (79 patients in ALADIN 1 and 100 patients in ALADIN 2). The DIPAK 1 study involved patients aged between 18 and 60 years and stage 3a and 3b of chronic renal failure, and the follow up had a duration of 2.5 years. Although the study confirmed the ability of Lanreotide to determine a reduction in the progression of increase in renal size, the primary outcome of the study, the slowing of the worsening of renal function, was not successful: there were no significant differences for incidence of worsening kidney function (hazard ratio, 0.87 [95%CI, 0.49 to 1.52]; P = .87) and change in eGFR (−3.58 vs −3.45; difference, −0.13 mL/min/1.73m^2 per year [95%CI, −1.76 to 1.50]; P = .88). For this reason, the authors concluded that Lanreotide was not indicated in the treatment of advanced stages of ADPKD.

The ALADIN studies focused on the analog of somatostatin Octreotide. ALADIN 1 recruited 79 patients at a relatively early stage of disease (GFR greater than 40 ml / min / 1.73m^2 -MDRD formula- and age above 18 years). The primary outcome was the evaluation of the effect on the renal volume, which was positive at the end of the first year of follow-up but statistically not significant by the third year. The most clinically significant outcome of glomerular filtration variation was assessed as secondary outcome and resulted not

significant in measured GFR (based on iohexol)(annual slope of GFR in Octreotide-LAR group –3·85 mL/min per 1·73m^2 per year (–6·20 to –1·92) vs –4·95 mL/min per 1·73m^2 per year (–7·49 to –1·97) in placebo group; p=0.13). The ALADIN 2 study recruited patients in a more advanced disease phase (estimated GFR between 15 and 40 ml / min / 1.73m2). The study showed a reduction effect on renal volume growth in the first and third years. However, the authors were not able to demonstrate efficacy based on the co-primary outcome of the reduction of renal function (GFR was measured by the iohexol method, reduction in the median (95% CI) rate of GFR decline (0.56 [−0.63 to 1.75] ml/min/1.73 m^2 per year) was not significant (p = 0.295)). In this study, an exploratory analysis based on a composite clinical outcome (initiation of replacement treatment and/or doubling of creatinine) would have suggested a protective effect of lanreotide as compared to placebo. However, in the study the two groups do not appear perfectly balanced as the treatment group with Octreotide shows lower renal volumes and more preserved renal function than the placebo group. Furthermore, as in Aladin 1, also in Aladin 2 do the outcomes of renal function appear to be discrepant between what is recorded with the iohexol method and what was observed on creatinine (in the Aladin 2 study, the mGFR is not significant while the composite clinical outcome based on the doubling of plasma creatinine is significant thus making a definitive evaluation at least problematic).

Ongoing Trial

The “Lanreotide In Polycystic kidney disease Study” (LIPS) is an ongoing trial that was recorded for the first time in ClinicalTrials.gov in April 2014. It will recruit 156 patients that will be randomized to either placebo or to the experimental arm. Patients of both sexes older than 18 years of CKD class 2 and 3 will be recruited for a 36-month follow-up period. The main outcome consists of evaluating the variations of GFR between the two groups. The study should be completed in September 2019. (source www.clinicaltrials.gov, NCT02127437)

SUBSTRATE REDUCTION THERAPY AGAINST SPHINGOLIPIDS

Rationale for the use of Venglustat in ADPKD

Sphingolipids, despite constituting a very modest proportion of all cell lipids, play a central role in the control of mechanisms that regulate critical cellular functions, including proliferation and apoptosis. Historically, attention to these molecules has originated from lysosomal storage diseases, such as Fabry's disease. More recently, attention to this class of molecules has involved research fields of diseases that are even more common, such as diabetic nephropathy and polycystic kidney disease (35). Sphingolipid synthesis is closely coupled with the availability of glucose metabolites from aerobic glycolysis (see the following paragraph "METABOLIC AND DIETETIC APPROACH") which is activated in conditions of stimulation of cell proliferation and growth. Polycystic kidney animal models have shown a significant increase in two central sphingolipids: glucosylceramide (GL-1) and ganglioside GM3 plasma levels (36, 37). In the treatment of animal models with glucosylceramide synthase (GCS) inhibitors, a key enzyme in the synthesis of sphingolipids of the globosid class, there have been important reductions in the progression of cystic disease (36, 37). Venglustat is a potent oral inhibitor of GCS, the enzyme that transforms ceramide into glucosylceramide (GL-1). GL-1 is the precursor of many important pathogenetic sphingolipids in a wide range of diseases (Gaucher disease Type 3, Parkinson's disease, acid β-glucosidase mutation, polycystic kidney diseases). The treatment with Venglustat is based on the strategy of ‘substrate reduction therapy’, which reduces the availability of an intermediate necessary for the biochemical synthesis of subsequent molecules directly involved in the disease of interest. According to the preclinical data of efficacy of Venglustat, the pharmaceutical pipeline of the molecule has been extended to several conditions, with ADPKD among them.

Ongoing Trial: Venglustat

"A Medical Research Study Designed to Determine if Venglustat Can be a Future Treatment for ADPKD Patients (STAGED-PKD)" is a worldwide Phase 3 clinical trial that will recruit 560 patients. The subjects will be randomized to the experimental product (at two different dosages) or to placebo and will have a follow-up of 24 months. Patients will be adults aged between 18 and 50 years of both sexes. Patients will be selected to have a CKD stage 2 and 3a. Patients will also be selected for having a rapidly progressive condition based on the Mayo Imaging Classification of ADPKD (38) (Class 1C, 1D, or 1E). The primary outcomes of the study concern the rate of kidney growth and the rate of glomerular filtration change. The study is currently recruiting and should be completed by January 2023 (source www.clinicaltrials.gov: *NCT03523728).*

METABOLIC AND DIETETIC APPROACH

Rationale of the interventions oriented to the correction of the metabolic derangement of ADPKD

Although many cellular pathways that are dysregulated in ADPKD have been identified, new pathways are still emerging. In recent years, convincing data have accumulated regarding the presence of profound alterations of cellular metabolism in ADPKD. In particular, these data suggest that cystic cells shift their energy metabolism from oxidative phosphorylation to aerobic glycolysis (39), an alteration of the energy metabolism previously described in neoplastic cells (Warburg effect) (40, 41). The role of aerobic glycolysis and its therapeutic potential in ADPKD has been extensively studied in pre-clinical models. In particular, at least three independent research groups have replicated the positive effect of the metabolic interference produced by the administration of 2-deoxy glucose in orthologous and non-orthologous rodent and rat models of ADPKD (42-45). The hypothesis is that, in ADPKD, the energetic metabolic derangement is related to the alteration of the activity of the metabolic sensors, such as the mTOR complex (46), AMPK (39, 43, 47), and Sirtuins (47-51). All these pathways are theoretically amenable for pharmacologic modulation: mTOR complex can be inhibited by the class of the mTOR inhibitors (everolimus and sirolimus); metformin, a common hypoglycemic drug, is an activator of AMPK; and finally, several natural polyphenols, including resveratrol, can modulate the sirtuin family. In addition to potential pharmacological interventions, recent preclinical experiences have suggested the possible role of dietary manipulations targeting the same metabolic sensors. Warner et al. applied a caloric restriction of 40% compared to an ad libitum feeding in a mouse model of ADPKD, and obtained an extraordinary reduction of the cystic growth (52). Kipp et al., in their preclinical mouse model, showed that a substantial benefit can be maintained, even with a small reduction of food intake (23% reduction of food intake) (53). Furthermore, the same group tested the hypothesis that the beneficial effects obtained by the diet are due to ketosis caused by intermittent starvation rather than caloric restriction per se (54).

Clinical trials with strategy active against the metabolic derangement of ADPKD

mTOR inhibitors

Despite a number of promising preclinical studies, the results of clinical trials on mTOR inhibitors in ADPKD have been extremely frustrating. Both the everolimus study (51) on a cohort of 433 patients characterized by a relatively advanced phase of the disease, as well as the study on sirolimus (50) of a lower number (100 subjects) of patients at an earlier stage had negative results. This discrepancy between the excellent preclinical results and the demoralizing clinical failure is probably due to a number of contributing factors that are not easy to identify. One of the hypotheses put forward regarding the Everolimus study concerns the possibility that the recruited population was in an advanced stage of disease, whereby the fibrotic processes were relatively irreversible. This hypothesis appears weaker in light of subsequent studies on Tolvaptan, and in particular, the REPRISE study (16), which showed therapeutic potential, even in the advanced stage of the disease. Another hypothesis is that, in the study by Walz et al., Everolimus-inhibited phenomena of compensatory hypertrophy and glomerular hyperfiltration would lead to a worsening of renal function. This hypothesis would leave room for hope that, in the long term, the therapy could have shown beneficial effects, precisely as a function of the long-term protective potential of the inhibition of

glomerular hyperfiltration, but this remains completely speculative. However, despite these considerations, the long-term tolerability profile of Everolimus appears low. In fact, in the study, the dropout of the experimental group was about 25%.

The evaluation of the Sirolimus study is more problematic, although, in fact, the study by Serra et al. had a smaller numerosity, this work was not even able to demonstrate an effect on renal volume reduction. It was hypothesized that the exposure concentrations of Sirolimus in mice in preclinical studies (55) were extremely superior to what was tolerable, and therefore, applied in clinical studies. Furthermore, it is possible that the Sirolimus blood dosages used for the inhibition of circulating leukocytes are not effective at the level of the renal tubule, as suggested by a case report (56) in which the researchers reported the outcome of the accidental renal transplantation in two recipients of the kidneys of a donor with a mild form of ADPKD. One recipient was treated with Sirolimus, while the other was treated with immunosuppressive therapy without Sirolimus: both subjects developed a similar progressive cystic disease despite the presence or absence of Sirolimus in the immunosuppressive regimen.

An important fact that emerged from the experience on Everolimus was the decoupling between the renal and kidney function data. In fact, until the study on Everolimus, renal volume and renal function were considered closely linked based on the seminal work of the CRISP group (57). Violation of this principle with uncoupling between renal volume and renal function has been clearly replicated in other subsequent experiences, and in particular, in the studies on somatostatin analogs (32-34, 58). Consequently, according to these data, the renal volumetric assessment alone is no longer accepted as the primary outcome by the drug regulatory agencies in the ADPKD registration studies.

METFORMIN

As described in the previous section, Metformin is a molecule capable of stimulating the 5' AMP-activated protein kinase (AMPK), a metabolic sensor that appears to be inhibited in ADPKD. AMPK, in turn, inhibits the CFTR channel, which is involved in intracystic fluid flows, and mTOR, another metabolic sensor implicated in the activation of cell proliferation. Preclinical studies based on metformin have shown a decrease in the cystic index in two mouse models of ADPKD (47). There are currently three clinical trials in progress to evaluate the role of metformin in ADPKD.

“Metformin as a Novel Therapy for Autosomal Dominant Polycystic Kidney Disease (TAME)” is a phase 2 controlled against placebo randomized study that will recruit 97 participants. In the study, the experimental drug (and the placebo in the control arm) will be uptitrated from 1 g to 2 g per day according to the patient’s tolerability. The inclusion criteria will select subjects of age 18-60 years, non-diabetic, of both sexes, with a GFR larger than 50 ml/min/1.73m^2. Because of the phase of the study, the primary outcomes regard compliance, tolerability, and toxicity of the drug; as secondary outcomes, renal volume and variation of GFR will be compared between the two arms. The study is expected to end in December 2020 (source ClinicalTrials.gov NCT02656017*).*

“Feasibility Study of Metformin Therapy in ADPKD” is a phase 2 controlled against placebo randomized study that will recruit 50 participants. The titration of the drug is closely related to the TAME study, starting from 1 g to a maximum of 2 g per day according to patient tolerance. The inclusion criteria are slightly different as they recruit an older population (30-60 years old non-diabetic patients) of both sexes and restricting the GFR between 50 and 80 ml/min/1.73m2. The study is expected to end in March 2020. (Source ClinicalTrials.gov , NCT02903511)

Finally, the “Metformin vs Tolvaptan for Treatment of Autosomal Dominant Polycystic Kidney Disease (METROPOLIS)” is an Italian study that, in contrast with the other metformin trials, will compare metformin against an active comparator: Tolvaptan. This is a phase 3 controlled study that will recruit 150 subjects.

The inclusion criteria are also different from those of the previous studies as a principle of genetic selection will be adopted in this work. The study will recruit non-diabetic patients with truncating mutations of the PKD1 gene, aged between 18 and 50 years and a GFR equal or larger than 45 ml/min/1.73m2. The study is expected to end in September 2021. (Source ClinicalTrials.gov , NCT03764605).

2 DEOXY GLUCOSE

2-deoxy glucose(2DG) is a glucose analog that can be internalized into the cells by the same plasma membrane carrier of the glucose. Inside the cell, like glucose, it is phosphorylated, but it cannot be further metabolized. It accumulates in the cell, causing the energetic metabolic paralysis of the glycolytic pathway. The use of 2-deoxy glucose in ADPKD is based on the strategy of directly targeting the energy demand of the cystic epithelia. This strategy takes advantage of the evidence that cystic cells are completely dependent on glucose metabolism for their energy needs because they do not have the possibility of switching to other metabolic fuels (amino acids or lipids) due to mitochondrial inhibition (39, 43). The dependence of cystic tubular cells from glucose and the inability to metabolize other energetic sources as fatty acids or amino acids makes them highly sensitive to the toxicity of 2DG. In this regard, not-cystic cells are protected from 2DG toxicity by their ability of switching to mitochondrial oxidative phosphorylation. This glycidic dependency is in accordance to the neoplastic paradigm of the aerobic glycolysis or Warburg Effect (40, 59-61). This approach effectively slowed down the disease progression in several distinct orthologous models of the disease (42, 43) and obtained similar results in the Han:SPRD rat model (44). The 2DG is not currently registered for any therapeutic indication, although it has been tested in oncology clinical trials (62-66). A phase 1 clinical trial coordinated by an Italian collaborative group has the aim of assessing the drug's toxicity, tolerability, and pharmacokinetics in an ADPKD cohort (67). The study will recruit 18 patients with a 3-month follow-up, with the study scheduled to end in July 2020 (Source: personal communication).

PIOGLITAZONE

As already anticipated, fatty acid oxidation (FAO) is inhibited in ADPKD in the process of the metabolic rewiring of cystic cells that preserve molecules, such as lipids from energy consumption, because useful in anabolic processes (68). The peroxisome proliferator-activated receptor family (PPARα and PPARγ) are nuclear hormone receptors that are activated by fatty acids or their prostaglandin derivatives. At the nuclear level, they promote the gene expression of several factors involved in metabolism, including elements of activation of lipid peroxidation. PPARα is mainly expressed in the organs in which FAO is most active and its downregulation has been identified in cystic tubular cells (69). The fibrates, a known class of molecules with lipid-lowering activity, have a PPARα-activating capacity and have shown a protective role in animal models of ADPKD (69). Also, altered levels of PPARγ have been identified in ADPKD and in analogy to what has been shown for PPARα, stimulation of PPARγ has demonstrated a protective effect in animal models (70-73). Thiazolidinediones are a family of molecules that have the ability to activate PPARγ. Some molecules of this family are in clinical use as hypoglycemic agents, such as Pioglitazone and Rosiglitazone (Rosiglitazone’s authorization has been suspended in Europe because of cardiovascular safety concerns).

Pioglitazone is the subject of the PIOPKD clinical trial (Use of Low Dose Pioglitazone to Treat Autosomal Dominant Polycystic KidneyDisease). PIOPKD is a phase 2 clinical trial that will evaluate the safety of 18 participants during one year of treatment. The study will be followed up for 2 years. The non-diabetic patients of 18-55 years of age and with a GFR above ≥ 50 ml/min/1.73 m2 will be enrolled. The patients will be randomized to PIOGLITAZONE at a 15 mg per day regimen or placebo. The primary outcome of the study is to evaluate the safety of this treatment, while secondary outcomes comprise the evaluation of renal

volume by MRI and bone marrow fat content through the use of MRI spectrometry. The study should be completed by October 2019. (Source: ClinicalTrials.gov: NCT02697617)

Caloric Restriction and Ketogenic Diet

In consideration of the important metabolic imprint highlighted in this disease, a dietary approach has been purposed (52, 54). In theory, although clinical efficacy is demonstrated, this approach has some indisputable advantages: a diet therapy has low toxicity and low costs. On the other hand, diet therapy clashes with a problematic compliance that is particularly exacerbated in the case of diets that implement important caloric restrictions. Finally, since these approaches go beyond specific industrial interests, it is difficult to obtain substantial funding in the development of these programs.

Our group recently published a small pilot trial (the GREASE 1 trial) to explore the feasibility of a ketogenic diet in ADPKD (74). The central idea of any ketogenic diet essentially consists of a high fat and restricted carbohydrate content; this dietetic regimen produces a metabolic response that mimics starvation, whereby ketone bodies become the main fuel for the energetic need of cellular metabolism. According to the glucose dependency of cystic cells, ketogenic promises to be another non-toxic approach for disease management. The pilot study involved three patients for three months. The patients showed a positive compliance to the dietetic regimen and glycemia was significantly reduced during the follow up. The largest side effect was the increase in cholesterol levels. A larger randomized trial will be organized in the months following the publication of this paper (GREASE 2 trial).

A Caloric Restriction strategy has been adopted in the study "Daily Caloric Restriction and Intermittent Fasting in Overweight and Obese Adults With Autosomal Dominant Polycystic Kidney Disease" which is currently in the active recruitment phase. This is a randomized trial that will be conducted on 40 overweight or obese patients (BMI 25-45 kg/m^2) suffering from ADPKD, 18-65 years old, and with a GFR equal or larger than 30 ml/min/1.73m2. Patients will be randomized to intermittent or continuous energy intake reduction (in both cases by a 34% weekly energy deficit). The primary outcomes of the study mainly regard weight loss, tolerability, and compliance. Renal volumes by MRI will be analyzed in a secondary outcome. The study should be concluded by September 2020 (Source ClinicalTrials.gov: NCT03342742)

TYROSIN KINASE INHIBITION

Rationale for the use of Tyrosin Kinase Inhibitors

Tyrosin kinases are enzymes capable of transferring a phosphate unit derived from a donor, often an ATP molecule, to an acceptor protein whose activity is modified by this covalent modification. Many cellular pathways are controlled through these enzymatic cascades, and in ADPKD, the role of tyrosin kinases is also central; for example, in the EGFR pathway that constitutes one of the central stimuli of cystic cell proliferation (75). C-src is a tyrosin kinase closely implicated in the EGFR cascade, although the molecular details have not yet been fully elucidated (76). Pre-clinical studies have supported the role of tyrosin kinase inhibitors in cystic diseases (75, 77, 78).

Trials of Tyrosin Kinase Inhbitors with results

Bosutinib (SKI-606) is an oral dual Src/Bcr-Abl tyrosine kinase inhibitor (TKI) approved for the treatment of Philadelphia chromosome–positive chronic myeloid leukemia. A phase two trial that enrolled 172 patients has been published (79). The treatment obtained a 66% reduction of renal volume for Bosutinib versus placebo. However, the eGFR decline was not statistically significant in the comparison of the placebo group versus the treatment groups in the two groups during the 3 years follow up of the trial (however, the decline was numerically higher in the treatment group). Furthermore, the treatment showed the known adverse effects for this class of oncologic drugs (diarrhea and nausea) in a large proportion of patients,

thereby raising doubts about the real potential of chronic treatment prolonged for many years, as needed in ADPKD.

Ongoing Trials of Tyrosin Kinase Inhbitors

Tesevatinib is a multi-kinase inhibitor that promotes the inhibition of c-Src, thereby decreasing the activity of the EGFR axis (80). The Trial "A Safety, Pharmacokinetic and Dose-Escalation Study of KD019 (Tesevatinib) in Subjects With ADPKD" is active but not yet recruiting. This is a non-randomized Phase 1/2 trial that will enroll 74 participants, with the age range between 22 and 62 years, a GFR higher or equal to 35 ml/min/1.73m^2, and a htTKV ≥ 1000 mL (Total Kidney Volume corrected for height). The primary outcomes of the study will be evaluation of safety, pharmacokinetics, maximum tolerated dose, and Glomerular Filtration Rate (Source: ClinicalTrials.gov: NCT01559363).

Concluding remarks

In recent years, a high number of molecules have been experimentally evaluated in ADPKD, with a number of these strategies promoted to advanced phase clinical trials. Figure 1 shows the temporal projection of the concluded trials and those that are on-going or in activation. Also documented in other fields, the attrition rate of these molecules is generally high, and to date, only one molecule (Tolvaptan) has obtained authorization for treatment in ADPKD (limited to the Italian territory Octreotide-LAR can be reimbursed by the Italian National Health System in adult ADPKD patients with stage 4 CKD and increased risk of rapid progression after the authorization of the Technical Scientific Advisory Board of the AIFA-CTS on the basis of the case-by-case assessment). The high failure rate in the clinical development of pharmaceutical molecules is well known and has been discussed in many previous reviews (81, 82), with ADPKD no exception. Many very promising approaches in preclinical models were not successful at the time of clinical validation. The reasons for these failures are heterogeneous and complex and should be taken into account in each individual case. Overall, we can draw a hypothesis that, in some cases, clinical studies have been undertaken after an excessively hasty preclinical evaluation. Further, pharmacokinetic and toxicity assessments have not been analyzed with sufficient depth in the preclinical phase and have, therefore, failed in the translational phase from animal to human. In this sense, the failure of Everolimus and Sirolimus in particular could be paradigmatic (83). A large number of clinical validation failures have predominantly matured in academic studies, with these studies only marginally supported by the pharmaceutical industry. Although academic research has the great advantage of complete intellectual freedom, on the other hand, it often does not have the sufficient resources to completely carry out expensive clinical protocols. This limitation of funds often results in studies with reduced sample sizes and/or reduced follow up, and ultimately, with low statistical power. Figure 2 shows the relationship between the length of the follow-up and the sample size of the phase III clinical trials in ADPKD in the concluded trials and in the on-going or in activation trials. The graph concerning the completed studies clearly shows how the studies on Tolvaptan are distinguished from the studies conducted on other molecules. Considering the trials conducted until now, the graph suggests that academic studies (all except those of Tolvaptan) have, most likely, been able to develop less statistical power due to a reduced sample size, among other factors. Studies on Tolvaptan have enrolled more than 2500 patients (points 6,7 and 8 of Fig.2). Even when considering the family of molecules most studied after Tolvaptan (i.e., the somatostatin analogs), the sample size did not exceed 600 patients (points 1,2 and 4 of Fig.2): one-fourth compared to what was expressed by Tolvaptan. It is possible that this reduced recruitment capacity has partly contributed to determining some of the failures in clinical trials in ADPKD. This trend does not seem to be different in either on-going or in-activation studies. Indeed, even an industrial study such as that on Venglustat does not express a sample size comparable to that of the Tolvaptan studies, a hint that suggests the expectation for a high efficacy compared to the previous experiences, an expectation that only the outcome of this trial can decree if well placed.

More than 10 years after the start of the first Phase III clinical trials in ADPKD, a drug active in slowing disease progression is finally available. It cannot be considered a goal but only the beginning of a journey: the significant side effects, especially those of the aquaretic type, as well as the high cost of the drug, make this therapeutic option applicable only in a modest fraction of the affected patients. Even in patients undergoing treatment, it is not possible to obtain a cure but only a slowing of the progression of the disease. All the other molecules considered in this review had a negative clinical outcome or at best they require the confirmation of new or ongoing clinical studies before a possible clinical adoption. An exuberant basic research activity in the field, together with the large number of ongoing protocols, keep doctors' and their patients positive with regard to the discovery of new and better therapies in a not-too-distant future.

Agent/	Type of Trial / status	Inclusion Criteria	Results/ Outcomes
Vaptans:			
Tolvaptan	TEMPO 3: 4 phase 3, double blinded placebo-controlled RCT 1445 patients , 36 months Published (10)	Age 18-50years eCrCL≥60ml/min TKV≥750ml (MRI)	TKV growth reduction by 49% eGFR loss protected by 26%
	TEMPO 4:4 phase 4, extension trials of TEMPO 3:4 871 patients, 24 months Published (15)	Patients from TEMPO 3:4	sustained disease-modifying effect of tolvaptan on eGFR
	REPRISE phase 3, double blinded placebo-controlled randomized withdrawal trial 1370 patients, 12 months Published (18)	Age 18-55 years with eGFR 25-65 ml/min/1.73m^2 Or Age 56-65 years with eGFR 25-45ml/min/1.73m^2 and progressive disease	eGFR loss significantly reduced , but not in patients >55y
Lixivaptan	ELISA phase 2, open-label 32 patients, 12 months Recruiting NCT03487913	Age 18-65 years and eGFR > 30 mL/min/1.73 m^2	Primary : toxicity and pharmacokinetic studies of the molecule, Secondary: TKV and creatinine
mTOR inhibitors:			
Everolimus	Phase 3, double blinded placebo-controlled RCT 431 patients, 24 months Published (51)	eGFR≥ 30 ml/min1.73 m^2 and TKV>1000ml	Significant reduction in TKV growth, no effect on eGFR
Sirolimus	Phase 3, open-label placebo controlled RCT 100 patients, 24 months Published (50)	Age 18-40years and eCrCl≥ 70 ml/min/1.73 m^2	No effect on TKV and eGFR
Somatostatin analogues :			
Octreotide	ALADIN 1 Phase 3, single-blind placebo-controlled RCT, 79 patients, 36 months Published (32)	Age >18 years and mGFR ≥ 40ml/min/1.73m^2	Positive effect on TKV at the end of the 1° year, not significant at the 3° year
Octreotide	ALADIN 2 Phase 3, double-blind placebo-controlled RCT, 100 patients, 36 months Published (33)	Age >18years and mGFR 15-40ml/min/1.73 m^2	Positive effect on TKV at 1° and 3° year, not significant on mGFR
Lanreotide	DIPAK 1 phase 3, open-label RCT, 309 patients , 30 months Published (34)	Age 18-60 years and eGFR 30-60ml/min/1.73m^2	No positive effect on eGFR, significant reduction in TKV growth
Lanreotide	LIPS Phase 3, double-blind RCT, 156 patients Active, not recruiting NCT02127437	Age > 18 years and eGFR 30-89ml/min/1.73m^2	Variations of GFR between the two groups

Substrate reduction therapy against Sphingolipids:			
Venglustat	Phase 3, double-blind placebo-controlled RCT 560 patients, 24 months, Recruiting NCT03523728	Age 18-50 years and eGFR 45-90ml/Min/1.73 m^2 TKV Mayo 1C-1E	Variation of kidney growth and change of glomerular filtration
Metabolic and Dietetic approach:			
Metformin	TAME Phase 2, double-blind placebo-controlled RCT, 97 patients, 26 months Active, not recruiting NCT02656017	Age 18 -60 years, non-diabetic and eGFR > 50 ml/min/1.73m^2	Compliance, tolerability and toxicity of the drug; secondary outcomes TKV and changes in eGFR
Metformin	phase 2, double-blind placebo-controlled RCT, 50 patients ,12 months Recruiting NCT02903511	Age 30- 60 years, non-diabetic with eGFR between 50 and 80 ml/min/1.73m^2	changes of TKV and eGFR
Metformin	METROPOLIS Phase 3, RCT Tolvaptan - controlled, 150 patients, 25 months Not yet recruiting NCT03764605	Age 18-50 with eGFR ≥45 ml/min/1.73m^2 non-diabetic with truncating mutations of the PKD1 gene.	changes of TKV and eGFR
2-Deoxy Glucose (2DG)	Phase 1, 18 patients, 3 months	Age 18-55 with eGFR > 45-ml/min/1.73m^2, TKV Mayo 1C-1E	tolerability and toxicity
Pioglitazone	PIOPKD Phase 2, double-blind placebo-controlled RCT, 18 patients, 24 months Active, not recruiting NCT02697617	Age 18-55 years with eGFR ≥ 50 ml/min/1.73 m^2 non-diabetic	safety; secondary: TKV by MRI and bone marrow fat content by MRI spectrometry
Caloric Restriction Diet	Randomized to intermittent or continuous energy intake reduction , 40 patients , 18 months, recruting NCT03342742	18-65 years, BMI 25-45 kg/m^2 with eGFR≥ 30 ml/min/1.73m^2	Primary: weight loss, tolerability and compliance. Secondary: TKV by MRI
Tyrosin Kinase Inhibition:			
Bosutinib (SKI-606)	Phase 2, RCT, 172 patients, 36 months Published (79)	Age 18-50 with eGFR ≥ 60 ml/min/1.73 m^2 TKV ≥ 750mL	66% reduction of TKV for bosutinib versus placebo the eGFR decline was not statistically significant
Tesevatinib	Non-randomized Phase 1/2 trial, 74 patients, 24months Active , not yet recruiting NCT01559363	Age 22-62 years with eGFR ≥35 ml/min/1.73m^2 and a htTKV ≥ 1000 mL	safety, pharmacokinetics, maximum tolerated dose and eGFR

Table 1: summary data of the main clinical trials completed, ongoing or activated in ADPKD. RCT = Randomized Clinical Trial, eGFR = estimated glomerular filtration rate, TKV = Total Kidney Volume, BMI = Body Mass Index

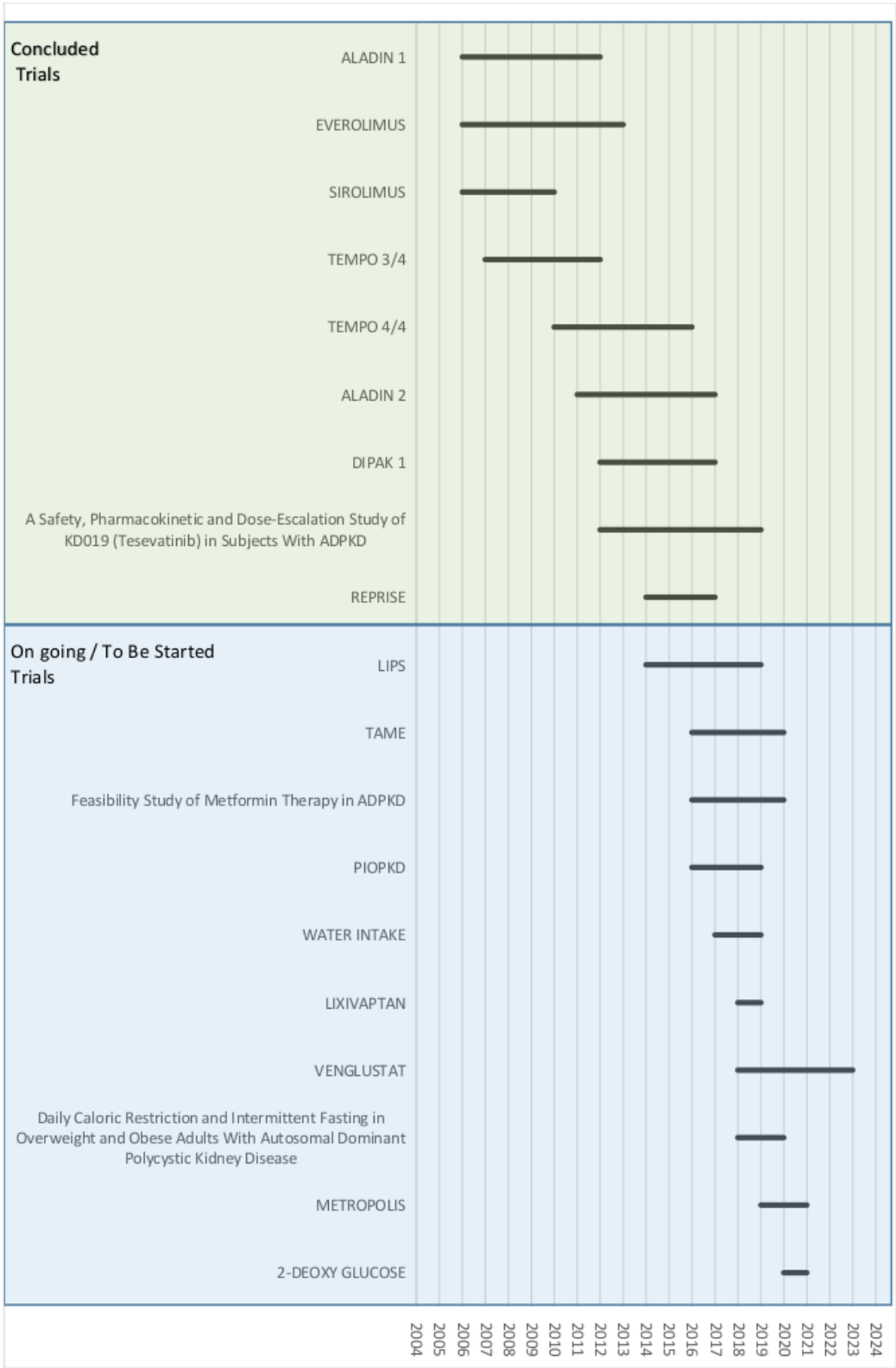

Fig. 1: representation of the temporal distribution of completed, on-going or in activation clinical trials

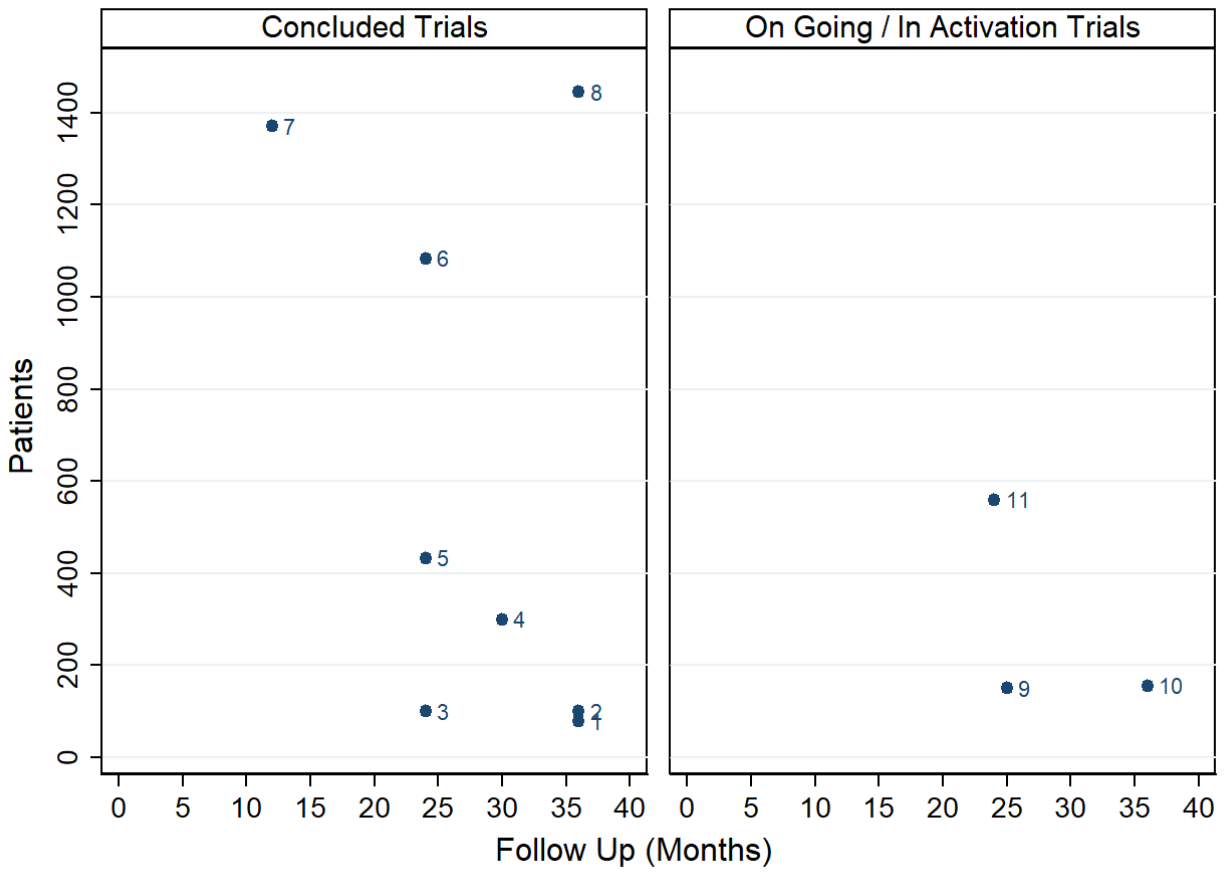

Fig. 2: graph representing the ratio between sample size and duration of the FUP of the main phase 3 clinical trials. The left panel shows thecompleted studies, while the right panel shows on-going and in activation clinical trials. 1 = Aladin 1 (32); 2 = Aladin 2 (33); 3 = Sirolimus (50); 4 = DIPAK 1 (34); 5 = Everolimus (51); 6 = Tempo 4:4 (15); 7 = REPRISE (18); 8 TEMPO 3:4 (10); 9 = METROPOLIS (NCT03764605); 10 = LIPS (NCT02127437); 11 = VENGLUSTAT (NCT03523728)

Disclosure of Financial Interests and Conflict of Interest

R.M. was involved in the trials of Tolvaptan sponsored by Otsuka Pharmaceutical as Principal Investigator. R.M. is scientific advisor of Otsuka Italia. F.T. does not declare any conflict of interest.

Compliance with Ethical Standards”

This paper did not involve Human Participants and/or Animals

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Figure 1

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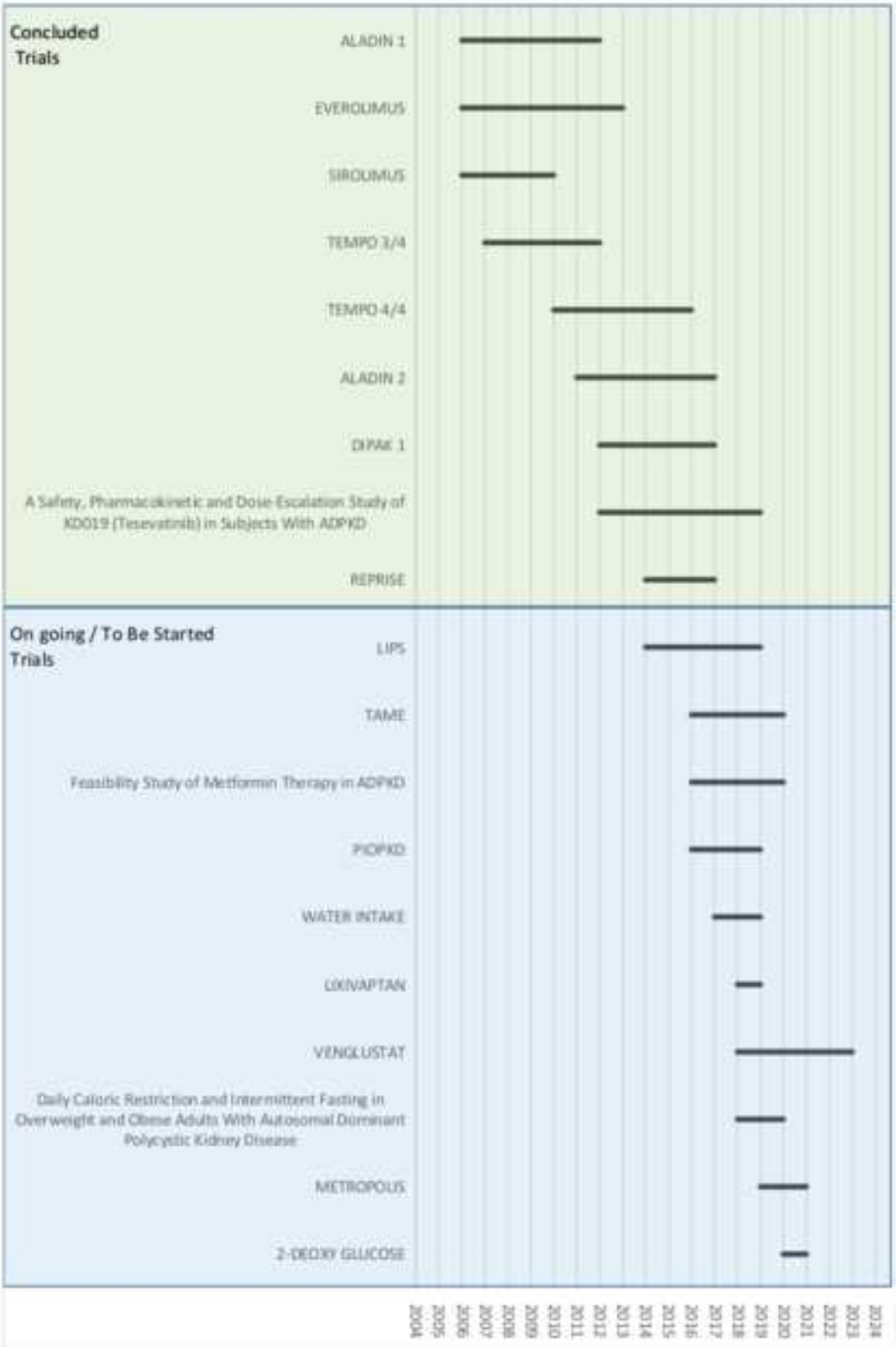

Figure 2

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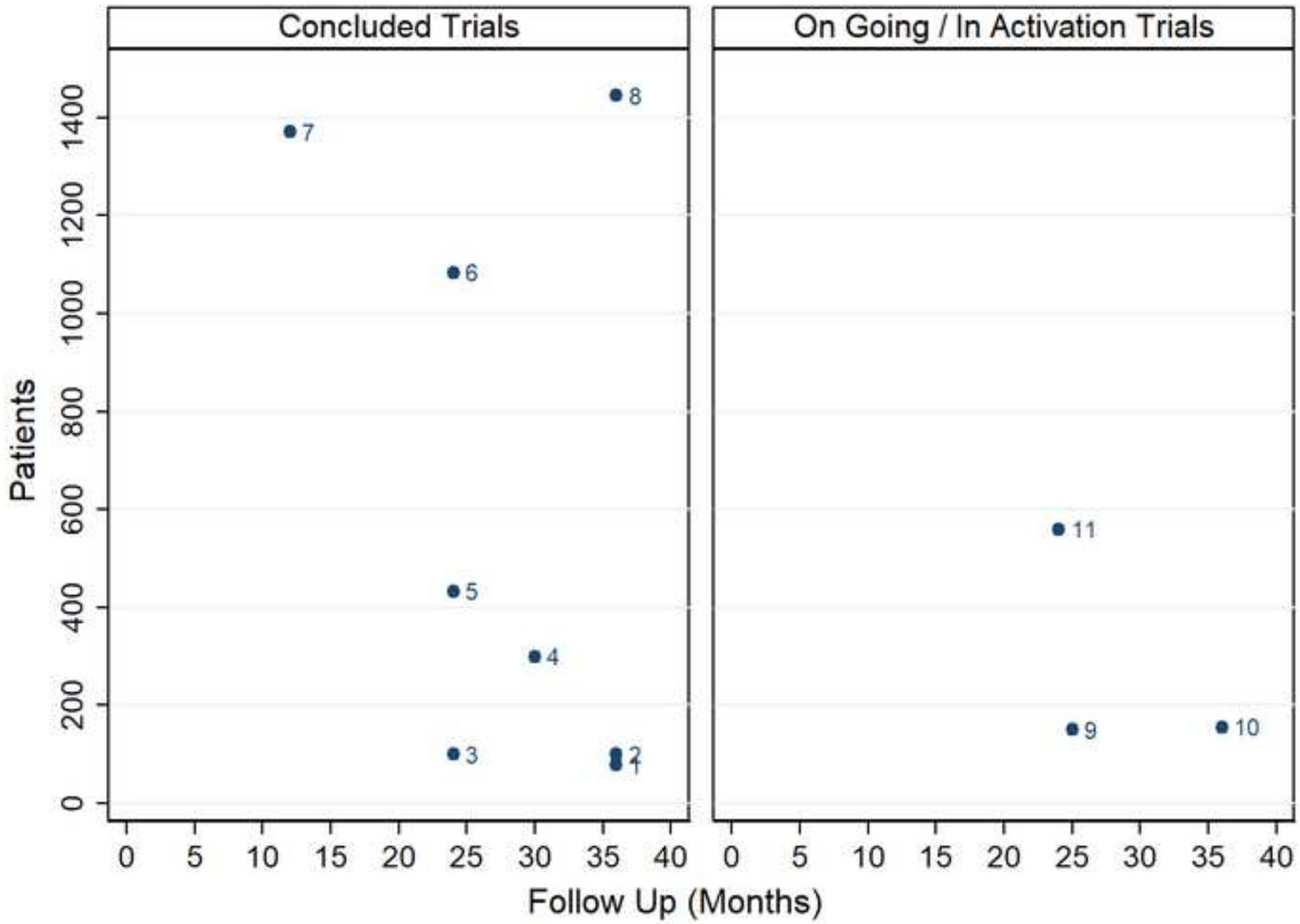

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UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

Divisione di Nefrologia Dialisi e Trapianto Renale
Direttore: Prof. Gianni Cappelli

Modena, 02/11/2019

Dear Editor,
we thank you for reviewing our work which has allowed us to improve the final quality of our paper. We have carefully read the comments of the reviewers to whom we replied promptly as indicated in the following lines.

Major Criticisms

1) *The results from studies are reported without the support of numbers but only with a description and some comments. I believe this setting should be amended. The Authors should report the results in detail, especially for more clinically significative studies (Tolvaptan). Otherwise, the reader must look for the original manuscript to appreciate the data reported in the manuscript. The numeric details of the results are particularly crucial for Tolvapatan studies while it is comprehensible that in ongoing studies it is not worth summarising in details the results*

Support of numbers was added for the main clinical trials in particular for the Tolvaptan and Somatostatin trials. These modifications can be checked in the main document by the 'review tracking feature' of Word.

Minor Criticisms

1) *The acronyms CFTR is used without an explanation. I suppose that it stands for fibrosis transmembrane conductance regulator*

The complete definition 'cystic fibrosis transmembrane conductance regulator' was reported at the first appearance of the CFTR acronym.

2) *The formula of eGFR is written not correctly; indeed the 2 has to in superscript ml/min/1.73m2*

'2 supercript' was corrected for all the occurrences of the eGFR formula

3) *In the Venglustat paragraph is reported "…increase in two central sphingolipids: glucosylceramide (GL-1) and ganglioside GM3 levels (34, 35)…" I suppose that levels refer to plasma levels. Please specify*

We specified plasma levels

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Direttore: Prof. Gianni Cappelli

4) *In "Concluding remarks" the Authors say: "…only one molecule (Tolvaptan) has obtained authorization for treatment in ADPKD…". This point should be checked because I think that also the analogs of somatostatin have this authorization*

Octreotide-LAR is not EMA neither FDA authorized for ADPKD, but limited to the Italian Territory is reimbursed according to a special authorization of the Italian Agency of Drugs AIFA (*'DETERMINA 3 agosto 2018. Inserimento del medicinale octreotide a lunga durata d'azione nell'elenco dei medicinali erogabili a totale carico del Servizio sanitario nazionale,ai sensi della legge 23 dicembre 1996, n. 648'*).
This particular condition for the Italian patients and physician was reported in the 'final remarks' section with the following sentence :
….
limited to the Italian territory Octreotide-LAR can be reimbursed by the Italian National Health System in adult ADPKD patients with stage 4 CKD and increased risk of rapid progression after the authorization of the Technical Scientific Advisory Board of the AIFA-CTS on the basis of the case-by-case assessment
…..

Best Regards
Riccardo Magistroni and Francesca Testa

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