Title page

Potential therapeutic value of urotensin II receptor antagonist in chronic kidney disease and associated comorbidities

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Abbreviations:

UII: Urotensin II; URP: urotensin related peptide; UT: urotensin II receptor; CKD: chronic kidney disease; SoC: standard of care; CHO: Chinese hamster ovary; SHR_SP: stroke-prone spontaneously hypertensive rat, SAD: single ascending dose; MAD: multiple ascending dose; ACE: Angiotensin-converting enzyme; ARB: Angiotensin II receptor blocker

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Abstract

Chronic kidney disease (CKD) remains a common disorder leading to growing health and economic burden without curative treatment. In diabetic patients, CKD may result from a combination of metabolic and non-metabolic-related factors with mortality mainly driven by cardiovascular events. The marked overactivity of the urotensinergic system in diabetic patients implicates this vasoactive peptide as a possible contributor to the pathogenesis of renal as well as heart failure. Previous preclinical studies with urotensin II (UII) antagonists in chronic kidney disease were based on simple endpoints that did not reflect the complex etiology of the disease. Given this, our studies revisited the therapeutic value of UII antagonism in CKD and extensively characterize 1-((6-(4-chloro-3-[3-(dimethylamino)propoxy]phenyl)-5-(2-methylphenyl)pyridin-2-yl)carbonyl}amino) cyclohexanecarboxylic acid hydrochloride (SAR101099), a potent, selective and orally long-acting UT competitive antagonist inhibiting not only UII but also urotensin related peptide activities. SR101099 treatment more than halved proteinurea and albumin/creatinine ratio in spontaneously-hypertensive stroke-prone- (SHR-SP) rats fed with salt/fat diet and Dahl-salt-sensitive rats, respectively, and halved albuminuria in streptozotocin-induced diabetes rats. Importantly, these effects were accompanied by a decrease in mortality by 50% in SHR-SP and by 35% in the Dahl salt-sensitive rats. SAR101099 was also active on CKD-related cardiovascular pathologies, and partly preserved contractile reserve in models of heart failure induced by myocardial infarction or ischemia/reperfusion in rats and pigs, respectively. SAR101099 exhibited a good safety/tolerability profile at all tested doses in clinical phase-I studies. Together, these data suggest that CKD patient selection considering comorbidities together with new stratification modalities should unveil the urotensin antagonists’ therapeutic potential.
Significance statement

CKD is a pathology with growing health and economic burden, without curative treatment. For years, the impact of UT antagonism to treat CKD may have been compromised by available tools/models to deeper characterize the urotensinergic system.

New potent, selective, orally long-acting cross species UT antagonist such as SAR101099 exerting reno- and cardio-protective effects could offer novel therapeutic opportunities. Its preclinical and clinical results suggest that UT antagonism remains an attractive target in CKD on top of current standard of care.
**Introduction**

CKD is a common pathology affecting around 10% of the total population (Lees et al. 2019). It has an indirect impact on global morbidity and mortality by increasing the risks associated with cardiovascular diseases (CVD), diabetes, hypertension, infection with human immunodeficiency virus (HIV) or malaria (Anavekar et al., 2004; Luyckx et al., 2018). However, there is no curative treatment for CKD. The current therapeutic strategy is based on risk factor management, but this is insufficient to curb the growing health and economic burden.

Cellular and molecular drivers of CKD pathogenesis remain unclear. Key features among others include focal segmental glomerulosclerosis with mesangial expansion and podocyte function impairment, tubulointerstitial inflammation, renal arteriolar lesions or necrosis. Beyond the standard of care limited to ACE inhibitors or angiotensin AT1 receptor blockers, identification of new targets for CKD together with improved patient stratification is becoming critical for the development of transformative treatments. In this respect the urotensinergic system might be re-considered for targeted therapy in some CKD subgroups. The kidney is the major source of urotensin II (UII) in humans and animal species, and the urotensin II receptor (UT, GPR14) is expressed in renal tubules. Elevation in UII endogenous tone has also been recently demonstrated to contribute to the deterioration of renal function in rat (Eyre et al., 2019). UII is an 11-amino acid vasoactive peptide that possesses a highly conserved cyclic hexapeptide region from fish to mammals (Conlon et al., 1996; Coulouaran et al., 1999). It is known as one of the most potent vasoconstrictors identified so far (Ames et al., 1999; Douglas et al., 2000) but its effect is dependent upon species and vascular beds (Gardiner et al., 2001; Behm et al., 2004; Tsoukas et al., 2011). UII and urotensin related peptide (URP) which share the same cyclic hexapeptide region, are the endogenous selective ligands of urotensin II receptor UT. Components of urotensinergic system including UII, URP and UT are widely expressed in the cardiovascular, renal and endocrine systems (Castel et al., 2017).

The role of the UII system in human (patho)physiology is not yet fully understood. In humans, increased plasma UII concentrations have been associated with diabetes, chronic heart failure or renal failure (Douglas et al., 2002; Richards et al., 2002; Totsune et al., 2003; Langham et al., 2004; Bousette and Giaid, 2006; Gruson et al., 2010). In human diabetic nephropathy, gene expression of UII and UT was markedly increased in comparison to controls in particular in tubular epithelial cells (Langham et al., 2004).

To elucidate the role of the urotensinergic system, many peptidic and non peptidic UT antagonists have been developed over the past decade. Unfortunately, few of them reached the clinical stages with disappointing results in patients despite promising preclinical results (Vaudry et al., 2015; Castel et al., 2017; Nassour et al., 2019). However, it is possible that limited preclinical studies may not have led to selection of the most appropriate patient population. For instance, palosuran was found ineffective in
hypertensive patients with diabetic nephropathy (Vogt et al., 2010) or in patients with type 2 diabetes (Sidharta et al., 2009) despite positive effects on glycated hemoglobin and albuminuria in rat models (Clozel et al., 2004). However, palosuran is now recognized as a very weak antagonist on rodent UT (IC50 > 10 µM) and more selective UT antagonists like SB-6474510 have been reported not to affect diabetic hyperglycemia (Watson et al., 2013). Therefore, it cannot be excluded that its beneficial effects in rodent models are related to some off-target effects and not to UT blockade. Moreover, no results with selective UT antagonists like SB-710411 and KR-36996 have been reported in models of CKD and therefore the use of UT antagonists in this pathology stills remains to be clarified.

In order to address this question as well as the cardiovascular co-morbidities associated with CKD and thus improve predictivity in clinical studies, SAR101099, a novel potent, orally available and selective competitive UT antagonist active across species (Altenburger A, 2008) was extensively studied in this setting. We here present a full characterization of its pharmacological properties and therapeutic benefits in multiple models of CKD and CVD, using relevant endpoints including survival. Results of the phase 1 trials after single or multiple ascending doses indicate a good safety and pharmacokinetics profile making this new drug suitable for investigating the role of the urotensinergic system in physiological and pathological conditions.
Materials and methods

A: Ethical approvals

Experiments in rats, pigs and monkeys were performed at Sanofi in AAALAC-accredited facilities in full compliance with the standards for the care and use of laboratory animals, according to French and European Community (Directive 2010/63/EU) legislation. All procedures were approved by the local Animal Ethics Committee (CEEA #24 and CEA#21) and the French Ministry for Research.

The clinical phase I trials complied with recommendations of the 18th World Health Congress (Helsinki, 1964) and all applicable amendments. The protocols also complied with the laws and regulations, as well as any applicable guidelines, in France where the studies were conducted. Informed consents were obtained prior to the conduct of any study-related procedures.

B: Reagents

Human UII (Bachem AG, Bubendorf Switzerland), rat and mouse UII (Sigma-Aldrich, St Louis, USA) and URP (Tocris Biosciences, Bristol, UK) were dissolved in distilled water at 1mM and 10 µL aliquots were stored at -20°C until used. MEM Earles (1X), gentamicin and geneticin were from Gibco. Fluo-4AM (Molecular Devices) was solubilized at 20 mM in DMSO and stored at -20°C until used. Pluronic acid (Molecular Probes) was solubilized at 200 mg/mL in DMSO and stored at -20°C.

SAR101099 and palosuran (ACT-058362) were synthesized at Sanofi R&D (Chilly-Mazarin, France). Flashplates PLUS and moniodinated human [125I-Tyr9]hUII for radioligand binding assays were from PerkinElmer Life Sciences (Boston, MA).

C: Binding studies

HEK-293_hUT-R cells stably expressing the cloned human UT were generated at Sanofi by transfection of HEK-293 with pEAK8-hUT-R construct to assess the affinity of SAR101099 for the human UT. [125I]Tyr9-UII was used as a probe for evaluating the binding characteristics on human UT as previously described (Brkovic et al., 2003). Briefly, cells were incubated with 25 nCi/well [125I-Tyr9]human UII (45 pM) in presence of increasing concentrations of SAR101099 (1 nM to 1 µM) in a total volume of 200 µL binding assay buffer for 2 hours at + 4°C. After washing the cells with ice-cold PBS, 100 µL UltimaGold MV mix (PerkinElmer, Waltham, USA) was added for 5 min. Bound radioactivity was counted using a MicroBeta counter (PerkinElmer, Waltham, USA). Total binding was determined in absence of SAR101099, and nonspecific binding was measured in presence of a final concentration of 1 µM unlabeled UII (n=2 experiments). The Ki was calculated according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973): $Ki = IC50 / (1+[radioligand concentration]/KD)$. 

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Geometric mean of Ki values and the respective 95% confidence interval was calculated using BIOST@T-SPEED V2.0 LTS internal application.

**D: Calcium mobilization experiments**

Chinese hamster ovary (CHO) cells expressing UT were obtained by stably transfecting the human UT (hUT-R, nm_018949), the rat (rUT-R, NM_020597.1) and the mouse (mUT-R, AF441863) into CHO-CRE-Luc-DHFR- cells. Transfected cells were seeded in 96-well plates coated with poly-D-lysine (Biocoat, BD) at a density of 4x10^5 cells/well. After 24 hours in culture, cells were rinsed and loaded for 1 hour at 37°C with the calcium dye Fluo-4AM (10 µM) in the presence of pluronic acid (0.1 mg/mL) in freshly prepared assay medium consisting in HBSS supplemented with HEPES 20 mM, MgSO4 8 mM, Na2CO3 33 mM, CaCl2 10 mM and BSA 1%, pH 7.4. Intracellular calcium mobilization experiments were performed at 37°C using the fluorometric reader FlipR (Molecular Devices, USA). Fluorescent emissions were recorded simultaneously in all wells at 516 nm following excitation at 494 nm. Serial dilutions of SAR101099 and palosuran were added 10 minutes prior to the addition of agonists (hUII, rUII, mUII or URP) and percent inhibition of UII-induced increase in [Ca] was calculated for each concentration of antagonists. IC50s were calculated using the Sanofi-developed software Biost@t-Speed V2.

**E: Selectivity profile of SAR101099**

An extended profiling of SAR101099 was performed by CEREP on 100 targets (listed in supplementary Table 1) using receptor-binding, ion channel-binding and enzyme assays. The inhibitory action against enzyme activity was assessed either by enzyme immunoassays, fluorimetric, photometric, HTRF or radiometric assays. The activity of SAR101099 at the 5-HT2B receptors was also determined in an in vitro rat stomach bioassay.

**F: In vitro electrophysiology**

The effect of SAR101099 (0.04 to 8.3 µM) on hERG currents was evaluated with the conventional patch-clamp method at room temperature, using whole-cell configuration, on CHO cells stably expressing hERG channels according to a method derived from that described by (Yang et al., 2001) and (Hamill et al., 1981) with a MultiClamp 700B amplifier along with the eCLAMP software (Molecular Devices Corporation, USA). SAR101099 electrophysiological effects were also studied in 6 Purkinje fibers by the microelectrode method (online supplement methods).

**G. Isolated rat aortic rings**
Thoracic aortae from male Lewis rats (n=8, 12-14 week-old, Charles River, France) were used to evaluate the competitive antagonist effects of SAR101099 on the contractile response induced by hUII or URP (online supplement methods).

**H: Isolated monkey coronary and renal arteries**

Coronary and renal arteries from adult cynomolgus monkeys (n=4, Macaca Fascicularis, 5-6 kg, Noveprim, Mauritius) were obtained following animal euthanasia (Zoletil® 50 at 8 mg/kg i.v.). Coronary (circumflex, right and left anterior descending) and renal arteries were collected, cleaned of fat and adhering connective tissue. Endothelium-denuded vessels were cut into circular rings of 2-3 mm in length and were manipulated in the same conditions as rat aortic rings except for the addition of 0.01 mmol/L indomethacin in the bathing solution. SAR101099 treatments and calculations are identical to the rat thoracic aorta study.

**I: Hemodynamic effect of SAR101099**

The effect of SAR101099 on mean arterial blood pressure (MAP) was evaluated in rodents, pigs and non-human primates (NHP). All hemodynamic data were collected with a telemetry system (Data Sciences International, USA) and analyzed with a HEM software (Notocord systems, France)

Spontaneously-hypertensive stroke-prone (SHR-SP) rat/salt fat diet:

Male spontaneously-hypertensive stroke-prone (SHR-SP) rats (Charles River, USA), 6 to 8-week old were submitted to a salt fat diet (SFD) consisting of 1% NaCl supplied in the drinking water and 24.5% fat supplemented standard NIH-07 diet. Rats were divided into 3 groups, based on body weight and age, to receive either the vehicle (control chow NIH-07), SAR101099 at 30 mg/kg/day per os (po) or ramipril at 3 mg/kg/day po. To evaluate the pathological state of SHR-SP rats fed with SFD, age-matched Wistar Kyoto (WKY) normotensive rats (Charles River, US) were included in the study. This group was not exposed to SFD and was fed with control chow NIH-07 throughout the study. The hemodynamic telemetry monitoring was continuously performed over 24 hours before and after 1, 3, 5, 7 and 9 weeks of treatment. Plasminogen activator inhibitor type 1 (PAI-1) concentrations were determined in plasma by ELISA (Imuclone®, American Diagnostica Inc. Greenwitch, CT, USA).

Conscious telemetered pig:

A first set of male farm pigs (Cormier, France), weighing between 20 and 30 kg, were prepared to measure the hemodynamic parameters in freely-moving animals using a telemetry device allowing recording of systemic and left ventricular pressure. On the day of the experiment, left ventricular pressure (LVP), arterial blood pressure (AP) was continuously recorded. Heart rate (HR) was derived from the LVP. Mean arterial pressure (MAP) and maximal value of the first derivative of the left ventricular pressure signal (dP/dtmax) were calculated by HEM software. Each pig was submitted to 2
experimental sessions, repeated at a 1-week interval. Treatments with SAR101099 (1, 3 or 10 mg/kg po) or its vehicle were given 4 hours before hUII i.v. administration. For each experiment, hemodynamic recording began 2 minutes before the injection of hUII and continued for 15 minutes post-dosing.

To confirm these results and to evaluate the duration of action of UTR antagonism by SAR101099, hUII- and URP-induced increase in arterial pressure were measured in a second set of conscious telemetered pigs (same characteristics and provider as above). Each pig was submitted to 2 experimental sessions, performed at one week interval to receive an oral treatments with SAR101099A (10 mg/kg) or its vehicle. After each treatment, pigs were assigned to receive either 1 injection of hUII (0.5μg/kg, i.v., 4 or 8 hours after treatment) or 2 injections of URP (0.5 μg/kg, i.v., 4 and 8 hours after treatment). The hemodynamic response to hUII or URP was recorded in conscious unrestrained pigs from 2 minutes before to 15 minutes after the injection of hUII and to 10 minutes after the injection of URP.

Telemetered cynomolgus monkey:
This study was performed on telemetered male or female cynomolgus monkeys (Macaca Fascicularis, 3.8-9.0 kg, Noveprim, Mauritius). For each monkey, arterial blood pressure (systolic, diastolic and mean in mm Hg) and heart rate (in bpm) were continuously recorded. The double product (systolic arterial pressure x heart rate) was calculated and expressed as mmHg.bpm. In each experimental session, data were collected from at least 2 hours prior to the first administration of SAR101099 (or vehicle) up to 6 hours after the UUI challenge. Bolus i.v. injections of SAR101099 (1 mg/kg and UUI (300 ng/kg) were performed in the cephalic or sapheno us vein. SAR101099 was administered to animals 1 hour prior to the UUI challenge.

J: Effect of SAR101099 in CKD models

Spontaneously-hypertensive Stroke-prone (SHR-SP) rat/salt fat diet:

Male SHR-SP rats (Charles River, USA) were submitted to a high salt fat diet consisting of 1% NaCl supplied in the drinking water and 24.5% fat supplemented in standard NIH-07 diet. They were divided in five groups, on the basis of body weight and age, to receive either the vehicle, SAR101099 at 30 mg/kg/day po or irbesartan at 10 mg/kg/day or the combination of both. To evaluate the pathological state of SHR-SP rats fed with high salt-fat diet, age-matched normotensive Wistar Kyoto rats (WKY) were included in the study. This group was not exposed to high salt-fat diet and was fed with regular standard NIH-07 diet throughout the study. Rats were observed daily to monitor the mortality rate. Animals exhibiting either a decrease of locomotor activity, convulsive movements, paralysis, self-mutilation or nosebleed were sacrificed and these rats were included in the mortality rate.

Dahl-salt sensitive (DS) rat:
Male Dahl-salt sensitive (DS) rats (Charles River laboratories, USA) were placed on a high salt diet (2% NaCl) supplied by Sniff (Soest, Germany) according to the AIN 76A formula from Dyets Inc. (Bethlehem, USA). Dahl-salt resistant (DR) sham rat (Charles River laboratories, USA) received a normal salt diet according to the same formula. The animals were weighed and randomly divided into 6 groups: 4 DS groups treated po either with SAR101099 at 10 mg/kg/day or 30 mg/kg/day or 50 mg/kg/day or irbesartan at 30 mg/kg/day, 1 DS group receiving vehicle (DS-placebo) and 1 DR group receiving vehicle. Renal function was evaluated by collecting 24-hour urine in metabolic cages, 8 weeks after the beginning of treatment. After collection, urine volumes were measured and urine aliquots were stored at –20°C after centrifugation. Urine analysis was performed by either a clinical chemistry analyzer (Pentra 400®, ABX Diagnostics, France) or Elisa kit. At 8 weeks, creatinine (Jaffé method) and albumin (Rat Albumin Elisa, Bethyl Laboratories, USA) were determined. Rats were observed daily to monitor the mortality rate as described above.

Diabetic rat:

Male Wistar rats (Charles River Laboratories, France) were anesthetized with isoflurane 2.5% (AErane®, Baxter, France) and then underwent an unilateral nephrectomy to accelerate the development of contralateral nephropathy. After a 2-week recovery period, diabetes was induced by an i.v. injection of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, USA; 65 mg/kg in 1 mL/kg citrate buffer, 10 mM, pH 4.5). Sham rats were subjected to unilateral nephrectomy but received the same volume of citrate buffer. Two days after STZ administration, the level of diabetes was determined by serum glucose measurements. Only animals with a hyperglycemia above 17 mmol/L were included in the study. One week after diabetes induction, the chronic oral treatments with compounds or vehicle were initiated for a period of 16 weeks according to a randomization based on serum glucose levels. Two groups received the vehicle (Sham and STZ-placebo), 3 other groups received either SAR101099 at 30 or 50 mg/kg/day or irbesartan at 10 mg/kg/day. The last group received the combination of SAR101099 at 30 mg/kg/day and irbesartan at 10 mg/kg/day. The renal function was evaluated as described above after 16 weeks of treatment. Plasma PAI-1 concentrations were determined as described above.

**L: Effect of SAR101099 in chronic heart failure models**

Left ventricular dysfunction post myocardial infarction in rats:

Adult male Lewis rats (260–300 g of body weight; IFFA CREDO, France) were subjected to a permanent left coronary artery ligation as described previously (Selye et al., 1960), Berthonneche et al., 2004) to produce myocardial infarction (MI). The same procedure was performed for sham-operated control animals, but the coronary ligation was not tied. Seven days after coronary ligation, rats were randomly allocated to one of the following 5 groups: sham-vehicle, MI-vehicle, MI-Ramipril.
1 mg/kg/day po, MI-SAR101099 30 mg/kg/day po, or MI-Ramipril 1 mg/kg/day po plus SAR101099 30 mg/kg/day po. Under anesthesia with 2% isoflurane, mean arterial blood pressure (MAP) was measured via a PE-50 arterial catheter inserted into the femoral artery and connected to a pressure transducer (Statham P23XL, Hugo Sacks Electronik, Germany). A second catheter was inserted into the left ventricle (LV) via the right carotid artery to monitor LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), heart rate (HR), maximal and minimal values of the first derivative of developed pressure (dP/dtmax and dP/dtmin) and to calculate LV developed pressure (LVDP). To assess the cardiac dysfunction under stress conditions, a PE-10 catheter was inserted into the jugular vein to deliver a continuous infusion of dobutamine at 10 µg/kg/min for 2 min.

After a stabilization period of 20 to 30 min, a 15-min basal hemodynamic period and cardiac response to dobutamine were recorded and analyzed with a hemodynamic software (IOX 1.8 and Datanalyst, Emka technologies, France).

Left ventricular dysfunction post myocardial infarction in pig:

The effects of SAR101099 were studied in a pig model of chronic heart failure induced by myocardial infarction. Male farm pigs (Cormier, France) weighing 22 to 25 kg, were used.

Each pig was submitted to 2 experimental sessions interspaced by a 7-day period. At day 0, MI was induced after thoracotomy by occluding for 45 min the left anterior descending (LAD) coronary artery below the first diagonal branch followed by reperfusion. The following treatments were given at the onset of reperfusion: SAR101099 (1 mg/kg infused over 20 minutes) (IR-SAR101099 group), its vehicle (methyl pyrrolydon) (IR-vehicle group) or ramipril (0.1 mg/kg) (IR-ramipril group). A sham group receiving the vehicle was used for comparison purpose. Then during the 7 days following this ischemia/reperfusion episode, animals were daily treated orally either with SAR101099 at 10 mg/kg/day bid or ramipril at 1 mg/kg/day or vehicle. At day 7, cardiac hemodynamics (MAP, HR, dP/dtmax) was performed under anesthesia to evaluate the cardiac function and the inotropic response to dobutamine (IV infusion of dobutamine at 1 and 3 µg/kg/min).

Clinical trials

Sequential single ascending dose (SAD) po administration of SAR101099 from 10 to 500 mg was performed in healthy young men to explore its tolerability, safety, and pharmacokinetics (PK) (Part 1) before assessing a potential food interaction (Food effect - Part 2) and to determine the most appropriate conditions of administration for subsequent studies. Tolerability, safety, and PK of SAR101099 was then assessed following multiple ascending oral doses (MAD) from 50 to 500 mg, once daily for 14 days in a sequential ascending dose design in healthy young males (Part 3). Detailed study designs are presented in the online supplement.
Statistical analysis

Statistical analyses were performed with an in-house software interface accessing SAS system release v9 and SAS system release 8.2 for SUN4 to carry out the calculations. Results are presented as mean ± SEM in tables and figures. The significance level was set to 0.05 for all analyses. Normality and homogeneity of variance hypotheses were checked with Shapiro-Wilk and Levene tests, respectively. Prior to testing the main hypothesis in each individual in vivo pharmacology study, the vehicle-treated group was compared to the sham-operated group in order to verify that a noteworthy pathology had indeed been induced in the model. As by definition only two groups were compared, this test was carried out by Student’s t-test or else Wilcoxon’s test in case the assumption of normality had to be rejected (*p<0.05, **p<0.01, ***p<0.001). For the actual analysis, SAR101099-treated groups were compared to the appropriate vehicle-treated group. Analyses were carried out by one-way ANOVA unless measurements at repeated time points were available. In this case repeated-measures ANOVA with time as repeated factor was used. When overall significance was established, Dunnett’s test was used to compare treatments to control or the Bonferroni-Holm method was used for multiple comparison-tests. When the assumption of normality or homogeneity of variance had to be rejected, data were first normalized by Rank transformation or the Kruskal-Wallis test was used. #p<0.05, ##p<0.01, ###p<0.001. The LogRank test was used to analyze survival rate.
**Results**

**UT Affinity and antagonistic properties**

SAR101099 (Figure 1) fully displaced [125I-Tyr9]hU-II binding in a concentration-dependent manner (1 nM to 1 μM) with an IC50 of 10.1 nM (Figure 2A). Using the published Kd value of 1.4 ± 0.2 nM for [125I-Tyr9]hU-II (Brkovic et al., 2003), mean geometric Ki value calculated for SAR101099 was 9.7 nM [2.2 nM-42.9 nM].

Human UII, tested in cultured CHO cells expressing hUT in the concentration range of 3 pM to 1 μM, induced a concentration-dependent increase in fluorescence signal which returned rapidly to the baseline value with a calculated EC50 of 3.3 nM. The other UT ligand, URP, was tested in the same concentration range as UII and induced an increase in intracellular calcium with an EC50 of 0.96 nM (Figure 2B). These calcium responses to UII and URP were blocked by SAR101099. To characterize SAR101099 mechanism of action and antagonistic properties at the UT level, its effects were evaluated in CHO cells expressing human or rat or mouse UTs. Palosuran, a highly “primate” UT-selective inhibitor (monkey and human), lacking appreciable affinity at other mammalian UT isoforms (rodent and feline) according to (Behm et al., 2008) was also tested in the same experimental conditions. SAR101099 is a potent competitive antagonist on hUT with an IC50 of 20 nM [15-28nM] but slightly less active on rat and mouse UT with IC50s of 67 nM [53-86 nM] and 97 nM [77-122 nM], respectively. In comparison with SAR101099, palosuran displayed a much lower antagonistic property on hUT with an IC50 of 177 nM [135-231 nM] and very weak activities UII-induced increase in [Ca2+] mobilization on rat and mouse UT isoforms, with IC50s of 22 μM [19-27 μM] and 55 μM [46-68 μM] respectively (Figures 2C, 2D).

**In vitro selectivity profile**

When assayed in 100 (mainly human) receptor-binding, ion channel-binding and enzyme assays, SAR101099, at concentrations up to 10 μM, was inactive (inhibition less than 50%) in most tested targets. It displayed a low affinity to bombesin BB (Ki of 1.5 μM, IC50 of 1.5 μM), histamine H3 (Ki of 1.0 μM, IC50 of 4.1 μM), δ2 (Ki of 7.9 μM, IC50 of 1.3 μM), vasopressin V1a (Ki of 1.7 μM, IC50 of 2.8 μM), and N (neuronal) (α-BGTX-insensitive) (α4β2) (Ki of 1.8 μM, IC50 of 3.6 μM) receptors.

All these results demonstrate a good selectivity profile of SAR101099 versus ion channels, G-protein-coupled receptors, enzymes, transporters and receptors assays and thus a low probability of off-target adverse events.

**In vitro electrophysiology**
The potential effects of SAR101099 on IKr current from human ether-à-go-go related gene (hERG) channels expressed in stably transfected CHO cells were assessed using the whole cell patch-clamp method. Based on calculations using the actual concentrations, SAR101099 concentration-dependently blocked hERG currents with a mean IC50 value of 1.2 µmol/L, far from its on-target IC50.

Using a glass microelectrode method, SAR101099 was tested on 6 rabbit Purkinje fibers at the concentrations of 0.17, 0.75, 2.4 and 10 µmol/L. At 0.17 µmol/L, SAR101099 did not affect resting membrane potential (RP) or action potential parameters whatever the stimulation rate. From 0.75 µmol/L, SAR101099 induced a statistically significant concentration- and reverse use-dependent increase in action potential duration (APD50 and APD90). At the basal rate of 1 Hz, APD50 was increased by 8±1.6%, 21±3.2% and 41±6.6% and APD90 was increased by 9±2.8%, 34±12.4% and 52±8.3% at 0.75, 2.4 and 10 µmol/L, respectively. The action potential prolongation was reverse use-dependent, for example at 2.4 µmol/L APD90 was increased by 12±1.8%, 34±12.4% and 68±39.2% at 3, 1 and 0.25 Hz, respectively. Early after depolarizations were observed at 0.25 Hz, in 1 out of 6 fibers at 2.4 and 10 µmol/L, which were still observed during the washout period. Whatever the pacing rate, SAR101099 did not alter resting membrane potential, action potential amplitude or maximal rate of rise (Vmax) of action potential suggesting that SAR101099 did not act on Na+ channel.

**In vitro anti-vasoconstrictor effects**

Whereas the contractile response of rat aorta to KCl was rapid and quickly reversible, the contraction elicited by hUII or URP was slow appearing and long-lasting, reaching its maximum 30 min or more after application and was irreversible. Results of experiments indicated a very similar potency of both UII and URP agonists with respective EC50s of 1.40 and 1.45 nM (data not shown). The pre-treatment of rat aortic rings with SAR101099 (0.1 to 10 µM) during 30 min, did not affect the basal tension, suggesting that the compound is devoid of any intrinsic activity in this tissue. Exposure of rat isolated aortic rings to SAR101099 (0.1 to 10 µM) resulted in concentration-dependent, rightward, parallel shifts in the hUII and URP concentration-response curves (Figures 3A and 3C). Maximal contractile responses remained unaltered (respectively 107–118% and 86–108% KCl response) showing that this inhibition was surmountable. Global nonlinear regression analysis gave equipotent pKb of respectively 7.2 [7.0; 7.4] and 7.1 [6.9; 7.3] (Figures 3B and 3D).

Given the high homology of the urotensinergic system between the monkey and human and to further characterize these properties and improve the translatability/predictivity of preclinical results, the anti-vasoconstrictor properties were also tested in monkey arteries coming from different vascular beds. Exposure of monkey isolated coronary and renal arteries to increasing concentrations of hUII resulted in a potent and sustained concentration-dependent contraction. Calculated EC50 values were 0.016 nM [0.006 nM - 0.041 nM] for coronary artery and 0.188 nM [0.079 nM - 0.447 nM] for renal artery with Emax values of 146% and 77% respectively when normalized to KCl-induced contraction.
(Figures 3E and 3F). A 30-minute pretreatment of monkey arteries with SAR101099 (0.3 to 3 μM) did not affect basal tension, but resulted in concentration-dependent, rightward and parallel shift in the hUII concentration-response curve (Figures 3E and 3F). Maximal contractile responses remained unaltered in coronary and renal arteries (respectively 146-162% and 77-113% of KCl response). Global nonlinear regression analysis gave a pKb of 7.7 [7.3 - 8.2] in coronary arteries, and 7.4 [7.3 - 7.4] in renal arteries.

Hemodynamic profile

These anti-vasoconstrictor properties were then investigated in conscious animals from various species to minimize the risk of bias due to a specific model and improve the translatability/predictivity to human.

For hemodynamic investigations in rats, SHR-SP rats under salt fat diet were selected because this regimen was known to aggravate hypertension and to accelerate the progression of CKD in this strain, making this model attractive to explore potential blood pressure lowering effects of UT antagonists in comparison with renin-angiotensin-aldosterone system (RAAS) inhibitors reported for their efficacy in this setting (Abrahamsen et al., 2002). Arterial blood pressure and heart rate were therefore determined before and after 1, 3, 5, 7 and 9 weeks of SAR101099 treatment (30 mg/kg/day, po) or ramipril (3mg/kg/day, po) in conscious freely moving rats. SHR-SP rats on SFD diet had a significantly higher blood pressure than normotensive WKY rats (Figure 4A), without modification of heart rate (data not shown). SAR101099 did not lower arterial pressure but blunted the progression of hypertension induced by SFD whereas ramipril 3 mg/kg significantly reduced the hypertension (Figure 4A).

Because of the similarities between pig and human hearts, pigs are considered good models to improve the translatability to the human cardiovascular responses. However, existing reports show substantial variability in the effects of UII in isolated pig vessels (Camarda et al., 2002, Douglas et al., 2000). It therefore appeared important to characterize the hemodynamic effects of UII and its cognate receptor antagonist in conscious animals before carrying out heart failure studies with UT antagonist. In conscious telemetered pigs, intravenous injection of hUII (0.5 µg/kg iv) significantly increased MAP without affecting heart rate. These hemodynamic effects lasted at least 15 minutes before returning to baseline. They were significantly and dose-dependently inhibited by SAR101099 administered orally 4 hours before hUII injection. SAR10109910 mg/kg fully antagonized the pressor response to hUII (Figure 4B). This effect was confirmed in a second set of pigs where hUII or URP (0.5 µg/kg, iv) were administered 4 or even 8 hours after oral SAR101099 treatment at 10 mg/kg (study 3) showing a full inhibition of hUII and URP pressor effects, highlighting the long duration of action of SAR101099 (Figures 4C and 4D).
No effect on heart rate was observed after SAR101099 administration (data not shown) in any of the studies performed in conscious rat or pigs.

Responses of telemetered cynomolgus monkey were also studied because of their major translational relevance and the dramatic cardiovascular collapse that UII has previously been reported to induce in this species (Zhu et al., 2004). Handling of conscious monkeys in restraining conditions to allow intravenous injections induced significant hemodynamic changes consisting of a marked and sudden increase in heart rate (from 132 to 222 bpm) and blood pressure (from 99 to 116 mmHg). These modifications were sustained and not reversed during the 15 minutes following injection (Figures 4E and 4F, vehicle/saline group). In these conditions, the UII challenge (0.3 µg/kg i.v.) induced a dramatic myocardial depression with a marked decrease in heart rate and blood pressure. The maximum decrease in blood pressure occurred 10 minutes post-injection from 105 mmHg in the control group to 69 mmHg in the UII group and remained low during the period of recording (Figure 4E, vehicle/UII group). The maximum decrease in heart rate occurred 5 minutes post injection with a drop from 207 bpm in the vehicle/saline group to 135 bpm in the UII group but returned to control value at 15 minutes (Figure 4F, vehicle/UII group). SAR101099 pre-treatment, one hour prior to the UII challenge, prevented both the decrease in blood pressure and heart rate (Figures 4E, 4F, 1 mg/kg/UII group). The maximal heart rate decrease observed 5 minutes post UII injection (135 bpm) was reverted to 174 bpm and at 15 minutes. In the presence of SAR101099 no drop in blood pressure was observed during the recording period. No gender effect was noted during the study. In one NHP whose results were excluded from the study, hUII produced a dramatic myocardial depression eventually evoking a life-threatening cardiovascular collapse which was rescued by an i.v. administration of SAR101099 at 1 mg/kg (Figure 1, online supplement).

**Chronic kidney disease**

Harmful effects of salt fat diet on CKD progression in SHR-SP rats have been well documented in the literature (Abrahamsen et al., 2002). SHR-SP rats fed with SFD developed a severe and progressive proteinuria 8.7-fold higher (p<0.0001) than WKY rats at 8 weeks (Figure 5A). Chronic treatment with SAR101099 (30 mg/kg/day) for 8 weeks prevented the rise in proteinuria compared to the SHR_SP SFD-Vehicle group (-50 %, p = 0.0455). The ramipril-treated group (3 mg/kg/day) also displayed a significant reduction in proteinuria compared to the SHR-SP SFD-Vehicle group (-79 %, p<0.0001).

In comparison with WKY rats, SHR-SP rats on SFD had severe glomerular and tubulointerstitial lesions including glomerulosclerosis, as well as renal arteriolar damage. SAR101099 treatment tended to reduce the severity of renal arteriolar lesions (score: -18%, p = 0.0504) (Figure 5B). The chronic treatment with ramipril limited both glomerular injury (-90%, p<0.05) and the severity of renal arteriolar lesions (-35%, p<0.01) which was also in line with the reduction observed in proteinuria in
this group. Both compounds prevented significantly the rise of PAI-1, a marker of inflammation and fibrosis (Ma and Fogo (2009), induced by the pathology (online supplement Figure 3).

In order to assess the renal protective effect of UT antagonist in a CKD model of a different hypertensive etiology than SHR-SP SFD SP (Rapp, 2000; Schulz and Kreutz, 2012), SAR101099 was tested in Dahl-salt sensitive (DS) rats which are exceptionally prone to develop renal dysfunction, particularly under high salt diet. As expected, DS rats submitted to high salt diet displayed a dramatic increase in urinary albumin-to-creatinine ratio when compared to Dahl-salt resistant (DR) rats: 7.60±1.06 versus 0.69±0.27 mg/μmol, p<0.0001; respectively. Chronic treatment of SAR101099 administered at 10, 30 or 50 mg/kg/day for 8 weeks reduced significantly the albumin-to-creatinine ratio in comparison with DS rat receiving the vehicle: 4.70±0.56 (p = 0.0266), 4.61±0.78 (p = 0.0157) and 4.45±0.67 (p = 0.0075) versus 7.60±1.06 mg/μmol, respectively. In comparison, irbesartan provided similar beneficial effects on this parameter (Figure 5C). This positive effect of SAR101099 on renal dysfunction was independent of any blood pressure change (Figure 4A).

Although both the SHR-SP and DS rats suffer from metabolic alterations (Schulz and Kreutz, 2012), it was important to investigate in addition the effects of UT antagonist in a CKD model of diabetic origin (Hewitson et al., 2009). In rats in which diabetic nephropathy was induced by unilateral nephrectomy and STZ, the urinary albumin excretion was dramatically increased in the STZ group in comparison to the sham group. SAR101099 at 50 mg/kg reduced significantly the STZ-induced albuminuria. Irbesartan administered alone or in combination with SAR101099 did not significantly improve renal function (Figure 5D).

SAR101099 significantly and consistently improved the renal function in these different models, without modifying either metabolism (glucose, cholesterol, triglycerides, body weight) or hemodynamic parameters (MAP and heart rate).

Survival in CKD models

The translation of renal function improvement into increase of survival rate has been evaluated in different rat models.

SHR-SP rats fed with SFD suffer from CKD but also from cardiac hypertrophy (Barone et al., 1996), suggesting that they share some of the cardiac complications observed in CKD patients. As such, they displayed a high mortality rate of 59.4% by 29 weeks, which differed significantly from the normotensive WKY rats (p = 0.003). Chronic administration of SAR101099 alone or in combination with irbesartan significantly improved survival (p = 0.0338 and p<0.0001, respectively). Chronic administration of irbesartan alone markedly and significantly reduced the mortality rate (p<0.0001) (Figure 6A).
In a CKD model with different etiology, the Dahl-salt sensitive rat model, where hypertension is the predominant pathogenic factor (Rapp, 2000; Schulz and Kreutz, 2012), a mortality rate of 45% was noted in DS-vehicle group. Without reaching a statistically significant level, SAR101099 at 10, 30 and 50 mg/kg tended to improve the survival rate in comparison with DS-vehicle treated group in a dose-dependent manner with 30%, 25% and 16% versus 45% of mortality, respectively. Irbesartan 10 mg/kg orally significantly improved the survival rate (95%) in comparison with DS-vehicle group (p = 0.0193) (Figure 6B).

Effect of SAR101099 in chronic heart failure models

Because the urotransinergic system is widely expressed in the cardiovascular system and CKD patients die mainly from cardiovascular complications we investigated the potential benefits of SAR101099 effects in various models of heart failure in comparison or on top of standard of care, namely an ACE inhibitor or an AT1 receptor blocker.

SAR101099 was first evaluated in a rat model of heart failure induced by myocardial infarction (MI). MI induced a marked dilatation of the left ventricle with an 213% increase in ventricular cavity surface area in the MI-vehicle group compared to the sham-operated group. However, SAR101099 or ramipril or the combination of both had no effect on infarct size (data not shown) or cardiac remodeling. The mean surface area of left ventricular cavity was similar to the MI-vehicle group (from 30.2 to 32.5 mm²) whatever the treatments. Without altering MAP (Figure 7A), seven days after MI, SAR101099 significantly preserved the cardiac contractile reserve as depicted in the Figure 7B. SAR101099 also reduced significantly the rise in LVEDP induced by MI from 14.5±1.7 mmHg in the MI-vehicle group to 8.9±1 mmHg in the MI-SAR101099 group (Figure 7C).

The same effect on cardiac contractile reserve was observed in another model of chronic heart failure developed in pigs which develop contractile and biochemical alterations more similar to those observed in humans (Milani-Nejad and Janssen, 2014). Seven days after ischemia/reperfusion injury in pigs, the cardiac function was significantly depressed as supported by the decrease in MAP and cardiac contractility (measured by dP/dt max) in the MI-vehicle group as compared to sham-operated group (-17% and -18%, respectively). MI led to a decrease in MAP which was not modified by any of the treatments (Figure 7D). Repeated administration of SAR101099 initiated at the onset of reperfusion improved the cardiac contractile reserve in pigs as assessed by the dobutamine response (Figure 7E, p=0.041 at 1µg/kg and p=0.057 at 3 µg/kg of dobutamine) whereas ramipril had no effect (p=0.10 and p=0.14 at 1 and 3 µg/kg of dobutamine). Neither of the drugs tested had an effect on infarct size.

Clinical trials
During the SAD study, no AE were observed. A few treatment-emergent adverse events without any specific pattern and no clinically significant abnormalities in biological tests and vital signs parameters were reported across dose groups (Table 2 – online supplement). Regarding ECG evaluation, no clinically significant abnormalities were reported.

SAR101099 was rapidly absorbed after a single oral administration of 10 to 500 mg. Elimination was characterized by a apparent elimination half-life (~12h) whatever the dose. Exposure increased in a dose proportional manner between 10 and 500 mg but more than dose proportionally between 200 and 400 mg (Table 3 – online supplement).

After repeated oral daily administration of SAR101099 from 50 to 500 mg for 14 days (MAD study), less than 1.3-fold accumulation of exposure was observed after 2 weeks of treatment (Figure 8). SAR101099 was generally absorbed with median tmax of 2 to 4h whatever the dose and the day. No deviation from dose proportionality was observed for Cmax and AUC0-24 on Day 14: for a 10-fold increase in dose from 50 to 500 mg, Cmax increased by 9.9-fold [90% CI: 9.26 to 12.22 and 8.34 to 11.64] and AUC0-24 by 10.2-fold [90%CI: 8.68 to 11.96] (Table 4 – online supplement). No significant effect of dose on terminal half-life (t1/2z) was shown across the dose range, with a geometric mean estimated t1/2z of 14.3 hours [90%CI: 13.45 to 15.23] for pooled doses. Overall, the within-subject variability of blood PK parameters (Cmax and AUC0-24) was low (estimates of within-subject CV = 14% and 11%, respectively).

There was one severe AE in this study, a tendinoplasty, not related to the study drug administration and one drop out due to an increased CPK following a trauma on one arm. A good overall safety/tolerability was observed at all doses of SAR101099 50 mg, 130 mg, 260 mg, 350 mg and 500 mg once a day for 14 days. No clinically relevant abnormalities were issued from laboratory tests and vital signs evaluations, including the hemodynamic parameters (Table 5 – online supplement). Regarding ECG evaluation, there was no individual clinically significant abnormality in any parameter. A mean elevation of QTcF from time-matched baseline was observed at 260 mg and above. No relevant changes were observed on ECG morphology neither on mean values of Heart Rate, QRS- or PR- intervals in any dose group.

Overall, the safety and tolerability profile of SAR101099 in the SAD or MAD studies conducted in healthy young male subjects was considered acceptable. In addition, after a single administration, no relevant effect of food was observed on SAR101099 PK parameters.
Discussion

Despite high unmet medical need in chronic kidney disease (CKD) few drug candidates are today in clinical development. This is mainly due to a high failure rate in phase 3 trials, that were unable to demonstrate an improvement in renal outcomes and associated cardiovascular risk, to an incomplete understanding of human disease biology as well as a poor translatability of the preclinical studies that often did not sufficiently consider the cardiovascular co-morbidities driving mortality in CKD patients.

The present report describes a novel, potent, non-peptidic UT antagonist, SAR101099, displaying a good selectivity and safety profile, orally active and long-acting. Its in vivo efficacy is demonstrated both in chronic renal and in cardiac dysfunction models developed in various preclinical species to improve its predictivity of clinical efficacy in humans.

Several non-peptidic antagonists have already been reported in the literature but often with a profile limited to their UII pattern blockade. Here, we show that SAR101099 consistently antagonized UT activation induced by UII or UPR both in vitro and in vivo, in various species or animal models. Compared to other UT antagonists, it shows a high binding affinity with an inhibition constant (Ki) of 9.7 nM and consistent ability to antagonize urotensin-induced vasoconstriction of isolated rat, pig and non-human primate arteries with pKb values in the range of 7.2 to 7.7 making it a best-in-class compound across species.

The poor selectivity profile of other non-peptidic UT antagonists may explain why only a few drug candidates entered clinical development. SAR101099 was selected for its UT selectivity, as it showed no relevant binding activity to a large panel of receptors, ions channels and enzymes (less than 50%) at concentrations up to 10 µM. Only a low binding affinity to bombesin, histamine H3, V1a and N (neuronal) (α-BGTX-insensitive)(α4β2) was reported but with no functional activity. This differs from palosuran, which has been shown to display significant off-target activity thus compromising the relevance of solely blocking UT in the interpretation of the preclinical and clinical studies reported (Strowski et al., 2006; Malagon et al., 2008).

Although UII does not alter blood pressure in rodents, it caused a significant hypertensive effect in conscious pigs. SAR101099 at 10 mg/kg po completely blocked the pressure response to both UII and URP up to 8 hours post treatment, highlighting the attractive properties of SAR101099 as a long acting UT antagonist. In comparison SAR101099 at 1 mg/kg po was more potent and long lasting at blocking the pressor response to UII in pigs than SB611812, another UT antagonist (Figure 2 – online supplement). No effect on heart rate was observed at pharmacological active doses in rodent and pigs. Interestingly, in one monkey where UII at 0.3 µg/kg iv induced a severe cardiovascular collapse likely related to a profound coronary vasoconstriction (also described by Zhu et al., 2004), iv administration
of SAR101099 at 1 mg/kg allowed to rescue the animal from dying with a prompt and full restoration of the hemodynamics (Figure 1 – online supplement). Taken together, the results across the different species show a consistent UT antagonist profile of SAR101099 despite diverging effects of UII. The UT antagonist SAR101099 has no effect of blood pressure but is able to inhibit any UII responses that can be elicited, suggesting that SAR101099 should be able to consistently reduce all pathological processes related to UT activation. These results also underline the attractive safety profile of the drug making its administration suitable on top of current standard of care with a good hemodynamic tolerance expected.

The extensive evaluation of SAR101099 in different models of chronic renal and cardiac impairment demonstrates that it is a potent UT antagonist devoid of species selectivity. The chronic CKD studies confirmed its strong renoprotective effect with a marked reduction in proteinuria, renal arteriole damage and inflammation whether the pathology was mainly induced by hypertension (DS rats), diabetes (STZ rats) or both (SHR-SP on salt-fat diet) and at doses that do not lower blood pressure. This latter point is critical because CKD trials have to be conducted on top of standard of care including RAAS inhibitors known for their anti-hypertensive and hemodynamic effects. That is why future treatments for CKD beyond renal protection should have limited impact on blood pressure in order to reduce the risk of renal hypoperfusion and hypotension. Interestingly, angiotensin II increased UII and UT expression in tubular cells and UII interacts in synergy with angiotensin II. UII gene polymorphism has been also associated with some renal diseases and could be considered to streamline more relevant CKD patient populations for UT blockade (Balat and Buyukcelik, 2012).

CKD translation from animals to humans is however challenging as RAAS is recognized as the main contributor in the pathogenesis of CKD in rodents. RAAS inhibition markedly suppresses proteinuria and improves survival in various CKD models but mainly at doses that lower blood pressure. In contrast, although ACEi/ARB treatments reduce proteinuria in patients, they do not halt progression toward end-stage renal disease. Ravera et al showed in the PRIME study that neither irbesartan (ARB) nor amlopidine (calcium channel blocker) provide total protection from both renal and cardiovascular events in patients (Ravera et al., 2005), while for instance in SHR-SP submitted to salt-fat diet irbesartan notably suppressed proteinuria and decreased the mortality rate in this rodent model (Figure 4A &B-online supplement).

The benefit of chronic treatment with SAR101099 on long term survival in rat models at doses from 30 to 50 mg/kg/day po deserves a special attention. Because no metabolism changes were observed in the different studies (cholesterol, glucose, triglycerides, body weight), and because of the potency and selectivity profile of SAR101099, we can hypothesize that this improvement is mainly driven by UT antagonism. In opposition, the 25% survival improvement observed by Clozel et al., 2006 in a STZ-induced diabetes rat model with palosuran at 300 mg/kg/day (a dose known to prevent the
development of renal failure (Clozel et al., 2004)) is difficult to interpret because at that dose an off-target effect towards somatostatin receptors which are involved in the glucose and albuminuria regulation has previously been demonstrated (Malagon et al., 2008, Strowski et al., 2006). If the improvement is driven by the positive change in metabolism, that might explain why these positive results were not reproduced in type 2 diabetes patients with nephropathy.

In addition, because CKD patients mostly die from CVD complications, it was important to evaluate the benefit of SAR101099 on cardiac outcomes. In rat and pig models of chronic heart failure, SAR101099 restored both the impaired contractile state as well the cardiac contractile reserve, i.e. the capability to adequately respond to inotropic support (dobutamine) together with a reduction in left ventricular filling pressure. The positive effects of SAR101099 in pigs are particularly interesting because RAAS inhibition with ramipril was much less beneficial than in rats, consistent with its limited protective effect in human CKD patients with heart failure (Lunney et al., 2020).

The results of phase I trials (SAD, MAD and food effect studies) confirmed the safe profile of SAR101099 in young healthy male subjects with PK properties suitable for clinical development. Following repeated once daily oral administration of SAR101099, less than 1.3-fold accumulation was observed after 2 weeks of treatment. On Day 14, SAR101099 exposure increased in proportion with the dose between 50 and 500 mg. The within- and between-subjects variability was limited and no notable food effect was observed. As of today, no clinical study has yet been conducted in CKD patients. The present data suggest that it will be important to confirm the promising preclinical data in CKD patients.

One particular challenge of the CKD indication is that the disease suffers from a large heterogeneity with sub-populations progressing at different rates regardless of the etiology and level of proteinuria. Diabetes-independent factors can cause CKD, even in patient with diabetes (Anders et al., 2018). New approaches for segmenting CKD, like deep phenotyping, may be needed to subsequently facilitate specific investigations of the underlying mechanisms that contribute to disease progression or cardiovascular complications in the different subgroups allowing more targeted therapies and improved CKD patient management. Several approaches have been initiated to better characterize the natural history of this heterogenous pathology, improve its diagnosis, identify the genetic factors involved (Levin et al., 2017) or better stratify the CKD patients using artificial intelligence (Elhoseny et al., 2019). The present data suggest that urotensinergic activity should be one of the parameters to be included in this analysis.

Altogether these preclinical and clinical results suggest that UT antagonism remains an attractive target in CKD on top of current standard of care. UT blockade is neutral on hemodynamics which would limit the risk of an additional blood pressure lowering effect when combined with ACEi or ARBs. Selective long-lasting UT blockade with drugs such as SAR101099 could address
complementary pathogenic pathway for the benefit of CKD patient subgroups which remain today to be defined. Its unique pharmacological properties, selectivity, PK and safety profiles offer new avenues for better appreciating the role of the urotensinergic system in CKD patients.

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Figure legends

Figure 1: Chemical structure of SAR101099

Figure 2: In vitro binding assay of SAR101099 to UII and antagonism assays of SAR10109 and palosuran to human, rat and mouse UT.

A: SAR101099A displacement curve of [125I]Tyr9-UII binding to human UT, B: Effects of UII and URP on intracellular calcium mobilization in CHO cells expressing hUT. Mean ± SEM. Effects of SAR101099 (C) and palosuran (D) on the intracellular calcium mobilization in CHO cells expressing human, rat and mouse UT.

Figure 3: In vitro antivasoconstrictor effects of SAR101099

Effect of increasing concentrations of SAR101099 (0.1 µM to 10 µM) on the concentration-response curves of hUII (A) and URP (C) in isolated rat thoracic aorta and in isolated coronary (E) and renal (F) monkey arteries. Results are expressed as mean ± SEM (KCl response taken as 100%) obtained from n=4 to 8 preparations in rat aorta and from n=8-13 monkey coronary and from n=3-5 monkey renal arteries. Shild-plot on UII (B) and on URP (D) responses in rat are depicted as example.

Figure 4: Hemodynamics effects of SAR101099, per os in conscious rats, pigs and monkeys

Mean ± SEM arterial blood pressure in SHR rat (A) where hypertension was induced by salt-fat diet, in pigs where hypertension was induced by hUII (B, C) or by URP (D) before SAR101099 treatment (E): Mean ± SEM arterial blood pressure and (F) mean ± SEM heart rate in monkeys where hypertension was induced by hUII before SAR101099 treatment.

Figure 5: SAR101099 effect in different rat models of renal impairment

(A): Mean+SEM proteinuria after 8 weeks of treatment in SHR-SP fed with salt-fat diet rat model.

(B) Mean + SEM score of arteriolar lesions severity in SHR-SP salt fat diet rat model.

(C): Mean+SEM albuminuria/creatininuria ratio after 8 weeks of treatment in Dahl-salt sensitive rat model.

(D) Mean +SEM albuminuria after 16 weeks of treatment in STZ rat with unilateral nephrectomy.

Figure 6: Survival improvement in rat models of renal impairment

(A) SHR-SP salt fat diet rat model.

(B) Dahl-salt sensitive rat model.
Figure 7: Effect of SAR101099 in chronic heart failure models

Mean+SEM MAP, LVEDP and dP/dtmax measured in rat model (A, B and C) mean SEM+MAP and dP/dtmax measured in pig model (D and E) of left ventricular dysfunction post-MI.

Figure 8: Mean + SD SAR101099 blood concentration-time profiles on Day 1 and Day 14 over 24h period in linear scale
Figure 1
Figure 2

A. Specific binding (%)

B. Fluorescence Units (FU)

C. % inhibition of U II-induced increase in [Ca^{2+}]

D. % inhibition of U II-induced increase in [Ca^{2+}]

SAR 101099 (M)

Agonists (M)

human n=7
rat n=7
mouse n=6
Figure 3

A. Rat

B. Rat

C. Rat

D. Rat

E. Monkey

F. Monkey

% E versus KCl 60 mM

U-II (M)

URP (M)

DMSO SAR101099 0.1 µM SAR101099 0.3 µM SAR101099 1 µM SAR101099 3 µM SAR101099 10 µM

DMSO SAR101099 0.3 µM SAR101099 1 µM SAR101099 3 µM

DMSO SAR101099 0.3 µM SAR101099 1 µM SAR101099 3 µM

pKm = 7.813  [7.002 ; 7.385]

pKc = 7.135  [6.930 ; 7.340]
Figure 4

A  Rat

B  Pig

hUII (0.5 µg/kg iv) 4 hours after SAR101099

Vehicle (n=5)  
1 mg/kg po (n=5)  
3 mg/kg po (n=4)  
10 mg/kg po (n=6)

C  Pig

D  Pig

hUII (0.5 µg/kg iv)

Vehicle (n=3)  
10 mg/kg – 8hr pre URP (n=3)

URP (0.5 µg/kg iv)

Vehicle (n=4)  
10 mg/kg po – 4 hr pre URP (n=4)  
10 mg/kg po – 8 hr pre URP (n=4)

E  Monkey

Vehicle/Saline (n=3)  
Vehicle/UII (n=3)  
f 1 mg/kg/UII (n=3)

F  Monkey

MAP (mmHg)  
Heart rate (bpm)
Figure 5

A

**SHR rat**

![Bar graph showing proteinuria (mg/24h)]

- WKY-vehicle (n=19)
- SHR_SP SFD-vehicle (n=34)
- SHR_SP SFD-rampirli: 3 mg/kg/day (n=19)
- SHR_SP SFD-SAR101099: 30 mg/kg/day (n=20)

B

**SHR rat**

![Bar graph showing score (AU)]

- WKY-vehicle (n=14)
- SHR_SP SFD-vehicle (n=27)
- SHR_SP SFD-rampirli: 3 mg/kg/day (n=27)
- SHR_SP SFD-SAR101099: 30 mg/kg/day (n=14)

C

**Dahl rat**

![Bar graph showing albumin/creatinine (mg/g)]

- DR (n=17)
- DS-Placebo (n=9)
- DS-SAR101099A: 30 mg/kg/day, PO (n=13)
- DS-SAR101099A: 30 mg/kg/day, PO (n=14)

D

**SIZ rat**

![Bar graph showing albuminuria (mg/100g/24h)]

- Shani (n=14)
- STZ-Placebo (n=15)
- STZ+Vehicle (n=13)
- STZ+SAR101099A: 30 mg/kg/day, PO (n=15)
- STZ+SAR101099A: 30 mg/kg/day, PO (n=17)
- STZ+SAR101099A: 30 mg/kg/day, PO (n=14)
Figure 6

A

SHR rat

Survival rate %

Time (Weeks)

DR-placebo (n=18)
DS-placebo (n=20)
DS-Irbesartan: 10 mg/kg/d PO (n=20)
DS-SAR101099: 10 mg/kg/d PO (n=20)
DS-SAR101099: 30 mg/kg/d PO (n=20)
DS-SAR101099: 50 mg/kg/d PO (n=19)

B

Dahl rat

Survival rate %

Time (Days)

DR-placebo (n=18)
DS-placebo (n=20)
DS-Irbesartan: 10 mg/kg/d PO (n=20)
DS-SAR101099: 10 mg/kg/d PO (n=20)
DS-SAR101099: 30 mg/kg/d PO (n=20)
DS-SAR101099: 50 mg/kg/d PO (n=19)
Figure 7

A

B

C

D

E
Figure 8

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