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Efficacy of a novel inhibitor of vascular adhesion protein-1 in reducing albuminuria in patients with diabetic kidney disease (ALBUM): a randomised, placebo-controlled, phase 2 trial

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Summary

Background Many patients with diabetic kidney disease have residual albuminuria and are at risk of disease progression. The ALBUM trial investigated the efficacy of a novel, orally active inhibitor of vascular adhesion protein-1, ASP8232, compared with placebo for reducing albuminuria in individuals with type 2 diabetes and chronic kidney disease.

Methods In this randomised, double-blind, placebo-controlled phase 2 trial, we randomly assigned individuals (aged 18–85 years) from 64 clinical sites in nine European countries to receive ASP8232 40 mg or placebo orally once daily for 12 weeks using a web-based randomisation schedule (block size 4), stratified by country. Eligible patients had a urinary albumin-to-creatinine ratio (UACR) of 200–3000 mg/g, an estimated glomerular filtration rate of at least 25 mL/min per 1·73 m² but lower than 75 mL/min per 1·73 m², HbA_{1c} less than 11·0% (97 mmol/mol), and stable treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and antidiabetic medication for 3 months or more. The primary endpoint was mean change from baseline to week 12 in log-transformed first morning void UACR, which was assessed in all patients who received at least one dose of study drug and had at least one post-baseline UACR measurement (full analysis set). Safety was assessed in all patients who received at least one dose of study drug. Participants and investigators were masked to treatment allocation. This trial is registered with ClinicalTrials.gov, number NCT02358096.

Findings 125 participants were randomly assigned to receive ASP8232 (n=64) or placebo (n=61), of whom 120 (60 in each group) were included in the full analysis set; all participants were assessed for safety endpoints. At 12 weeks, UACR decreased by 17·7% (95% CI 5·0 to 28·6) in the ASP8232 group and increased by 2·3% (–11·4 to 18·1) in the placebo group; the placebo-adjusted difference between groups was –19·5% (95% CI –34·0 to –1·8; p=0·033). 39 (61%) patients in the ASP8232 group and 34 (56%) patients in the placebo group had a treatment-emergent adverse event, of which 16 in the ASP8232 group and four in the placebo group were drug-related. The most frequently reported adverse events that were possibly drug-related in the ASP8232 group were renal impairment (five patients) and decreased eGFR (three patients); in the placebo group, no single drug-related treatment-emergent adverse event was reported by more than one participant.

Interpretation ASP8232 is effective in reducing albuminuria in patients with diabetic kidney disease and is safe and well tolerated. These findings warrant further research to ascertain the effect of ASP8232 on delaying progression of diabetic kidney disease.

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Introduction

Drugs that block the renin-angiotensin-aldosterone system, such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), are recommended as first-line antihypertensive therapy for patients with diabetic kidney disease and overt albuminuria.[1] Although these medications are effective, many patients still have residual albuminuria and are at increased risk of disease progression.[2] Therefore, new drugs targeting albuminuria are needed to address an unmet need in the management of diabetic kidney disease.

Vascular adhesion protein-1 (VAP-1) is an endothelial amine oxidase that belongs to the semicarbazide-sensitive amine oxidase family of enzymes; it catalyses the oxidative deamination of primary amines to produce aldehydes, hydrogen peroxide, and ammonia, resulting in oxidative stress and cellular toxicity.[3] VAP-1 is expressed in the vascular endothelium of renal and retinal capillaries, smooth muscle cells, and adipose

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Research in context

Evidence before this study

Vascular adhesion protein-1 (VAP-1) is implicated in various conditions associated with oxidative stress and inflammation. The role of VAP-1 in diabetic kidney disease is unknown, although epidemiological data suggest an association between higher circulating concentrations of VAP-1 and rapid progression of diabetic kidney disease. We searched PubMed and ClinicalTrials.gov without language restrictions for studies published between 1998 and 2018 using the search terms "VAP-1" and "vascular adhesion protein-1". No clinical trial has investigated a therapeutic intervention for patients with diabetic kidney disease that specifically targets VAP-1.

Added value of this study

To our knowledge, this is the first clinical trial to investigate the safety and efficacy of a VAP-1 inhibitor in a human disease. Participants with type 2 diabetes and chronic kidney disease were randomly assigned to a novel, specific, orally active inhibitor of VAP-1 (ASP8232) or placebo, which were administered over 12 weeks in conjunction with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. We found that ASP8232 significantly reduced albuminuria, as measured with the urinary albumin-to-creatine ratio, after 12 weeks of treatment compared with placebo and was safe and well tolerated, with no drug-related serious adverse events reported. This study provides the first clinical evidence that VAP-1 activity is involved in the pathophysiology of human diabetic kidney disease and that VAP-1 inhibition could improve disease status.

Implications of all the available evidence

Owing to its novel mechanism, ASP8232 might have the potential to provide clinical benefit to patients with type 2 diabetes and chronic kidney disease when used in conjunction with the current standard of care. Further studies are needed to ascertain whether ASP8232 delays progression of diabetic kidney disease.

tissue where it promotes an inflammatory response by modulating various steps of leucocyte trafficking between blood and tissues.[4,5] VAP-1 activity is implicated in multiple disorders and pathological processes that involve oxidative stress or inflammation, such as primary sclerosing cholangitis,[6] tumour growth,[7] graft-versus-host disease,[8] multiple sclerosis,[9] ischaemic brain injury,[10] ophthalmological disorders,[11] atherosclerosis,[12] and acute kidney injury.[13]

A causative role for VAP-1 in diabetic kidney disease has not yet been shown but is plausible because of the enzyme's effects on oxidative stress and inflammation. Additionally, epidemiological studies have shown that circulating concentrations of VAP-1 are associated with albuminuria and estimated glomerular filtration rate (eGFR) in patients with diabetes,[14,15] and that VAP-1 independently predicts cardiovascular mortality and progression of diabetic kidney disease to end-stage renal failure in these patients.[16,17] Yet, clinical proof-of-concept studies supporting a beneficial effect of VAP-1 inhibition on human diseases are currently lacking.

ASP8232 is a potent, orally active, specific VAP-1 inhibitor that is currently being investigated in phase 1 trials (NCT02218099 and unpublished) for patients with diabetic kidney disease. In vitro, ASP8232 non-competitively inhibited the activity of rat and human VAP-1, with inhibition constants of 3·55 nmol/L for rat VAP-1 and 4·66 nmol/L for human VAP-1 (unpublished; company data on file). Binding assays showed that ASP8232 is specific for VAP-1 and does not inhibit any other monoamine oxidases (unpublished). Pharmacological effects for ASP8232 were confirmed in several rat models of diabetes and kidney injury, including reduced albuminuria and improved renal function and tissue damage (unpublished). Phase 1 clinical data (unpublished) indicated that ASP8232 was safe and well tolerated across a wide dose range in healthy individuals and in people with renal impairment. The bioavailability of ASP8232 appeared unchanged under fasted versus fed conditions (unpublished data from first-in-man study [NCT02218099]). Thus, owing to its novel mode of action, ASP8232 has promise as a novel therapy for patients with diabetic kidney disease, potentially as an adjunct to ACE inhibitors, ARBs, or other renal protective drugs.

We aimed to investigate the efficacy of ASP8232 compared with placebo in reducing albuminuria after 12 weeks of treatment in patients with type 2 diabetes and chronic kidney disease. The study also included 6 months of follow-up to further assess the drug's efficacy, safety, and pharmacokinetic profile; the results of these analyses are also reported here.

Methods

Study design and participants

The phase 2, double-blind, randomised, parallel-group, placebo-controlled, proof-of-concept ALBUM trial was done at 64 clinical sites in nine countries (Czech Republic, Denmark, Germany, Hungary, Italy, Poland, Spain, the Netherlands, and the UK). Eligible participants (aged 18–85 years) had type 2 diabetes, an eGFR of at least 25 mL/min per 1·73 m² but lower than 75 mL/min per 1·73 m², HbA_{1c} less than 11·0% (97 mmol/mol), and a urinary albumin-to-creatinine ratio (UACR) in first morning void of 200–3000 mg/g. Additionally, they had to have been treated with antidiabetic medications for at least 1 year before screening and on stable therapy (ie, no changes in dose or type of medication) with an ACE inhibitor or ARB and antihypertensives, oral antidiabetic medications, or vitamin D receptor activators for 3 months or more before screening. Women of

non-childbearing potential were post-menopausal before screening or surgically sterile or had a post-hysterectomy status at 1 month before screening. Women of childbearing potential were required to use two forms of birth control (including one barrier method), starting at screening and maintained throughout the study, and to have a negative pregnancy test at screening. Women were not breastfeeding and must have not donated ova or had a male partner donate sperm at screening, throughout the study period, and for 24 weeks after the final study drug administration. Participants agreed to not participate in any other interventional study from signing of informed consent to the end of the study, and had the ability and willingness to return for all scheduled visits and perform all assessments.

Exclusion criteria were renal replacement therapy, known renal disease due to conditions other than diabetes, autoimmune disorder or requirement for immunosuppressive therapy, active urinary tract infection, type 1 diabetes or diabetes with unclear aetiology, and any other condition that, in the investigator's opinion, made the participant unsuitable for the study. Moreover, individuals were excluded if they had a sitting systolic blood pressure of less than 90 mm Hg or greater than 160 mm Hg or a diastolic blood pressure of greater than 90 mm Hg; received any investigational therapy 28 days before screening; significant obstructive uropathy, other renal impairment not related to parenchymal renal disorder or disease of the kidney, or current or previous renal disease secondary to malignancy; known or suspected hypersensitivity to ASP8232 or any components of the formulation used; or were an employee of Astellas or the clinical research organisation involved in the study. Women were excluded if they were lactating or had a positive pregnancy test within 72 h before screening, had been pregnant within 6 months before screening or breastfeeding within 3 months before screening, or were planning to become pregnant within the study period.

The date of first enrolment was Sept 17, 2015, and the date of last evaluation (including the 6 months of follow-up) was Sept 15, 2017. Following a 1-week screening period, eligible participants initiated a 5-week pretreatment period to ensure that they had a stable baseline UACR.

The study was done in accordance with the Declaration of Helsinki, the International Conference on Harmonisation, and local laws and regulations. The study protocol and informed consent forms were approved by the ethics committees for each site. All participants provided written informed consent to participate in the study.

Randomisation and masking

Participants were randomly assigned in a 1:1 ratio to receive oral ASP8232 or placebo once daily for 12 weeks. Randomisation was done according to a randomisation schedule stratified by country, with a block size of four. Randomisation numbers were generated in accordance with the randomisation schedule stored in an interactive web response system. All participants, investigators, and trial staff were masked to treatment allocation.

Procedures

ASP8232 was self-administered as 40 mg capsules. This dose was selected because it had been predicted to result in complete inhibition of plasma VAP-1 activity in phase 1 studies (NCT02218099 and unpublished) without exceeding target exposure concentrations guided by toxicity studies (unpublished). Placebo was supplied as a matching capsule of microcrystalline cellulose (indistinguishable to active study drug). During the 12-week treatment period, ASP8232 or placebo were taken every morning, with or without food. For site visits in which pharmacokinetic sample collection was scheduled, participants were instructed to not take the study drug before the visit.

Patients visited clinics at weeks –2 and –1 during the 5-week run-in period; at weeks 2, 4, 6, 8, and 12 during treatment; and at weeks 4, 12, and 24 after treatment (ie, 16, 24, and 36 weeks after randomisation; see appendix for trial overview). Patients were asked to provide a single first morning void sample at screening and then three first morning void samples collected on three consecutive days before each clinic visit during the 5-week run-in period, treatment, and off-drug follow-up. 24-h urine collections were provided at baseline and at then week 4 and week 12 visits during treatment.

See Online for appendix

All samples were transported to a central laboratory (Bio Analytical Research Corporation, Ghent, Belgium) for measurement of serum biochemistry parameters and urinalysis. Urine albumin was measured with an immunoturbidimetric assay (Tina-quant Albumin Gen.2; Roche Diagnostics, Rotkreuz, Switzerland) on a Cobas 8000 c502 modular chemistry system (Roche Diagnostics). Creatinine was measured with a Jaffé-compensated, rate-blanked, kinetic colorimetric assay (Creatinine Jaffé Gen.2; Roche Diagnostics) on a Cobas 8000 c702 modular chemistry system (Roche Diagnostics). Plasma concentrations of ASP8232 were measured by liquid chromatography–tandem mass spectrometry. Soluble VAP-1 concentration in plasma was measured with an ELISA (Human sVAP-1 ELISA kits; Immuno-Biological Laboratories International, Hamburg, Germany), and VAP-1 activity was measured in plasma using a radioactive substrate enzymatic conversion assay with scintillation counting (in-house assay).

Outcomes

The primary efficacy endpoint was the mean change in log-transformed first morning void UACR from baseline to week 12. Least square means were calculated to establish the treatment effect and subsequently back-transformed to percentage change in UACR.

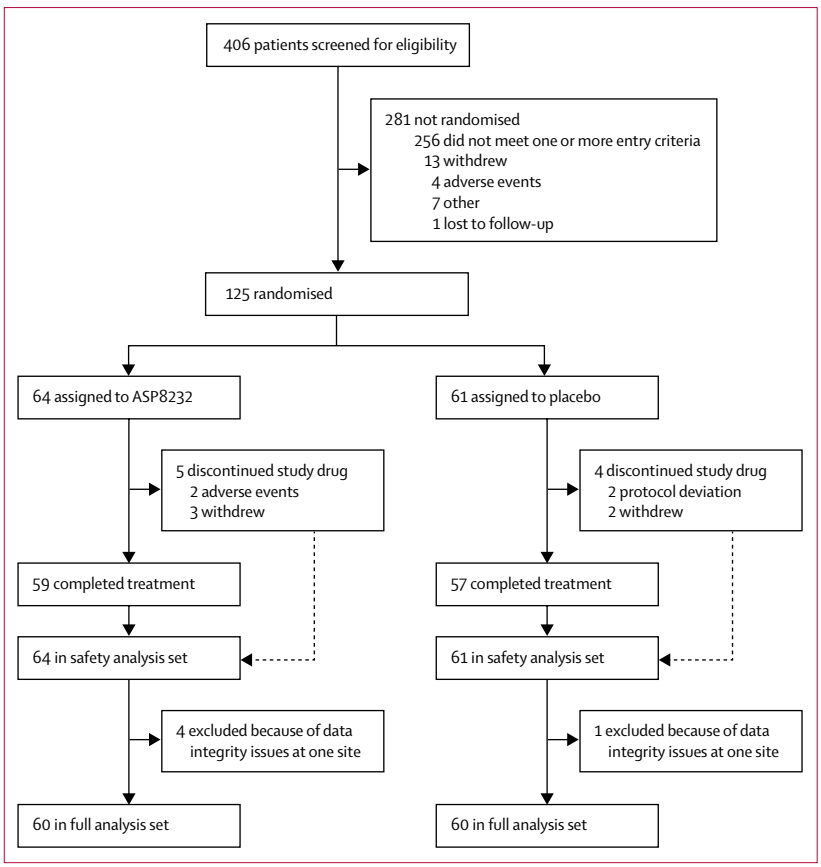

Figure 1: **Trial profile**

Secondary efficacy endpoints were the change in log-transformed 24-h albuminuria from baseline to week 12 and the proportion of participants with 30% or more reductions in UACR or 24-h albuminuria. Renal function, as measured by eGFR, was assessed as an exploratory efficacy endpoint and calculated with both the Chronic Kidney Disease Epidemiology Collaboration (based on creatinine) and cystatin C equations.[18,19]

Safety was assessed by monitoring the nature, frequency, and severity of adverse events, vital signs (blood pressure and pulse rate), 12-lead electrocardiogram (ECG) measurements, and clinical laboratory tests (biochemistry, haematology, and urinalysis). Adverse events were classified according to Medical Dictionary for Regulatory Activities (version 15.1) preferred terms.

Statistical analysis

Assuming a two-sided significance level of 0·05 and an SD of 0·6, we calculated that 46 patients in each group would provide at least 80% power to detect a 0·356 difference in mean change in log-transformed UACR from baseline to week 12 between ASP8232 and placebo groups (equivalent to a 30% difference in geometric mean percentage change). Assuming a 15% dropout rate, a sample size of 55 participants for each treatment group was planned for randomisation.

The efficacy analyses were done in the full analysis set, which included all randomised participants who received at least one dose of study drug and had at least one post-baseline UACR measurement. Observations after treatment discontinuation were excluded from the primary analysis but reported separately as part of the post-treatment follow-up period. We also assessed the primary endpoint in the per-protocol population, which included all patients who received treatment for 52 days or more, were 70% or more compliant with the study treatment, had no treatment interruption lasting more than 7 days, and did not deviate from any inclusion or exclusion criteria that might affect the efficacy of ASP8232. Safety analyses included all participants who received at least one dose of study drug.

We used a (marginal) covariance-pattern mixed model for repeated measures for the analyses of UACR and 24-h albuminuria. The model included treatment, visit, visit by treatment interaction, and country as fixed class factors and baseline log-transformed UACR values as continuous covariates. The proportion of participants with 30% or more reduction in UACR was analysed with a logistic regression model that included treatment as a fixed factor and country and log-transformed UACR at baseline as covariates. All data analyses were done with SAS version 9.3.

We did a post-hoc analysis of the slope of eGFR, disaggregated by treatment group, using a conditional mixed model for repeated measures (in particular, we used a random intercepts and slopes model). We used eGFR calculated with the cystatin C rather than the creatinine method in this model because ASP8232 has been shown to interfere with tubular creatinine secretion (unpublished results of in-vitro transporter inhibition experiments and phase 1 studies). The model was constrained to have the same prediction for both groups during the screening period. We allowed the slope for ASP8232 to vary at each of the study visits because of the drug's observed acute haemodynamic effect, whereas the slope was assumed to be constant for placebo.

This trial is registered with ClinicalTrials.gov, number NCT02358096.

Role of the funding source

The funder of the study had a role in study design and was responsible for data collection, data analysis, data interpretation, writing the report, and the decision to submit for publication. The corresponding author had full access to all the study data and had final responsibility for the decision to submit for publication.

Results

We screened 406 individuals for eligibility, of whom 125 were randomly assigned to receive ASP8232 (n=64) or placebo (n=61; figure 1). All randomised individuals

received at least one dose of study drug. Five participants (four in the ASP8232 group and one in the placebo group) from one clinical site were excluded from the full analysis set because of site-specific issues with data integrity; no participants were excluded because of missing data. Thus, 120 participants (60 in each group) were included in the full analysis set.

Demographic and baseline characteristics in the full analysis set were generally similar between the treatment groups, except that there were more women in the ASP8232 group than in the placebo group (table 1). All participants had previously received medications for chronic kidney disease and diabetes and continued to receive these medications during the study; the types of previous and concomitant medications were similar between groups. The median treatment duration was 85 days for both groups.

Geometric mean percentage changes in UACR over time are shown in figure 2, and median UACR values at each visit are in the appendix. At week 12, the geometric mean percentage change in UACR from baseline was greater in the ASP8232 group (–17·7%; 95% CI –28·6 to –5·0) than in the placebo group (2·3%; –11·4 to 18·1; placebo-adjusted difference –19·5%, 95% CI –34·0 to –1·8; p=0·033). The largest placebo-adjusted difference in treatment effect was observed 4 weeks after the end of treatment, and a difference between groups remained but subsided during follow-up. A prespecified per-protocol analysis of the primary endpoint demonstrated quantitatively similar results, although the difference between groups was not significant (appendix).

Creatinine concentrations in first morning void samples were similar across treatment groups at baseline (table 1) but consistently lower in the ASP8232 group than in the placebo group during treatment and until the end of follow-up (data not shown). To rule out potential bias in the primary outcome due to differences between first morning void samples in urinary creatinine concentrations, we did a post-hoc analysis of albuminuria (unadjusted for creatinine) in first morning void samples. The albumin fraction of first morning void samples was significantly reduced with ASP8232 (–24·1%, 95% CI –34·2 to 12·3) compared with placebo (2·2, –11·5 to 18·0) at week 12 (placebo-adjusted difference –25·7%, 95% CI –39·0 to –9·4; p=0·004), consistent with the primary analysis.

The change in 24-h albuminuria from baseline to week 12 was not significantly different between the ASP8232 group (–26·7%, 95% CI –39·4 to –11·3) and the placebo group (–8·4%, –24·3 to 11·02; placebo-adjusted difference –20·0%, 95% CI –38·5 to 4·0; p=0·094). 22 (37%) of 60 patients in the ASP8232 group and 13 (22%) of 60 patients in the placebo group achieved a 30% or more reduction from baseline in first morning void UACR at week 12 (odds ratio 2·05, 95% CI 0·85 to 4·94; p=0·109).

	ASP8232 (n=60)	Placebo (n=60)
Sex		
Male	43 (72%)	50 (83%)
Female	17 (28%)	10 (17%)
Race		
White	56 (93%)	57 (95%)
Black or African American	1 (2%)	0
Asian	2 (3%)	2 (3%)
Other	1 (2%)	1 (2%)
Age (years)	69·0 (7·2)	68·5 (6·6)
Bodyweight (kg)	92·8 (19·0)	94·8 (20·5)
Height (cm)	169·5 (9·1)	171·4 (8·7)
BMI (kg/m^2)	32·3 (5·8)	32·2 (6·5)
Duration of chronic kidney disease (years)*	5·0 (4·4)	5·5 (4·2)
Duration of diabetes (years)*	16·3 (7·7)	16·2 (7·0)
Systolic blood pressure (mm Hg)	140·6 (13·1)	138·9 (14·8)
Diastolic blood pressure (mm Hg)	75·6 (9·0)	75·1 (10·3)
Serum albumin (g/L)	41·7 (2·7)	42·3 (2·8)
Serum creatinine (μmol/L)	138·5 (34·4)	139·6 (32·5)
eGFR (mL/min per 1·73 m^2)	44·0 (11·6)	44·8 (11·2)
Haemoglobin (g/L)	134·1 (18·3)	141·2 (20·0)
HbA_{1c} (%)	7·5 (1·4)	7·4 (1·3)
HbA_{1c} (mmol/mol)	58·4 (NA)	57·6 (NA)
Total cholesterol (mg/dL)	4·2 (1·0)	4·4 (1·1)
Triglycerides (mmol/L)	2·3 (1·7)	2·4 (1·6)
Serum potassium (mmol/L)	4·7 (0·6)	4·7 (0·5)
UACR (mg/g creatinine)†	785·5 (418·0–1230·0)	640·0 (388·5–1062·5)
Antihypertensive medications		
RAAS inhibitors	60 (100%)	59 (98%)
ACE inhibitors	26 (43%)	27 (45%)
Angiotensin receptor blockers	28 (47%)	20 (33%)
β blockers	39 (65%)	40 (67%)
Calcium channel blockers	30 (50%)	41 (68%)
α-receptor blockers	13 (22%)	19 (32%)
Diuretics	38 (63%)	39 (65%)
Antidiabetic medications		
Metformin	27 (45%)	28 (47%)
DPP-4 inhibitors	11 (18%)	7 (12%)
Insulin for inhalation	9 (15%)	11 (18%)
Fast-acting insulin	25 (42%)	26 (43%)
Long-acting insulins or insulin combinations	29 (48%)	32 (53%)
Sulfonylureas	16 (27%)	10 (17%)
Thiazolidinediones	0	1 (2%)
SGLT 2 inhibitors	0	0
Other medications		
Aspirin	32 (53%)	29 (48%)
Allopurinol	23 (38%)	23 (38%)
HMG-CoA reductase inhibitors	48 (80%)	48 (80%)

Data are presented as n (%) or mean (SD), unless otherwise noted. eGFR=estimated glomerular filtration rate. NA=not available. UACR=urinary albumin-to-creatinine ratio. RAAS=renin-angiotensin-aldosterone system. ACE=angiotensin-converting enzyme. DPP-4=dipeptidyl peptidase-4. SGLT 2=sodium-glucose co-transporter 2. HMG-CoA=3-hydroxy-3-methyl-glutaryl-coenzyme A. *Duration in years was calculated as (randomisation date – diagnosis date + 1) / 365·25. †Data are median (IQR).

Table 1: **Participant demographics (full analysis set)**

As an exploratory endpoint, we assessed the effect of the study drug on eGFR. The changes in eGFR from baseline at each study visit are shown, by method used, in table 2. A small reduction in eGFR in the ASP8232 group compared with the placebo group was observed from the first study visit after randomisation and throughout treatment and resolved during follow-up. At each timepoint, the decline in eGFR calculated with the creatinine method was greater than that calculated with the cystatin C method (table 2), consistent with interference of tubular creatinine secretion. A slope analysis of the change over time in eGFR, as calculated with the cystatin C method, is shown in the appendix. These data suggest complete and quick reversal of the initial decrease in eGFR with ASP8232 after discontinuation of treatment.

The observed plasma concentrations of ASP8232 at the presumed time to maximum plasma concentration (about 1–2 h) were consistent with phase 1 data (unpublished) and indicated good absorption. The mean pre-dose concentration of ASP8232 ranged from 74·97 ng/mL (SD 67·25) to 85·14 ng/mL (77·89) over weeks 4–12 and remained steady throughout treatment (figure 3). Plasma concentrations decreased rapidly after discontinuation of treatment to 1·73 ng/mL (0·50) at week 16 and 0·70 ng/mL (0·23) at week 36. Inhibition of VAP-1 activity was nearly complete (98·6%) after 2 weeks of treatment and remained high until week 12. Following discontinuation of ASP8232, VAP-1 activity gradually returned to baseline levels.

Throughout the study, there were no clinically significant differences between groups in changes from baseline in clinical laboratory parameters, ECGs, and vital signs. Median results of vital sign assessments and clinical laboratory tests at baseline and week 12 are shown in the appendix. Briefly, there were no significant differences between groups in changes in systolic blood pressure (p=0·974), diastolic blood pressure (p=0·116), and bodyweight (p=0·926). Additionally, effects on glycaemic control were not different between groups, as measured with HbA_{1c} and glucose concentrations (appendix).

39 (61%) of 64 patients in the ASP8232 group and 34 (56%) of 61 patients in the placebo group had a treatment-emergent adverse event (appendix). Drug-related treatment-emergent adverse events were reported by 16 (25%) participants in the ASP8232 group and by four (7%) participants in the placebo group. The most frequently reported drug-related treatment-emergent adverse events in the ASP8232 treatment group were renal impairment (in five patients) and decreased eGFR (in three patients); in the placebo group, no single drug-related treatment-emergent adverse event was reported by more than one participant. In the ASP8232 group, two participants had treatment-emergent adverse events that led to study drug discontinuation (decreased eGFR in one and renal impairment in the other), both of which were considered to be possibly drug-related, whereas no

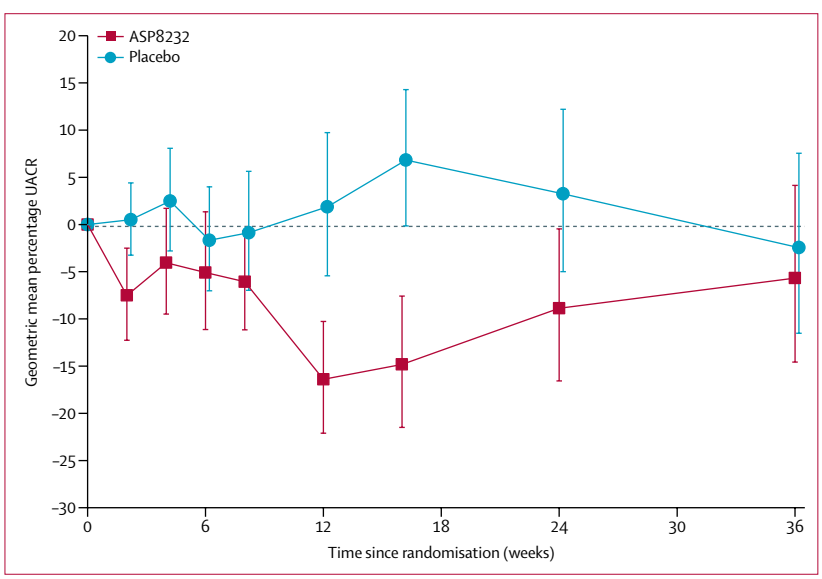

Figure 2: **Geometric mean percentage change in UACR from baseline to week 36 (full analysis set)**
Error bars are SEs. UACR=urinary albumin-to-creatinine ratio.

	$eGFR_{creatinine}$ (mL/min per 1·73 m²)			$eGFR_{cystatin\ C}$ (mL/min per 1·73 m²)		
	ASP8232	Placebo	Difference	ASP8232	Placebo	Difference
Week 2	−4·7 (−6·9 to −2·4)	−0·2 (−2·2 to 1·7)	−4·4 (−5·7 to −3·2)	−2·1 (−4·3 to 0·2)	−0·2 (−2·2 to 1·8)	−1·9 (−3·0 to −0·8)
Week 4	−3·7 (−5·9 to −1·4)	−0·5 (−2·5 to 1·5)	−3·2 (−4·5 to −1·9)	−1·4 (−3·6 to 0·9)	−0·4 (−2·4 to 1·7)	−1·0 (−2·1 to 0·1)
Week 6	−3·4 (−5·6 to −1·1)	−0·7 (−2·7 to 1·3)	−2·6 (−3·9 to −1·3)	−1·5 (−3·7 to 0·7)	−0·5 (−2·6 to 1·5)	−1·0 (−2·1 to 0·2)
Week 8	−4·2 (−6·4 to −1·9)	−1·0 (−3·0 to 1·0)	−3·2 (−4·6 to −1·8)	−1·8 (−4·1 to 0·4)	−0·7 (−2·8 to 1·3)	−1·1 (−2·3 to 0·1)
Week 12	−5·4 (−7·7 to −3·1)	−1·5 (−3·5 to 0·6)	−3·9 (−5·3 to −2·5)	−2·7 (−4·9 to −0·5)	−1·1 (−3·2 to 1·0)	−1·6 (−2·8 to −0·4)
Week 16	−2·9 (−5·3 to −0·6)	−2·0 (−4·1 to 0·1)	−1·0 (−2·5 to 0·6)	−2·5 (−4·8 to −0·3)	−1·5 (−3·6 to 0·6)	−1·1 (−2·4 to 0·3)
Week 24	−2·8 (−5·2 to −0·4)	−2·9 (−5·2 to −0·7)	0·1 (−1·7 to 2·0)	−2·1 (−4·4 to 0·2)	−2·2 (−4·4 to 0·0)	0·1 (−1·5 to 1·7)
Week 36	−3·8 (−6·4 to −1·2)	−4·4 (−6·9 to −1·9)	0·6 (−1·7 to 3·0)	−3·7 (−6·2 to −1·2)	−3·3 (−5·7 to −0·9)	−0·5 (−2·5 to 1·6)

Data are least square mean (95% CI). Differences might not equal ASP8232 minus placebo because of rounding. A random intercepts and slopes model was used for each parameter. The model was constrained to have the same prediction for both groups during the screening period. The slope was allowed to vary at each of the study visits for ASP8232 due to acute haemodynamic effects, whereas it was assumed to be constant for placebo. eGFR=estimated glomerular filtration rate.

Table 2: **Change in estimated GFR from baseline at each visit, by method used (full analysis set)**

treatment-emergent adverse event led to study drug discontinuation in the placebo group. Most treatment-emergent adverse events were mild to moderate in severity. Overall, three severe treatment-emergent adverse events were reported during the study: two in the ASP8232 group (dry mouth and cerebrovascular accident in different patients) and one in the placebo group (erysipelas; table 3 and appendix). Among them, dry mouth was thought to be related to the study drug. Three patients in each group had a serious treatment-emergent adverse event (table 3 and appendix). No drug-related serious treatment-emergent adverse events or treatment-emergent adverse events that resulted in death were reported. One death occurred in the ASP8232 group due to cardiac arrest and respiratory failure. This event occurred at the end of the follow-up period, 205 days after the last dose of ASP8232, and was not deemed to be related to the study drug.

Discussion

In this randomised, placebo-controlled, phase 2 trial, we found that ASP8232, a specific VAP-1 inhibitor, was effective in reducing residual albuminuria, a surrogate marker for disease progression, when administered alongside a stable ACE inhibitor or ARB regimen in participants with type 2 diabetes and chronic kidney disease. We quantified the change in albuminuria between baseline and week 12 of treatment by measuring the UACR, which decreased by 19·5% (95% CI 1·8 to 34·0) with ASP8232 compared with placebo. Moreover, a third of participants in the ASP8232 group (37% *vs* 22% in the placebo group) had a 30% or more reduction in first morning void UACR between baseline and week 12, which is a clinically relevant decrease associated with long-term renal protection.[20]

The true effect of ASP8232 on albuminuria might have been underestimated in this study in view of the consistently lower urine creatinine concentrations in the ASP8232 group than in the placebo group after randomisation. This difference was sustained throughout follow-up and could not be attributed to pharmacodynamic effects of ASP8232. Indeed, first morning void UACR was reduced by 25·7% (95% CI 9·4 to 39·0) when not adjusted for urine creatinine concentration.

The largest decline in UACR was noted at the end of treatment, indicating the potential for further improvement when administered for longer than 12 weeks. Although speculative, further reduction beyond 12 weeks of treatment would be consistent with the drug's anti-oxidative stress and anti-inflammatory activities, rather than its primary renal haemodynamic mechanisms, having an effect on albuminuria. The albuminuria-lowering effect of ASP8232 occurred without changes in systemic blood pressure or body-weight, indicating that the drug has local effects on the glomeruli and podocytes rather than systemic effects, such as altered renal perfusion or fluid status.

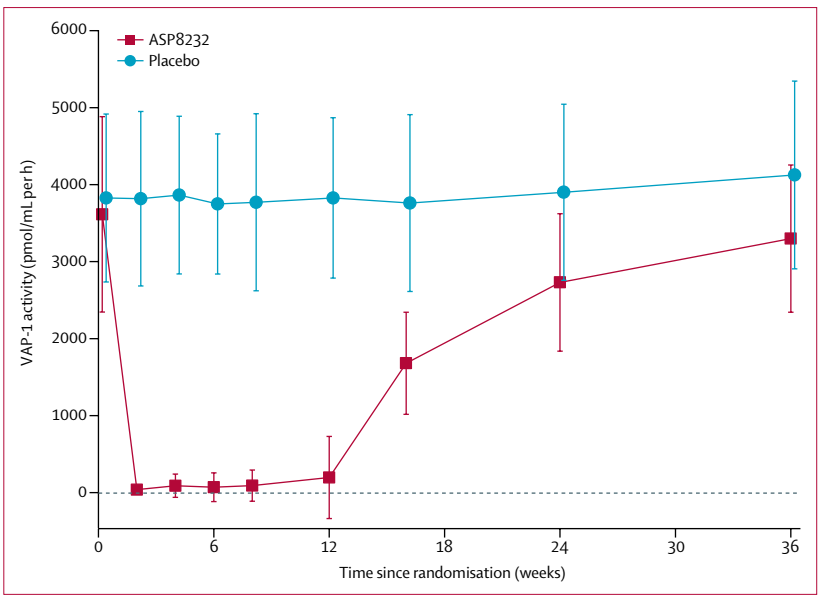

Figure 3: **Plasma VAP-1 activity**
Means and SDs are presented. VAP-1=vascular adhesion protein-1.

ASP8232 was associated with a small decline in eGFR compared with placebo, which was observed at the first visit after randomisation (week 2) and lasted throughout treatment. This difference, which resolved during follow-up, indicates that ASP8232 has acute haemodynamic effects. A similar effect on eGFR has been noted for several renal protective drugs, such as ACE inhibitors, ARBs, and sodium-glucose co-transporter 2 inhibitors.[21,22] The relatively greater decline in eGFR measured with creatinine than eGFR measured with cystatin C is consistent with an inhibitory effect of ASP8232 on tubular creatinine secretion (ie, inhibition of tubular creatinine transporters MATE1, MATE2-K, and OCT2). We previously identified this effect in vitro (unpublished), and phase 1 studies (NCT02218099 and unpublished) showed that ASP8232 elicited a dose-dependent increase in serum creatinine concentrations. This effect has also been observed with other drugs, such as cimetidine, and does not reflect an actual change in renal function.[23]

In phase 1 studies (NCT02218099 and unpublished) in healthy individuals and people with renal impairment, ASP8232 was safe and well tolerated at doses and exposure concentrations far above those that were explored in this study. Consistent with these findings, we found that ASP8232 was well tolerated and had a good safety profile. No drug-related serious adverse events were reported, although one severe adverse event was deemed possibly related to ASP8232 (dry mouth). The higher incidence of adverse events related to renal impairment in the ASP8232 group than in the placebo group was assessed in a case-by-case examination by the sponsor's pharmacovigilance representative and medical safety monitor, and is thought to be due to the combined

	ASP8232 (n=64)	Placebo (n=61)
Any TEAE*	39 (61%)	34 (56%)
TEAEs reported in ≥5% of participants in any treatment group		
Renal impairment	9 (14%)	1 (2%)
Mild	4 (6%)	1 (2%)
Moderate	5 (8%)	0
Peripheral oedema	6 (9%)	1 (2%)
Mild	5 (8%)	1 (2%)
Moderate	1 (2%)	0
Anaemia	4 (6%)	0
Mild	3 (5%)	0
Moderate	1 (2%)	0
Back pain	2 (3%)	4 (7%)
Mild	2 (3%)	3 (5%)
Moderate	0	1 (2%)
Hypertension	2 (3%)	6 (10%)
Mild	2 (3%)	3 (5%)
Moderate	0	3 (5%)
Serious TEAEs†	3 (5%)	3 (5%)
Anaemia	1 (2%)	0
Complete atrioventricular block	0	1 (2%)
Congestive heart failure	0	1 (2%)
Nausea	1 (2%)	0
Pyrexia	1 (2%)	0
Erysipelas	0	1 (2%)
Escherichia coli urinary tract infection	1 (2%)	0
Aphasia	1 (2%)	0
Cerebrovascular accident	1 (2%)	0
Grand mal convulsion	1 (2%)	0
Headache	1 (2%)	0
Homonymous hemianopia	1 (2%)	0
Severe TEAEs	2 (3%)	1 (2%)
Dry mouth	1 (2%)	0
Cerebrovascular accident	1 (2%)	0
Erysipelas	0	1 (2%)

Data are number of patients (%). TEAE=treatment-emergent adverse event. *The complete list of TEAEs in the safety analysis set can be found in the appendix. †Patients could have more than one serious TEAE.

Table 3: **Summary of frequent, serious, and severe TEAEs**

haemodynamic and creatinine transporter effects of the drug. The higher incidence of anaemia reported in the ASP8232 group than in the placebo group (6% *vs* 0%) was biased by the higher number of participants in that group who were reported by the investigator to have anaemia in their medical history at baseline (data not shown). Importantly, there was no difference between the groups in change in haemoglobin concentrations from baseline to week 12 (appendix). The imbalance in the occurrence of peripheral oedema between the groups (9% in the ASP8232 group *vs* 2% in the placebo group) was similarly confounded by differences in baseline characteristics, comorbidities, and concomitant medications. There were no other relevant safety signals based on vital signs, 12-lead ECG measurements, or clinical laboratory tests.

This study has several strengths. We ensured that participants had stable albuminuria at baseline by requiring them to have been on the same ACE inhibitor or ARB (and other albuminuria-lowering drugs, such as antihypertensives) for 3 months or more before screening and by performing UACR measurements in triplicate on first morning void samples collected on three consecutive days before each visit during the 5-week run-in period. Moreover, all samples were analysed at a central laboratory, and the sponsor and investigators were masked to the results of these analyses.

This study also has some limitations. First, the short treatment duration does not allow inferences about the long-term effects of ASP8232 on albuminuria and its potential to modify disease. Second, we did not record dietary intake of protein or salt, and so we could not assess whether changes in intake of these during the study affected the efficacy of the drug or led to variation in treatment responses between participants. However, the randomised study design should have mitigated such effects, and the study protocol prohibited any changes to clinician-prescribed diets to minimise their potential confounding effects on albuminuria. Finally, five participants from a single study site were excluded from the primary analysis because of data integrity issues. However, in a sensitivity analysis of the primary endpoint that included these five participants, the reduction in first morning void UACR in the ASP8232 group was greater than that observed in the primary analysis (data not shown), strengthening our confidence in the findings.

In conclusion, VAP-1 inhibition with ASP8232 reduces albuminuria when administered in combination with ACE inhibitor or ARB therapy in patients with type 2 diabetes and chronic kidney disease. In view of the high unmet need for renal protection in patients with diabetic kidney disease and the large variability in response to, and toleration of, existing therapies,[24] an alternative treatment option with a novel mechanism could provide added clinical benefit for patients. Future studies investigating the effect of ASP8232 on endpoints related to eGFR or hard renal outcomes (eg, dialysis initiation) are warranted to verify whether the drug delays progression of diabetic kidney disease.

Contributors

All authors were involved in study design and contributed to writing of the manuscript. RWR, AG-H, and TEL were involved in data collection. DdZ, RWR, HJLH, AG-H, and TEL were involved in data analysis.

Declaration of interests

DdZ is a consultant for and receives honoraria from AbbVie, Astellas, Bayer, Boehringer Ingelheim, Fresenius, Janssen, and Mitsubishi-Tanabe. GB reports grants from Vascular Dynamics, Janssen, and Bayer and is a consultant for Merck and KBP BioSciences. PR is a steering group member for Astellas, AstraZeneca, and Bayer; a member of an expert committee for Astellas; and an advisory board member for Novo Nordisk, AstraZeneca, Boehringer Ingelheim, Bayer, and Bristol-Myers Squibb. He also reports grants from Novo Nordisk and AstraZeneca and

lecture fees from Eli Lilly. All of PR's fees are paid to Steno Diabetes Center, Copenhagen, Denmark. VP is a steering committee member for studies funded by Astellas, AbbVie, GlaxoSmithKline, Boehringer Ingelheim, Pfizer, Retrophin, Tricida, and Janssen and an advisory board member for Amgen, Bristol-Myers Squibb, Eli Lilly, AstraZeneca, Novo Nordisk, Pharmalink, Relypsa, Baxter, Merck, Gilead, Novartis, Durect, and Janssen. He also reports honoraria for speaking at scientific symposia from Bayer, Pfizer, Servier, Boehringer Ingelheim, AstraZeneca, Novo Nordisk, and Sanofi; financial support for clinical trials from Pfizer; grants and personal fees from the National Health and Medical Research Council; and an extramural grant for clinical trials from Baxter. Any honoraria that VP receives are paid to his employer. FFH is a consultant for and has received consulting fees from Astellas, AbbVie, and AstraZeneca. MN reports grants and personal fees from Astellas. KS is on advisory boards for Boehringer Ingelheim, Sanofi, and Janssen. HJLH is a consultant for Astellas, AbbVie, AstraZeneca, Boehringer Ingelheim, Fresenius, Gilead, Janssen, and Merck, and reports research grants from AstraZeneca and Boehringer Ingelheim. He has a policy that all honoraria are paid to University Medical Center, Groningen, the Netherlands. RWR, TEL, and AG-H are employees of Astellas.

Data sharing

Access to anonymised individual participant-level data collected during the trial, in addition to supporting clinical documentation, is planned for trials conducted with approved product indications and formulations, as well as compounds terminated during development. Conditions and exceptions are described under the sponsor-specific details for Astellas on ClinicalStudyDataRequest.com. Study-related supporting documentation is redacted and provided if available, such as the protocol and amendments, statistical analysis plan, and clinical study report. Access to participant-level data is offered to researchers after publication of the primary manuscript (if applicable) and is available as long as Astellas has legal authority to provide the data. Researchers can submit a proposal to conduct a scientifically relevant analysis of the study data to the corresponding author. The research proposal is reviewed by an independent research panel. If the proposal is approved, access to the study data is provided in a secure data sharing environment after receipt of a signed data sharing agreement.

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