Received: 26 September 2018 | Revised: 15 January 2019 | Accepted: 16 January 2019

DOI: 10.1111/dom.13639

WILEY

ORIGINAL ARTICLE

Effects of valsartan, benazepril and their combination in overt nephropathy of type 2 diabetes: A prospective, randomized, controlled trial

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Funding information
This trial was sponsored by The Italian Medicines Agency (AIFA, Rome, Italy). Novartis Italia (Varese, Italy) supplied the study drugs free of charge. Neither the sponsor nor the

Aims: To evaluate whether angiotensin-converting enzyme (ACE) inhibitor and angiotensin II receptor blocker (ARB) combination therapy is more nephroprotective than ACE inhibitor or ARB monotherapy in people with type 2 diabetes and overt nephropathy.

Materials and methods: In this prospective, randomized, open, blind-endpoint phase III trial sponsored by the Italian Drug Agency, 103 consenting patients with type 2 diabetes, aged >40 years, with serum creatinine levels 159 to 309 μmol/L, spot morning urinary albumin–creatinine ratio > 1000 mg/g (or > 500 mg/g in those on ACE inhibitor or ARB therapy at inclusion) were stratified by centre and randomized to 4.5-year treatment with valsartan 320 mg/d (n = 36),

company had any role in study design, data collection, data analysis, data interpretation, or writing the report.

benazepril 20 mg/d (n = 34) or halved doses of both medications (n = 33). The primary endpoint was end-stage renal disease (ESRD). Modified intention-to-treat analyses were performed.

Results: Recruitment took place between June 2007 and February 2013 at 10 centres in Italy and one in Slovenia. A total of 77 participants completed the study and 26 were prematurely withdrawn. During a median (interquartile range) of 41 (18–54) months, 12 participants on benazepril (35.3%) and nine on combination therapy (27.3%) progressed to ESRD, versus five on valsartan (13.9%). Differences between benazepril (hazard ratio [HR] 3.59, 95% confidence interval [CI] 1.25–10.30; $P = 0.018$) or combination therapy (HR 3.28, 95% CI 1.07–10.0; $P = 0.038$) and valsartan were significant, even after adjustment for age, gender and baseline serum creatinine, systolic blood pressure and 24-hour proteinuria (HR 5.16, 95% CI 1.50–17.75, $P = 0.009$ and HR 4.75, 95% CI 1.01–22.39, $P = 0.049$, respectively). Adverse events were distributed similarly among the groups.

Conclusions: In people with type 2 diabetes with nephropathy, valsartan (320 mg/d) safely postponed ESRD more effectively than benazepril (20 mg/d) or than halved doses of both medications.

KEYWORDS

diabetic nephropathy, phase III study, type 2 diabetes

1 | INTRODUCTION

Nephropathy associated with type 2 diabetes is the leading cause of end-stage renal disease (ESRD). Nearly half of new incident cases requiring chronic renal replacement therapy (RRT) are affected by type 2 diabetes.[1–3] Most people with type 2 diabetes and nephropathy die from cardiovascular events before progressing to ESRD.[4] Moreover, treatment of terminal kidney failure and related complications imposes large direct and indirect costs on patients and healthcare providers.[5]

Pioneering studies have found that progressive renal function loss is slowed by blood pressure (BP)-lowering therapy in patients with long-lasting type 1 diabetes and proteinuria.[6] A seminal study found that angiotensin-converting enzyme (ACE) inhibitor limited progression to ESRD more effectively than BP-lowering medications that do not directly interfere with the renin-angiotensin-system (RAS) in patients with type 1 diabetes and overt nephropathy, an effect that was achieved at a similar degree of BP control between treatment groups.[7] In more recent years, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study (RENAAL) and the Irbesartan Diabetic Nephropathy Trial (IDNT) found that RAS inhibition with angiotensin II receptor blockers (ARBs) slowed renal disease progression in patients with type 2 diabetes and macroalbuminuria.[8,9] However, despite ARB therapy, ~25% of people with type 2 diabetes and overt nephropathy progress to ESRD over 4 to 5 years.[8,9] More effective nephroprotection could probably be achieved by combining ARBs with ACE inhibitors to maximize RAS blockade by affecting both the bioavailability and the activity of angiotensin II.[10] To test this hypothesis, the Veterans Affairs Nephropathy in Diabetes (VA NEPHRON-D) trial compared 5-year outcomes in people with type 2 diabetes and proteinuric chronic kidney disease randomly allocated to the combination of losartan with the ACE inhibitor lisinopril or to losartan alone. The study was stopped after a median follow-up of 2.2 years because of safety concerns.[11] The PRONEDI trial found that maximized inhibition of the RAS with 40 mg/d of lisinopril, 600 mg/d of irbesartan or their combination, at halved doses, in people with type 2 diabetes and mild renal dysfunction and proteinuria, was associated with a very high incidence (35%) of hyperkalaemia, without evidence of more effective nephroprotection with dual therapy.[12] Consistent with those results, a 1-year study in 90 patients with type 2 diabetes found more proteinuria reduction with captopril and losartan dual therapy than with the two drugs used alone, but failed to detect a difference in creatinine clearance between the three treatment groups.[13] Conversely, a network meta-analysis of 157 studies comprising 43 256 participants, mostly with type 2 diabetes and chronic kidney disease, found that ESRD was significantly less likely to occur in patients receiving ACE inhibitor and ARB dual therapy or ARB monotherapy than in those treated with placebo. Both regimens, however, were associated with borderline increases in estimated risks of hyperkalaemia and acute kidney injury.[14]

To formally test the risk–benefit profile of dual versus single drug RAS blockade, we designed the VALID study, a randomized controlled trial to evaluate whether, at similar BP control, combined therapy with the ACE inhibitor benazepril and the ARB valsartan reduces progression to ESRD more effectively than benazepril or valsartan alone in high-risk patients with type 2 diabetes and overt nephropathy. To avoid the risks and confounding effects of excess RAS inhibition, we compared the effect of standard (recommended by the manufacturer) anti-hypertensive doses of benazepril (20 mg/d as compared to the 40-mg/d dose of lisinopril used in the VA NEPHRON-D and PRONEDI trials) and valsartan with the effect of halved doses of the two drugs combined.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

In this phase III, multicentre, parallel, Prospective, Randomized, Open-Label Blind Endpoint (PROBE) trial,[15] we enrolled patients referred to the Aldo e Cele Daccò Clinical Research Centre for Rare Diseases

(Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy), and to ten diabetology or nephrology units in Italy and one diabetology unit in Slovenia (see Appendix). The trial included participants aged >40 years with type 2 diabetes mellitus diagnosed according to the World Health Organization criteria, serum creatinine concentration between 159 and 309 μmol/L and spot morning urinary albumin–creatinine ratio (UACR) >1000 mg/g for participants not receiving RAS inhibition therapy, or > 500 mg/g for participants given ACE inhibitor or ARB therapy. Participants had to be followed for a median of 4.5 years. Those who could not start RAS inhibitor therapy for safety/tolerability reasons or those who could not replace dual RAS blockade with ACE inhibitor or ARB monotherapy because of specific cardiovascular indications, were excluded. We also excluded people with: serum potassium levels ≥6 mEq/L despite diuretic therapy, and optimized metabolic and acid/base control; bilateral renal artery stenosis; evidence of immunologically mediated renal disease, systemic disease or cancer; drug or alcohol abuse; or any chronic clinical conditions that could affect completion of the trial or confound data interpretation. People who were unable to provide informed consent, and pregnant, lactating or potentially childbearing women without effective contraception were also excluded (for further details see http://www.villacamozzi@marionegri.it).

The study protocol and its amendments were approved by the Ethics Committee of each site and written informed consent was obtained from all participants in compliance with the Declaration of Helsinki. The study was coordinated and monitored by the Department of Renal Medicine of the Clinical Research Centre according to Good Clinical Practice guidelines. Data were recorded locally on dedicated paper case report forms and centralized into the database at the coordinating centre. Consolidated Statement of Reporting Trials (CONSORT) guidelines were adhered to. The trial is registered with ClinicalTrial.gov: NCT00494715 and EudraCT n. 2006–005951-14.

2.2 | Randomization and masking

Eligible participants were randomly assigned (1:1:1) to treatment with benazepril, valsartan or a combination of both at the centralized treatment assignment secretariat (Appendix) by an independent investigator (G.A.G.) according to a web-based, computer-generated randomization list created using SAS (version 9.2). Blocking was used to ensure balance in the number of patients in each group at any time during the trial. The block size was varied randomly in order to increase the unpredictability of the sequence. Neither participants nor care-providers were masked to group assignment. An adjudicating group, whose members were not aware of the treatment assignments, reviewed the data to determine which patients had reached study endpoints and to evaluate safety.

2.3 | Procedures

Participants were randomly given equivalent doses (half the full dose recommended by the manufacturer for BP control)[16,17] of benazepril (10 mg/d) or valsartan (160 mg/d), or one-quarter of the full doses of both agents in combination. BP, serum creatinine, serum potassium, blood glucose and venous pH were monitored within 7 to 10 days after randomization. In participants without symptomatic hypotension, serum creatinine increase <30% and serum potassium <5.5 mEq/L, treatment was uptitrated to the full dose of benazepril (20 mg/d) or valsartan (320 mg/d), or one-half of the standard dose of both agents in combination. BP, serum creatinine, serum potassium, blood glucose and venous pH were monitored within 7 to 10 days after uptitration of the study treatments. When tolerated well, treatment continued to study end. If an adverse event, possibly related to treatment, was observed, the dose of benazepril, valsartan, or the combination, was back-titrated to the previous step. To achieve and maintain the target BP of <130/80 mm Hg, additional antihypertensive medications were allowed in the following steps: (a) thiazide or loop diuretics, (b) β- and/or α-blockers or clonidine, and (c) dihydropyridine calcium channel blockers or minoxidil. Back-titration of concomitant treatments and, secondarily, of the study drugs were allowed in order to avoid symptomatic hypotension or severe hyperkalaemia (serum potassium ≥6 mEq/L despite diuretic therapy, and optimized metabolic and acid/base control). The general aim was to maintain target BP with the highest dose of the study drugs and the lowest doses of the concomitant medications. Potassium-sparing diuretics, aldosterone antagonists and RAS inhibitors different from the study drugs were not allowed. Antidiabetic and statin therapy were targeted to glycated haemoglobin (HbA1c) 53 mmol/mol and total cholesterol 5.18 mmol/L, respectively. Low-dose aspirin was recommended to all participants who did not have specific contraindications. All participants were maintained on diet recommended by their reference centres and no systematic change in calorie, protein and sodium intake was introduced during the study. Glomerular filtration rate (GFR) measured by iohexol plasma clearance,[18] 24-hour proteinuria, HbA1c, lipids and routine clinical and laboratory variables were assessed at randomization, at 3 and 6 months after randomization, and every 6 months thereafter. The GFR measurements were centralized at the Clinical Research Centre for Rare Diseases "Aldo e Cele Daccò". At each time point, proteinuria was measured in three consecutive 24-hour urine collections, and the median value of the three measurements was recorded for analyses.

2.4 | Outcome measures

The primary endpoint was progression to ESRD, defined as the need for chronic RRT by dialysis or kidney transplantation. The secondary endpoint was doubling of serum creatinine compared to baseline, confirmed in at least two consecutive measurements or followed by progression to ESRD. Other endpoints included fatal and major non-fatal cardiovascular events (sudden cardiac death or cardiac resuscitation, fatal and non-fatal acute myocardial infarction or stroke, unstable angina, coronary or peripheral artery revascularization, first hospitalization for heart failure, or amputation because of critically ischaemic limb), the rate of GFR decline, and changes in 24-hour proteinuria and UACR. Safety variables included serious and non-serious adverse events, and any clinical or laboratory abnormality possibly related to the study drugs. Data on adverse events were collected and coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 19.1.

2.5 | Sample size and statistical analysis

The primary efficacy comparison was between valsartan monotherapy and benazepril and valsartan combination therapy. Based on the results of the RENAAL study,[8] we estimated that 74.7% of the patients in the ARB group would progress to ESRD over the 4.5 years of the study period. Similar figures were expected for patients in the ACE inhibitor group. On the basis of the results of a previous trial, which was subsequently retracted,[19,20] and of preliminary evidence in type 2 diabetes,[21] the incidence of ESRD was predicted to decrease by 50% (from 74.7% to 37.4%) through combined therapy. To give the trial an 80% power to detect as statistically significant ($\alpha = 0.05$, two-tailed test) the expected reduction in ESRD events, and accounting for a 15% drop-out rate, 34 participants per group had to be included for a total of 102 participants. All statistical analyses were performed using SAS (version 9.4) and STATA (version 12) according to the intention-to-treat principle for endpoint analyses and the modified intention-to-treat approach for continuous variables in all participants who had at least one efficacy measurement after randomization, without imputation of missing data. Cumulative event curves were constructed using the Kaplan–Meier procedure and the Cox regression model was used to estimate the hazard ratio (HR) for the renal endpoints with the corresponding 95% confidence interval (CI). The model was adjusted for age, gender and baseline serum creatinine, systolic BP and 24-hour proteinuria. Non-prespecified, explorative analyses were performed, replacing (alternatively in separate models) age with diabetes duration or HbA1c levels and considering only participants who completed treatment uptitration after randomization and considering separately participants who, during the uptitration period, achieved or did not achieve treatment with full doses of the two drugs in monotherapy or combined. Participants who did not reach the outcome of interest were considered as right censored. Proportionality assumptions were assessed using Schoenfeld residuals. Further exploratory models for the primary endpoint included follow-up systolic or diastolic BP, HbA1c and 24-hour proteinuria. GFR decline was assessed with a linear regression analysis and compared with the Wilcoxon rank-sum test. For renal endpoints, competing risk models were carried out exploratively to account for risk of death. For binary outcomes the number of participants needed to treat was calculated using SAS Proc Freq.

One interim analysis was performed by the independent Data Safety Monitoring Board after the completion of 3-year follow-up. A further evaluation was carried out at the end of the enrolment period to assess whether the study could continue as planned, or whether the sample size and/or follow-up duration had to be adapted to achieve adequate statistical power. Data were expressed as mean (SD), median (interquartile range [IQR]), or number (%) unless otherwise stated. *P* values <0.05 (two-sided) were taken to indicate statistical significance.

3 | RESULTS

Of the 158 patients assessed for eligibility, 38 did not fulfil the eligibility criteria, nine withdrew consent, seven were lost to follow-up and one died (Supporting Information Figure S1); thus, 103 patients were enrolled between June 2007 and February 2013. Baseline demographic characteristics and the distribution of RAS inhibitors and other medications were balanced between groups (Table 1). Overall, 28 participants were treated with an ARB, 29 with an ACE inhibitor and 38 with an ACE inhibitor and an ARB combined. Eight participants were not receiving RAS inhibitors at inclusion.

At randomization all participants stopped previous treatment (if any) with RAS inhibitors. Then, 36 participants were randomly assigned to valsartan (160 mg/d), 33 to valsartan (80 mg/d) plus benazepril (5 mg/d) and 34 to benazepril (10 mg/d). After 1 week the treatment doses were uptitrated to full maintenance doses in 65 participants; thus, 28, 18 and 19 participants received valsartan 320 mg/d; valsartan 160 mg/d plus benazepril 10 mg/d; or benazepril 20 mg/d, respectively. The remaining 38 participants continued with previous doses of valsartan (n = 8), combination therapy (n = 15) or benazepril (n = 15) as a result of hyperkalaemia, worsening kidney function or for other reasons. Overall 26 patients withdrew from the study because of consent withdrawal (n = 13), loss to follow-up (n = 7), serious adverse events (n = 1, lung cancer) or other reasons (n = 5). They were followed for a median (IQR) of 18 (8–36) months after randomization. Only four were withdrawn within 6 months of randomization, while 22 (85%) completed at least 6 months and 19 (73%) at least 12 months of follow-up.

3.1 | Renal endpoints

3.1.1 | Main renal endpoints

During a median (IQR) follow-up of 41 (18–54) months, nine participants (27.3%) on dual therapy, 12 (35.3%) on benazepril and five (13.9%) on valsartan progressed to ESRD. The hazard for ESRD was more than threefold higher with dual therapy ($P = 0.038$) or benazepril ($P = 0.018$) than with valsartan, while it was similar in the dual therapy and the benazepril groups (Figure 1). Progression to ESRD was significantly different between dual therapy ($P = 0.049$) or benazepril ($P = 0.009$) and valsartan even after adjusting for age, gender and baseline systolic BP, serum creatinine and proteinuria, whereas it was similar between dual therapy and benazepril even after these adjustments (Figure 1). Overall, five participants had to be treated with valsartan to save one ESRD event, compared with either dual therapy or benazepril. Similar findings were obtained for the composite endpoint of ESRD or doubling of serum creatinine (Figure 3A).

3.1.2 | Exploratory analyses for the renal endpoints

The incidence of ESRD was significantly higher in regimens that included benazepril alone or combined with valsartan, compared with valsartan monotherapy ($P = 0.014$), even after adjusting for baseline covariates (Figure 2A; $P = 0.020$). Consistent with this finding, numerically more events were reported in the study treatment that did not include valsartan than in valsartan-based regimens ($P = 0.076$), and the difference attained nominal significance ($P = 0.012$) after adjusting for the aforementioned baseline characteristics (Figure 2B). Similar findings were obtained for the composite endpoint of ESRD or doubling of serum creatinine (Figure 3B,C). Similar findings were also observed with non-prespecified exploratory analyses restricted to the

TABLE 1 Participant characteristics at baseline according to treatment group

	Benazepril (n = 34)	Valsartan (n = 36)	Combination (n = 33)
Demographic characteristics			
Age, years	66.3 ± 7.1	63.9 ± 9.2	63.1 ± 9.0
Men/women, n	30/4	31/5	27/6
Smoker: never	12 (35.3)	18 (50.0)	12 (36.4)
Smoker: former	13 (38.2)	13 (36.1)	8 (24.2)
Smoker: current	9 (26.5)	5 (13.9)	13 (39.4)
Clinical history			
Coronary artery disease	11 (32.4)	6 (16.7)	13 (39.4)
Peripheral artery disease	6 (17.6)	7 (19.4)	7 (21.2)
Stroke or transient ischaemic attack	4 (11.8)	2 (5.6)	3 (9.1)
Diabetic retinopathy	14 (41.2)	13 (33.3)	16 (48.5)
Known duration of diabetes, years	19.5	17.7	18.1
Clinical features			
BMI, kg/m^2	31.7 ± 5.4	32.6 ± 6.5	30.8 ± 6.3
Systolic BP, mm Hg	143.8 ± 16.6	149.2 ± 21.2	149.6 ± 22.2
Diastolic BP, mm Hg	79.2 ± 10.8	80.4 ± 12.2	79.1 ± 11.3
MAP, mm Hg	100.7 ± 9.9	103.3 ± 12.6	102.6 ± 12.5
Laboratory variables			
HbA1c, mmol/mol	72.1 ± 12.1	71.9 ± 16.0	70.5 ± 17.8
Serum glucose, mg/dL	160.9 ± 60.2	167.6 ± 80.9	169.5 ± 73.3
Serum potassium, mg/dL	4.36 ± 0.54	4.52 ± 0.80	4.57 ± 0.64
Haemoglobin, g/dL	13.3 ± 2.3	13.4 ± 1.8	13.1 ± 2.0
Total cholesterol, mmol/L	4.72 ± 1.14	4.76 ± 1.13	4.63 ± 1.18
HDL cholesterol, mmol/L	1.14 ± 0.25	1.07 ± 0.32	1.08 ± 0.36
LDL cholesterol, mmol/L	2.53 ± 0.71	2.72 ± 0.98	2.36 ± 0.98
Triglycerides, mmol/L	2.51 ± 1.71	2.37 ± 1.15	2.75 ± 1.99
Kidney function variables			
Serum creatinine, μmol/L	203.3 ± 70.7	185.6 ± 53.0	212.2 ± 70.7
Measured GFR[a], mL/min/1.73 m^2	39.9 (29.7–47.5)	42.0 (34.4–69.1)	39.7 (31.9–49.0)
24-hour proteinuria, g	3.01 (2.13–5.25)	2.98 (1.90–4.45)	4.17 (2.29–6.20)
Participants with medications, n (%)			
ACE inhibitors and/or ARB			
ACE inhibitor alone	12 (35.3)	8 (22.2)	9 (27.3)
ARB alone	5 (14.7)	13 (36.1)	10 (30.3)
ACE inhibitors and ARBs in combination	15 (44.1)	13 (36.1)	10 (30.3)
Neither ACE inhibitor nor ARB	2 (5.9)	2 (5.6)	4 (12.1)
Other antihypertensive agents			
Diuretics	28 (82.4)	24 (66.7)	28 (84.8)
Calcium-channel blockers	24 (70.6)	20 (55.6)	22 (66.7)
β-blockers	17 (50.0)	14 (38.9)	20 (60.6)
α-blockers	9 (26.5)	10 (27.8)	14 (42.4)
Others	4 (11.8)	2 (5.6)	5 (15.2)
Lipid-lowering agents			
Any	29 (85.3)	24 (66.6)	27 (81.8)
Statins	27 (79.4)	22 (61.1)	23 (69.7)
Omega-3 fatty acid	3 (8.8)	3 (8.3)	4 (12.1)
Fibrates	0 (0)	0 (0)	1 (3.0)
Ezetimibe	0 (0)	0 (0)	2 (6.1)
Ezetimibe and statins in combination	2 (5.9)	1 (2.8)	3 (9.1)

TABLE 1 (Continued)

	Benazepril (n = 34)	Valsartan (n = 36)	Combination (n = 33)
Hypoglycaemic agents			
Any	33 (97.1)	35 (97.2)	33 (100.0)
Oral hypoglycaemic agents alone	7 (20.6)	13 (36.1)	13 (39.4)
Insulin and/or other hypoglycaemic agents	26 (76.5)	22 (61.1)	20 (60.6)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index. GFR, glomerular filtration rate; HbA1c, glycated haemoglobin; IQR, interquartile range; MAP, mean arterial pressure.
Data are mean ± SD, median (IQR) unless otherwise indicated.
[a] Measured by iohexol plasma clearance.

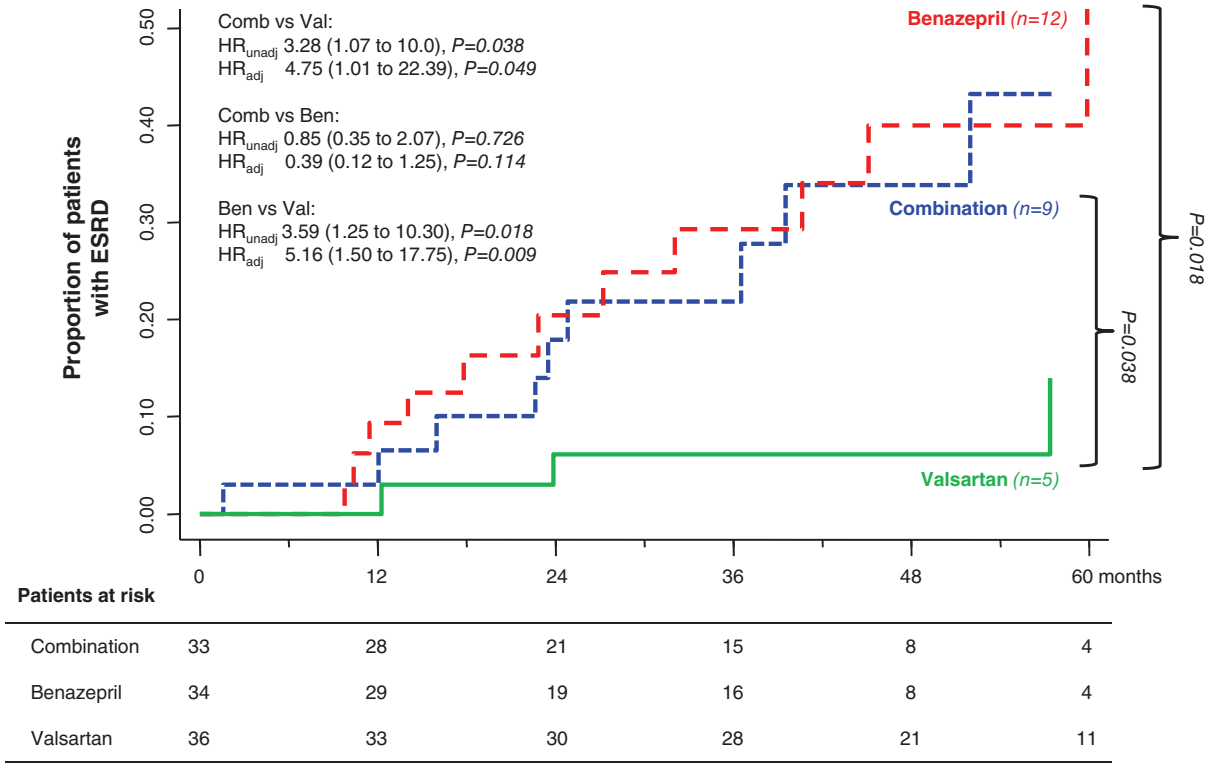

FIGURE 1 Kaplan–Meier curve for the primary endpoint of progression to end-stage renal disease (ESRD). Kaplan–Meier curve shows the proportion of patients who reached the primary endpoint of ESRD in the combination therapy, valsartan and benazepril groups during a median follow-up of 4.5 years. Hazard ratios (HRs) and 95% confidence intervals are crude (unadjusted) and adjusted for age, gender, baseline systolic blood pressure, serum creatinine and proteinuria. Adj, adjusted; ben, benazepril; comb, combination; Unadj, unadjusted; Val, valsartan

65 participants completing treatment uptitration (Supporting Information Figures S2 and S3) or in separate adjusted multivariable models where age was replaced with either diabetes duration or baseline HbA1c levels (Supporting Information Table S1). A trend towards fewer ESRD events with valsartan than with benazepril or combination therapy was observed also in the subgroup of participants who did not achieve treatment at full doses. In this subgroup, no participant on valsartan progressed to ESRD. The number of participants and events, however, was too low to draw conclusions from this.

The difference in progression to ESRD between the benazepril and valsartan groups was significant (although to a lower level of significance) even after adjustment for follow-up systolic BP (HR 4.50 [95% CI 1.31–15.4]; P = 0.017), whereas the difference between dual therapy and valsartan (HR 2.74 [95% CI 0.53–14.20]; P = 0.230) was not significant after this adjustment. Both differences were not appreciably affected by adjustments for follow-up diastolic BP, HbA1c or proteinuria (data not shown).

3.2 | Other outcomes

Eleven participants (33.3%) on dual therapy, 10 (27.8%) on valsartan and seven (20.6%) on benazepril experienced fatal or non-fatal major cardiovascular events, with no significant differences among the groups (Supporting Information Figure S4A, Table 2). Results were similar when the analyses were adjusted for baseline covariates. The event rate was similar according to treatment with or without benazepril or with or without valsartan, even after adjusting for baseline features (Supporting Information Figure S4B,C). Every month, measured GFR declined by a median (IQR) of 0.61 (0.21–0.82), 0.48 (0.27–0.72), and 0.39 (0.19–0.70) mL/min/1.73m^2 with dual therapy, benazepril or valsartan, respectively. Despite the trend towards slower GFR loss on valsartan, the rate of GFR decline was not significantly different among the three groups.

On follow-up, median (IQR) 24-hour proteinuria tended to be lower with valsartan (2.4 [1.7–3.6] g/24 h) than with combination therapy (3.5 [2.1–5.4] g/24 h) or benazepril (3.9 [1.9–6.9] g/24 h),

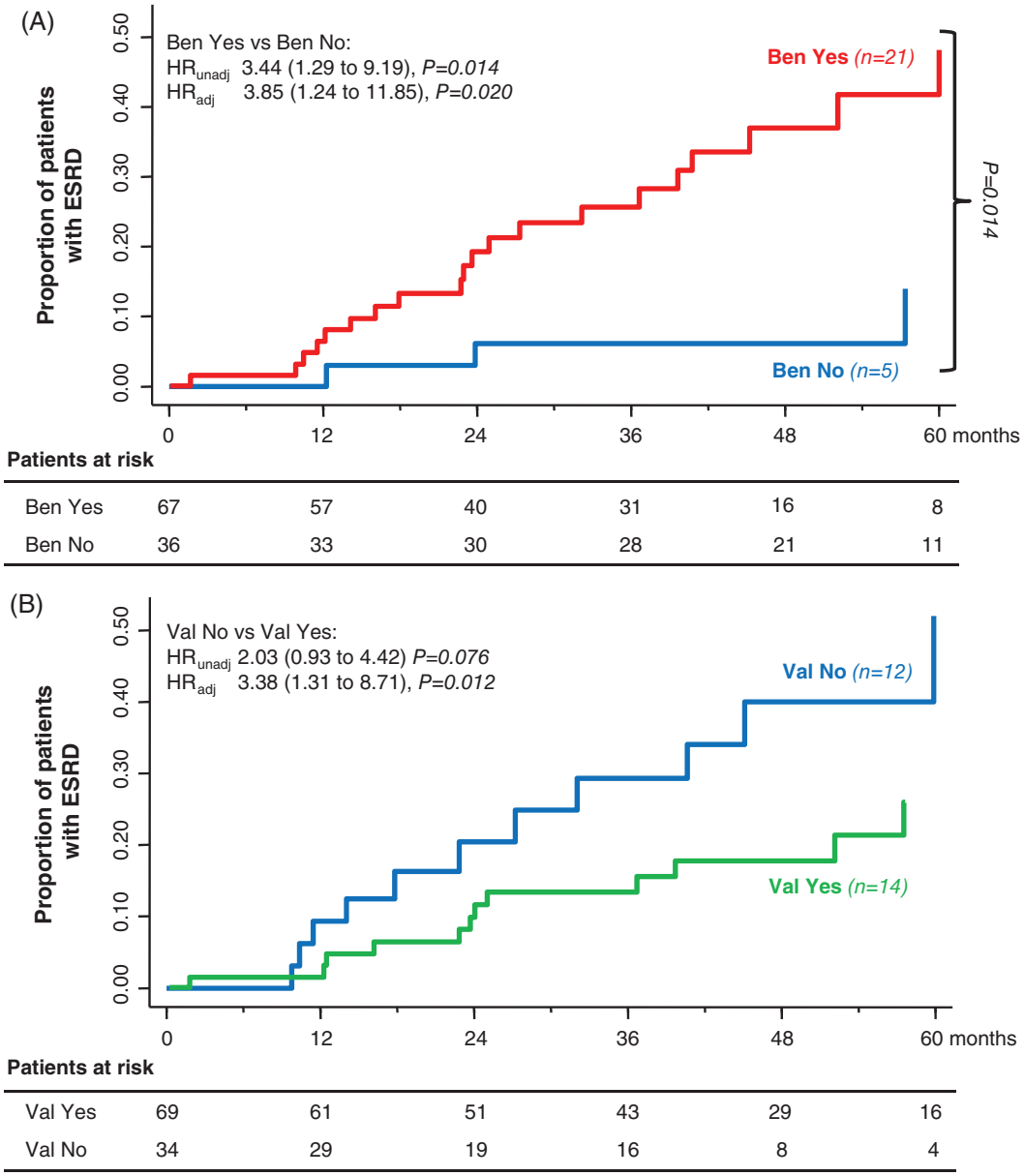

FIGURE 2 Kaplan–Meier curves for the primary endpoint of progression to end-stage renal disease (ESRD) according to treatment with or without angiotensin-converting enzyme (ACE) inhibitor, and with or without angiotensin receptor blocker (ARB). Kaplan–Meier curves show the proportion of participants who reached the primary endpoint of ESRD according to treatment with or without benazepril A, and the proportion of participants who reached the primary endpoint of ESRD according to treatment with or without valsartan B, during a median follow-up of 4.5 years. Hazard ratios (HRs) and 95% confidence intervals are crude (unadjusted) and adjusted for age, gender, baseline systolic blood pressure, serum creatinine and proteinuria. Adj, adjusted; ben, benazepril; Unadj, unadjusted; Val, valsartan

but between-group differences failed to achieve the nominal significance. Compared with baseline, 24-hour UACR fell by a median of 44.8% ($P < 0.01$) with valsartan, 11.2% with combination therapy (P = nonsignificant) and 10.2% with benazepril (P = nonsignificant). Despite the trend towards a greater UACR decline with valsartan, differences among groups did not attain statistical significance.

3.3 | BP and glycaemic control

Compared with baseline, mean systolic BP decreased in the valsartan group at 3-month follow-up, so that at this time point it was significantly lower than that observed in the dual therapy and in the benazepril groups ($P < 0.01$ for both comparisons). Systolic BP remained significantly lower with valsartan compared with dual therapy up to 18-month follow-up, while it was similar between the groups afterwards. The mean diastolic BP did not change over the follow-up and never differed among the three groups (Supporting Information Figure S5). Similarly, HbA1c never differed among groups.

3.4 | Safety

Nineteen participants (58%) on dual therapy, 15 (42%) on valsartan and 18 (53%) on benazepril had at least one serious adverse event. Overall, the distribution of serious (Table 2) and non-serious

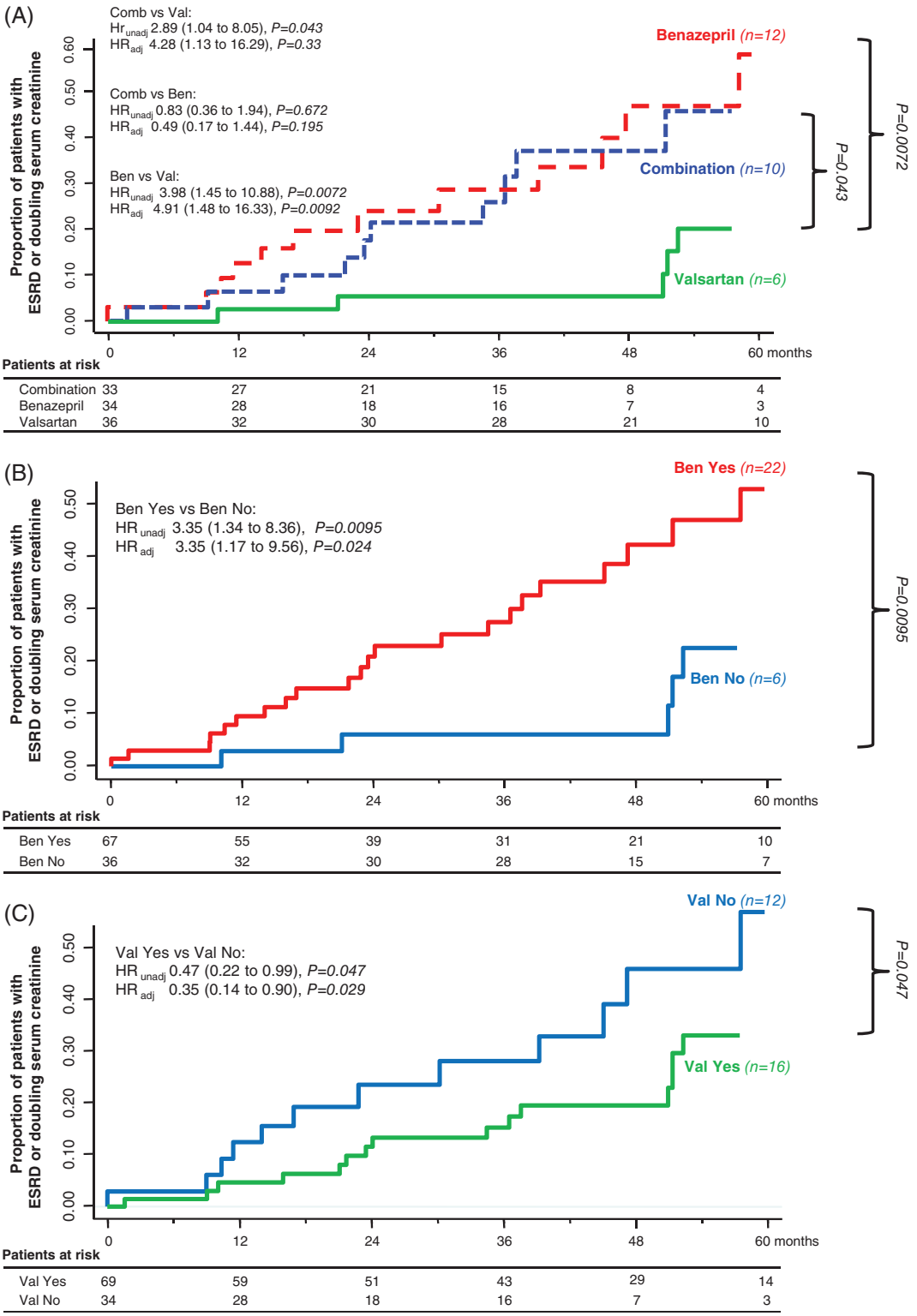

FIGURE 3 Kaplan–Meier curves for the composite endpoint of doubling of serum creatinine or dialysis. Kaplan–Meier curves show the proportion of participants who reached the composite endpoint of doubling of serum creatinine or dialysis in the combination therapy, valsartan and benazepril groups A, the proportion of participants who reached the composite endpoint of doubling of serum creatinine or dialysis according to treatment with or without benazepril B, and the proportion of participants who reached the composite endpoint of doubling of serum creatinine or dialysis according to treatment with or without valsartan C, during a median follow-up of 4.5 years. Hazard ratios (HRs) and 95% confidence intervals are crude (unadjusted) and adjusted for age, gender, baseline systolic blood pressure, serum creatinine and proteinuria. Adj, adjusted; ben, benazepril; comb, combination. ESRD, end-stage renal disease; Unadj, unadjusted; Val, valsartan

(Supporting Information Table S2) adverse events was similar in the three treatment groups. Treatment-related adverse events were not serious and all were transient and the participant fully recovered. There were eight cases of hyperkalaemia: three with valsartan and five with dual therapy (Supporting Information Table S2). Study treatment had to be withdrawn because of hyperkalaemia in one

TABLE 2 Number of patients (%) with at least one serious adverse event (SAE) and total number of SAEs according to treatment group

Patients with SAEs, n (%) and total SAEs, n	Benazepril (n = 34)	Valsartan (n = 36)	Combination (n = 33)
Overall	18 (53) 36	15 (42) 37	19 (58) 35
Fatal	5 (15) 5	4 (11) 4	1 (3) 1
Major cardiovascular events[a]	3 (9) 3	2 (6) 2	1 (3) 1
Myocardial infarction	1 (3) 1	0	1 (3) 1
Stroke	1 (3) 1	0	0
Sudden cardiac death	0	1 (3) 1	0
Heart failure	1 (3) 1	1 (3) 1	0
Sepsis	2 (6) 2	0	0
Cerebral haemorrhage (trauma)	0	1 (3) 1	0
Stomach cancer	0	1 (3) 1	0
Non-fatal	17 (50) 31	15 (42) 33	18 (55) 34
Cardiovascular	8 (24) 12	9 (25) 13	12 (36) 16
Major cardiovascular events[a]	5 (15) 5	8 (22) 11	10 (30) 14
Myocardial infarction	1 (3) 1	2 (6) 3	4 (12) 4
Stroke	1 (3) 1	1 (3) 1	2 (6) 2
Unstable angina/revascularization	2 (6) 2	1 (3) 2	1 (3) 1
Coronary revascularization	0	3 (8) 3	3 (9) 3
Peripheral revascularization	0	1 (3) 1	3 (9) 3
Unstable angina	0	1 (3) 1	0
Hospitalization for heart failure	1 (3) 1	0	1 (3) 1
Minor cardiovascular events[b]	0	1 (3) 1	0
Transitory ischaemic attack	0	1 (3) 1	0
Other cardiovascular events	5 (15) 7	1 (3) 1	2 (6) 2
Renal	6 (18) 6	6 (17) 7	6 (18) 6
Transient kidney function worsening	5 (15) 5	5 (14) 6	6 (18) 6
Hydronephrosis	0	1 (3) 1	0
C-Anca vasculitis	1 (3) 1	0	0
Cancer	1 (3) 1	3 (8) 3	2 (6) 2
Multiple Myeloma	1 (3) 1	0	0
Melanoma	0	1 (3) 1	0
Prostate carcinoma	0	1 (3) 1	1 (3) 1
Hepatocarcinoma	0	1 (3) 1	0
Lung cancer	0	0	1 (3) 1
Gastrointestinal	1 (3) 1	1 (3) 1	1 (3) 1
Haemorrhagic gastroenteritis/duodenitis	1 (3) 1	1 (3) 1	0
Haemorrhagic duodenal ulcer	0	0	1 (3) 1
Respiratory	5 (15) 5	2 (6) 2	2 (6) 2
Pneumonia	2 (6) 2	1 (3) 1	2 (6) 2
Acute bronchitis	2 (6) 2	1 (3) 1	0
COPD reacutization	1 (3) 1	0	0
Other infections	1 (3) 1	2 (6) 3	3 (9) 4
Other serious adverse events	4 (12) 5	4 (11) 4	1 (3) 2

Abbreviation: COPD, chronic obstructive pulmonary disease.
No statistically significant difference observed across treatments.
[a] Major CV events: acute myocardial infarction, stroke, cardiac death, unstable angina, first hospitalization for heart failure, coronary and peripheral artery revascularizations and amputations.
[b] Minor CV events: transient ischaemic attack, stable angina pectoris and coronary and peripheral artery disease without revascularization procedures.

participant on benazepril, worsening of kidney function in one participant on valsartan and hypotension/lipothymia in one on combination therapy. Treatment dose was back-titrated in three participants on benazepril because of hyperkalaemia, cough or worsening of kidney function and in five participants on valsartan because of hypotension (in three cases), hyperkalaemia or worsening of kidney function. No patient on combination therapy required dose back-titration because of treatment-related side effects.

Two participants died from stomach cancer or traumatic intracerebral haemorrhage in the valsartan group and two owing to severe

sepsis in the benazepril group. These events were deemed not related to the study treatments.

4 | DISCUSSION

We found that 4.5 years of treatment with standard (manufacturer-recommended) anti-hypertensive doses of valsartan reduced the incidence of ESRD, the primary efficacy variable of the trial, more effectively than standard doses of benazepril or halved doses of both drugs in 103 participants with type 2 diabetes at high risk of fast renal disease progression because of renal insufficiency and overt proteinuria at inclusion, despite concomitant RAS inhibitor therapy. Similar findings were observed when doubling of serum creatinine or progression to ESRD were considered as a combined endpoint. The above findings are unlikely to be explained by an unbalanced distribution of risk factors for a more severe outcome because demographic, clinical and laboratory variables at inclusion were similar in the three treatment groups. Moreover, no systematic changes in diet and concomitant medications that might affect patient outcomes were introduced throughout the study. Finally, the protective effect of valsartan against progression to ESRD considered as a single endpoint or in combination with doubling of serum creatinine was confirmed after adjusting the analyses for potential confounders, such as age, gender and baseline systolic BP, serum creatinine and proteinuria. The robustness of the findings was further confirmed by comparative analyses showing that the incidence of ESRD was significantly lower in the two groups of participants who were treated with valsartan, either alone or in combination, than in the group that was never exposed to valsartan during the study. In addition, ESRD events were significantly more frequent in participants who received benazepril, alone or in combination, compared with those who never received this drug.

Notably, a larger proportion of participants completed the uptitration in the valsartan group than in the combination or benazepril groups. Hyperkalaemia or worsening kidney function were the most frequent obstacles to completion of treatment uptitration; however, the fact that similar treatment effects on the primary outcome ESRD were observed with the non-predefined explorative analyses considering patients who completed treatment uptitration after randomization separately from those who did not achieve this target, confirmed that the superior nephroprotective effect of valsartan was explained by a specific treatment effect, rather than by different participant exposure to different study treatments. Taken together, these findings indicate that, in people with type 2 diabetes and renal insufficiency, valsartan is better tolerated and more effective than benazepril and combination therapy. The robustness of the findings were confirmed by analyses showing that valsartan retained its superior protective effect against progression to ESRD, even when diabetes duration or, alternatively, HbA1c levels at baseline were included in the multivariable models instead of participant age. Proteinuria (defined as spot-morning and, secondarily, as 24-hour UACR) and the rate of GFR loss tended to be lower on valsartan than with dual therapy or benazepril, but between-group differences in both outcomes failed to achieve the nominal significance, possibly because of insufficient statistical power. Similar considerations may apply to the incidence of fatal and major non-fatal cardiovascular events, which was similar in the three treatment groups. Interestingly, however, in the RENAAL and IDNT trials too, which had a remarkably larger sample size compared to VALID, losartan and irbesartan had no significant effect on the composite endpoint of morbidity and mortality from cardiovascular causes.[8,9] Thus, in participants with type 2 diabetes and overt nephropathy, effective cardioprotection with ACE inhibitor or ARBs is harder to achieve than in earlier stages of renal disease, probably because vascular changes may be too advanced and at least partially irreversible; thus, early intervention appears to be the best strategy to prevent cardiovascular events in people with diabetes.

To assess whether the superior nephroprotective effect of valsartan could be explained by better systolic BP control, in particular, during the first part of the trial, we performed exploratory multivariable analyses, adjusting the HR for ESRD for systolic BP levels. Finding that the difference between valsartan and benazepril was significant, although to a lower level of significance, even after the adjustment, can be taken to suggest that at least part of the superior protective effect of valsartan against progression to ESRD might be explained by a drug-specific effect beyond better BP control. Consistent with this, diastolic BP and HbA1c were similar in the three treatment groups during the study and did not change appreciably at follow-up compared to baseline.

We compared the effects of valsartan and benazepril at standard doses that were recommended by the manufacturer. Whether higher doses of benazepril, in monotherapy or combined with valsartan, could have been more effective is not known; however, data from the VA NEPHRON-D trial suggest that further benazepril uptitration would have been unsafe.[11] Benazeprilat, the active moiety of benazepril, is primarily cleared by the kidney[22] and may accumulate in patients, such as those included in the present study, with a GFR close to, or lower than, 30 mL/min/1.73m^2. Consistently, the VA NEPHRON-D trial,[11] which compared the effects of forced lisinopril dose uptitration from 10 to 40 mg/d versus placebo on top of the same background treatment with 100 mg/d of losartan in people with type 2 diabetes and macroalbuminuria, was closed prematurely because of a significant excess of adverse events, including acute kidney injury and hyperkalaemia, in participants on dual therapy. This finding was to some extent expected, since lisinopril, like benazepril and most ACE inhibitors, has a predominant renal clearance and in patients with reduced GFR who are receiving doses that are forcedly uptitrated, it may accumulate.[23] The risk of treatment-related side effects was further enhanced by combination therapy with an ARB at the full dose. The excess RAS inhibition could also explain the worrying findings of the PRONEDI trial, which compared the effects of 40 mg/d lisinopril with those of 600 mg/d irbesartan and of dual therapy with the two drugs at halved doses in people with type 2 diabetes and mild renal disease.[12] Despite much less severe renal insufficiency and proteinuria to start with, the yearly incidence of ESRD in the participants of the PRONEDI trial (8%) was higher than in those of the VALID study (6%) and did not differ between treatment groups. Moreover, the incidence of hyperkalaemia was more than fourfold higher than in the present study (35% vs. 8%) and required treatment with cation exchange resins in 18% of study participants. Notably, in the PRONEDI study, participants were exposed to the same dose of

lisinopril as that used in the VA NEPHRON-D trial and the dose of irbesartan was twofold higher compared to the dose used in the IDNT trial.[8,9] Both lisinopril and irbesartan were administered at doses that were twofold higher than recommended by the manufacturers.[24,25]

With regard to safety, in the present study, no wash-out period from previous RAS inhibitor therapy was planned before randomization because even transient withdrawal of ACE inhibitor or ARBs is unsafe in this high-risk population. Valsartan and benazepril dual therapy was tolerated well and the incidence of serious adverse events, including acute renal failure, was similar in the three treatment groups. Moreover, treatment-related adverse events were rare, non-severe and transient. Study treatment had to be withdrawn in one participant on benazepril, one on valsartan and one on combination therapy owing to hyperkalaemia, worsening of kidney function or hypotension/lipothymia, respectively. These reassuring findings are most likely explained by the fact that we used lower doses of ACE inhibitor and ARB than previous studies.[11,12] These doses were halved when valsartan and benazepril were used in combination. Moreover, at inclusion, all participants received halved doses that were uptitrated to full maintenance doses 1 week later only if their initial treatment was tolerated well. These precautions prevented the excess risk of symptomatic hypotension, acute renal function deterioration or hyperkalaemia that would have been associated with RAS inhibition that was too forced, with higher than recommended doses of the two drugs and their use in combination.[11,12]

The results of the present study should be interpreted with caution since, because of resource restriction, it was impossible to design a double-blind trial and the sample size was relatively small; however, the PROBE design allowed us to perform blinded analyses, in spite of open patient allocation, to different treatments. The sample size was estimated based on data from a paper indicating that ACE inhibitor and ARB dual therapy reduced the incidence of ESRD by ~50% compared with either type of agent alone in people with proteinuric nondiabetic nephropathy.[19] That paper was then retracted in 2009[20]; however, subsequent data from the VA NEPHRON-D trial,[11] showing a (borderline-significant [$P = 0.07$]) 34% ESRD incidence reduction with lisinopril and losartan dual therapy compared to losartan monotherapy in people with type 2 diabetes with overt nephropathy at the time of premature study closure (over only 2.2 years rather than the originally planned 5-year follow-up), confirmed that the hypothesis of a 50% treatment effect on the primary outcome over 5-year follow-up was realistic.

Intentional inclusion of patients at high risk of events who were expected to benefit the most from study treatments was the main strength of the present study.[26] Those with mild renal insufficiency, expected to have a low incidence of events, and those with severe renal insufficiency, expected to have no benefit from study treatments because of too advanced and irreversible renal structural changes, were excluded. This approach increased the power of the statistical analyses, which allowed us to assess the effects of valsartan, benazepril and their combination in the framework of a clinical trial with affordable sample size and follow-up duration. This is a crucial issue for fully academic, independent trials with resource restrictions and limited access to the study drugs. The inclusion of participants with two different levels of proteinuria according to concomitant treatment with or without RAS inhibitors was based on consistent evidence that RAS inhibitors reduce proteinuria by ~50% as compared to other medications that do not directly block the RAS.[27] This was aimed to select patients with similar disease severity and homogeneus risk of renal disease progression. Moreover, the use of the median of three proteinuria measurements in three consecutive 24-hour urine collections reduced the confounding effect of erroneous urine collections and random data fluctuations at each evaluation. Another strength was the centralized, direct measurement of GFR using a "gold standard" technique.[18] The decision to initiate chronic RRT was made based on standard clinical criteria by physicians who were blinded to both treatment assignment and GFR measurements,[15] which enhanced the robustness of the results and their generalizability to everyday clinical practice. The use of an online statistical program for the central generation of the randomization schedule by a researcher not otherwise involved in the trial ensured that investigators and participants could not predict treatment allocation and the PROBE design guaranteed that all data assessors were blinded to treatment allocation.

Because of resource restriction we did not measure 24-hour ambulatory BP profile, therefore, we had no information on nocturnal BP control, which is a major predictor of cardiovascular events in this population; however, evidence that both valsartan[28] and benazepril[29] provide similarly effective 24-hour BP control in hypertensive patients with type 2 diabetes, can be taken to suggest that, in all treatment groups, BP reduction was sustained and similar over the entire daily dosing interval. However, this was a renal study primarily aimed to test treatment effect on renal outcome. A few baseline characteristics slightly differed among groups, but randomization in a clinical trial does not guarantee that participants allocated to the different treatment groups will be similar with respect to all characteristics evaluated at baseline, with potential differences among groups being attributable to chance.[30,31] Thus, the slight differences, which were never significant, most likely reflected random fluctuations that were very unlikely to have had any appreciable effect on study outcomes. Finally, despite the highly labour-intensive design and the fragility of the study population, enrolled participants showed good adherence to the study interventions. Notably, active follow-up exceeded 6 months in 22 of the 26 participants who were prematurely withdrawn from the study. The long-duration of the active observation period in this subgroup, who represented the large majority of participants who were prematurely withdrawn from the study, was very unlikely to have affected the possibility to capture cases at higher risk of progression to ESRD (or other study outcomes) and therefore did not appreciably reduce the power of the analyses to detect a treatment effect in the study population considered as a whole.

In conclusion, treatment with 320 mg/d of the ARB valsartan delayed the onset of ESRD in high-risk patients with type 2 diabetes and overt nephropathy compared to treatment with 20 mg/d of benazepril or halved doses of both medications, and was safe. Thus, consistent with the results of previous studies,[11,12] the present data confirm that dual RAS blockade is not indicated in every day clinical practice, even when reduced doses of ACE inhibitors or ARBs are used. These findings may have implications as slowing or even preventing the progression of diabetic renal disease to ESRD is expected to translate into

improved patient quality and expectancy of life, and reduced direct and indirect costs for RRT and related complications.

ACKNOWLEDGMENTS

This trial was sponsored by the Italian Medicines Agency (AIFA, Rome, Italy). Novartis Italia (Varese, Italy) supplied the study drugs free of charge. Neither the sponsor nor the company had any role in study design, data collection, data analysis, data interpretation, or writing the report. We thank the patients in the VALID study for their participation and contribution, the trial investigators, the diabetologists, nephrologists and nurses of the participating centres for their invaluable assistance, the laboratory and regulatory affairs staff, trial monitors, data managers and statisticians, and everyone at the Clinical Research Centre for Rare Diseases (*Aldo e Cele Daccò* at the *Istituto di Ricerche Farmacologiche Mario Negri* IRCCS) for their efforts in making this study possible. We thank Norberto Perico for supervision of the study and critical revision of the final manuscript, and Giuseppe Maiocchi and Federico Bertocchi (Novartis Farma, Origgio, Varese, Italy) for continuous support for the study and their major contribution to all the administrative and operational aspects concerning the supply and distribution of the study drugs.

CONFLICT OF INTEREST

A.J. has received consulting fees from MSD, Novo Nordisk, Sanofi, AstraZeneca and lecture fees from MSD, Novo Nordisk, Sanofi and AstraZeneca. A.C.B. has received consulting fees from Lilly, Novo Nordisk and Artsana, lecture fees from Boehringer Ingelheim, travel support from AstraZeneca, current grant support from Bayer and Lilly. F.A. has received consulting fees from Amgen. A.B. has received consulting fees from Bruno Farmaceutici and Novo Nordisk. G.R. has received consulting fees from Alnylam, Boehringer Ingelheim and Handoc Ink. The remaining authors have no competing interests to declare.

Author contributions

G.R. and P.R. had the original idea and wrote the study protocol; M.T., D.P.B., A.Pi., A.Pa., I.P.I., B.R., S.R., M.C.A., A.J., N.G., E.R., A.C.B., R.T., P.M., G.B., S.D., F.A., A.B. and A.S. identified, treated and monitored the study participants and contributed to data recording; F.G., F.C. and N.S. performed laboratory analyses and centralized assays of the plasma clearance of iohexol. P.R., M.T., M.C. and G.R. contributed to data analyses and interpretation, A.P. and F.P. performed the statistical analyses, D.M. prepared the database and contributed to data handling, O.D. monitored the study, M.C. and P.R. wrote the first draft and with G.R. the final version of the manuscript. All the authors had direct access to data, and critically revised and approved the final manuscript. No medical writer was involved in the creation of the manuscript. The corresponding author had full access to the data in the study and final responsibility for the decision to submit for publication.

DATA-SHARING

On request the authors will provide access to individual participant data, which will be made available to study participants, further patients not enrolled in the present study, patients' associations and to researchers whose proposals meet methodologically sound research criteria to be discussed and shared with the study authors. Data may be requested up to 24 months after study publication. Requests for access to the study data can be submitted via email to Dr Annalisa Perna (annalisa.perna@marionegri.it), head of the Laboratory of Biostatistics of the Department of Renal Medicine of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Ruggenenti P, Trillini M, P. Barlovic D, et al. Effects of valsartan, benazepril and their combination in overt nephropathy of type 2 diabetes: A prospective, randomized, controlled trial. *Diabetes Obes Metab*. 2019;21:1177–1190. https://doi.org/10.1111/dom.13639

APPENDIX

VALID STUDY ORGANIZATION (NUMBER OF INCLUDED PATIENTS IN BRACKETS)

Coordinating Centre: Mario Negri Institute for Pharmacological Research IRCCS, Clinical Research Centre for Rare Diseases Aldo e Cele Daccò, Villa Camozzi, Ranica (Bergamo); Chief Investigator: Giuseppe Remuzzi (Bergamo); Study coordinator: Piero Luigi Ruggenenti (Bergamo).

Centres including patients: Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana-Slovenia (Andrej Janez, Drazenka Pongrac Barlovic, Nadan Gregorič, n = 30); Clinical Research Centre for Rare Disease Aldo e Cele Daccò, Ranica (Norberto Perico, Stefano Rota, Matias Trillini, Barbara Ruggiero, Silvia Prandini, Monica Cortinovis, Giulia Gherardi, n = 26); Cattedra di Nefrologia, Università Federico II, Napoli (Antonio Pisani, Eleonora Riccio, Ivana Capuano, Gennaro Argentino, n = 13); Unità Malattie Endocrine - Diabetologia, ASST Papa Giovanni XXIII, Bergamo (Roberto Trevisan, Elena Mondo, Maria Carolina Aparicio, Sergio Brescianini, n = 9); U.O.C. Malattie Endocrine e Centro regionale per il Diabete Mellito (Diabetologia), ASST Bergamo Ovest, Treviglio and Romano di Lombardia (Antonio Carlo Bossi, Aneliya Ilieva Parvanova, Ilian Petrov Iliev, Svitlana Yakymchuk, n = 7); Unità Operativa di Nefrologia, Ospedale San Raffaele, Milano (Paolo Manunta, Maria Teresa Sciarrone Alibrandi, Marialuisa Querques, Elena Brioni, n = 5); U.O.C. Nefrologia e Dialisi, Presidio Ospedaliero *S. Marta* e S. Venera, Acireale (Giovanni Giorgio Battaglia, Maurizio Garozzo, Anna Clementi, n = 4); Struttura Complessa di Nefrologia, Azienda Ospedaliero-Universitaria di Parma (Salvatore David, Riccado Bertolini, Chiara Cantarelli, n = 4); Poliambulatorio extra-ospedaliero, ASST Bergamo Ovest, Brembate di Sopra (Antonio Belviso, Matias Trillini, Veruska Lecchi, n = 2); Nefrologia e Dialisi, Casa Sollievo della Sofferenza, San Giovanni Rotondo (Filippo Aucella, Carmine Antonio Maria Stallone, Rachele Grifa, Matteo Piemontese, n = 2); Istituto di Patologia Speciale Medica, Università degli Studi di Sassari (Andrea Ercole Satta, Giovanna Pisanu, Elisabetta Carta, Giacomina Loriga, n = 1).

Centres not including patients: U.O.C. Nefrologia e Dialisi, ASL di Teramo (Goffredo Del Rosso, n = 0); Medicina Generale di Seriate (Diabetologia), ASST Bergamo Est, Seriate (Ruggero Mangili, Manuela Abbate, n = 0).

Activities of the Clinical Research Centre: Monitoring, Drug Distribution and Pharmacovigilance (Nadia Rubis, Wally Calini, Olimpia Diadei, Alessandro Villa, Davide Villa); Database and Data Validation (Davide Martinetti, Sergio Carminati); Randomisation (Giovanni Antonio Giuliano); Data Analysis (Annalisa Perna, Francesco Peraro); Centralised Laboratory Measurements (Flavio Gaspari, Fabiola Carrara, Silvia Ferrari, Nadia Stucchi, Antonio Nicola Cannata); Regulatory Affairs (Paola Boccardo, Sara Peracchi).