Zibotentan in combination with dapagliflozin compared to dapagliflozin alone in patients with chronic kidney disease: A randomised active-controlled clinical trial

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Abstract (300/300 words)

Background In patients with chronic kidney disease (CKD), sodium-glucose co-transporter-2 inhibitors (SGLT2is) and endothelin A receptor antagonists (ERAs) can reduce albuminuria and glomerular filtration rate (GFR) decline. We assessed the albuminuria-lowering efficacy and safety of the ERA zibotentan combined with the SGLT2i dapagliflozin.

Methods In this multicentre, randomised, double-blind, active-controlled clinical trial (NCT04724837), adults with an estimated GFR (eGFR) of >=20 mL/min/1·73m^2 and a urinary albumin-to-creatinine ratio (UACR) of 150–5000 mg/g, as adjunct to ACE-inhibitors or angiotensin receptor blockers if tolerated, were randomised to 12 weeks daily treatment with combined zibotentan 1·5 mg/dapagliflozin 10 mg, zibotentan 0·25 mg/dapagliflozin 10 mg, or dapagliflozin/placebo 10 mg. The primary endpoint was a change from baseline in log-transformed UACR (zibotentan 1·5 mg/dapagliflozin versus dapagliflozin/placebo) at week 12. Fluid retention was an event of special interest, defined as a >3% increase in body weight (at least 2.5% must be from total body water) from baseline or a >100% increase in B-type natriuretic peptide (BNP) and either BNP >200 pg/mL if without atrial fibrillation (AF) or BNP >400 pg/mL if with AF.

Findings Of 1492 participants assessed for eligibility, 447 (mean age 62·8 years [standard deviation (SD) 12·1]; mean eGFR 46·7 mL/min/1·73m^2 [SD 22·4] and median UACR 565·5 mg/g) were randomised and received treatment with zibotentan 1·5

mg/dapagliflozin (n=179), zibotentan 0·25 mg/dapagliflozin (n=91), or dapagliflozin/placebo (n=177). Zibotentan 1·5 mg/dapagliflozin and zibotentan 0·25 mg/dapagliflozin reduced UACR versus dapagliflozin/placebo throughout the treatment period of the study. At week 12, the difference versus dapagliflozin/placebo was –33·7% (90% CI –42·5 to –23·5; p<0·001) for zibotentan 1·5 mg/dapagliflozin and –27·0% (90% CI –38·4 to –13·6; p=0·002) for zibotentan 0·25 mg/dapagliflozin. Fluid retention events were observed in 18.4% (33/179) in the zibotentan 1·5 mg/dapagliflozin group, 8·8% (8/91) in the zibotentan 0·25 mg/dapagliflozin group, and 7·9% (14/177) in the dapagliflozin/placebo group.

Interpretation Zibotentan combined with dapagliflozin reduced albuminuria with an acceptable tolerability profile and is an attractive option to reduce CKD progression in patients already receiving currently recommended therapy.

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ZENITH-CKD Trial registration number NCT04724837.

Research in context

Evidence before this study

We searched PubMed for randomised controlled trials published between January 1, 2010, and February 1, 2023, with the terms “Chronic Kidney Disease” AND “Albuminuria” AND “Endothelin Receptor Antagonist” AND “randomized controlled trial”.

Clinical practice guidelines recommend renin-angiotensin-system (RAS) inhibitors and sodium glucose co-transporter 2 inhibitors (SGLT2i) to slow the progression of kidney function decline in patients with chronic kidney disease (CKD). Despite these guideline-recommended therapies, progressive kidney function loss occurs in many patients and is associated with persistently high albuminuria. Novel albuminuria lowering therapies may further slow CKD progression. Increased expression of endothelin-1 (ET-1) is thought to contribute to progression of CKD through several pathophysiologic effects, including injury to the vasculature, podocytes, tubulointerstitium, and mesangium.[1] In the Study Of diabetic Nephropathy with AtRasentan (SONAR) trial, the ERA atrasentan slowed decline of kidney function in adults with type 2 diabetes. In people with Immunoglobin A Nephropathy or focal segmental glomerulosclerosis, the dual endothelin angiotensin receptor antagonist sparsentan reduced proteinuria. High doses of non-selective endothelin receptor antagonists increase risk of fluid retention which can lead to heart failure. Because SGLT2 inhibitors exert natriuretic and diuretic effects, combining a SGLT2 inhibitor with a selective endothelin receptor antagonist holds promise to augment nephroprotection while potentially mitigating fluid retention. Zibotentan is a highly selective endothelin receptor antagonist originally developed for the treatment of prostate cancer. We conducted a prospective randomized controlled clinical trial to characterise the effects of the combination zibotentan/dapagliflozin versus dapagliflozin alone on albuminuria and fluid retention in order to select the appropriate zibotentan dose for further investigation in clinical outcome trials.

Added value of this study

ZENITH-CKD, an international, randomised, double-blind, active-controlled clinical trial, is the first prospective study of a fixed dose-combination of an endothelin receptor antagonist (zibotentan) with a SGLT2 inhibitor (dapagliflozin) on top of maximum tolerated RAS inhibition (if tolerated) in adults with CKD. The primary albuminuria efficacy endpoint demonstrated that 12-week treatment with low doses of 0.25 mg/day and 1.5 mg/day zibotentan in combination with dapagliflozin 10 mg/day led to meaningful and statistically significant reductions in albuminuria versus dapagliflozin 10 mg/day alone. The reduction in albuminuria was greater for zibotentan and dapagliflozin compared to dapagliflozin alone from the first post-randomization assessment at week 3 through to week 12. The albuminuria levels returned to baseline values two weeks after discontinuation of study medication. No clinically meaningful changes in B-type Natriuretic Peptide, body weight or total body water, as proxies for fluid retention, were observed during 12 weeks' treatment with zibotentan 0.25mg/dapagliflozin, whereas modest increases were observed in these parameters with zibotentan 1.5 mg/dapagliflozin compared to dapagliflozin alone.

Implications of all the available evidence

The ZENITH-CKD trial demonstrated the efficacy and safety of combining a low dose of the selective endothelin receptor antagonist zibotentan with dapagliflozin in adults with CKD. The results showed a robust and clinically meaningful reduction in albuminuria and an acceptable safety profile. These findings support the conduct and inform the design of a long-term phase 3 clinical trial to demonstrate the efficacy and safety of zibotentan/dapagliflozin combination in reducing the risk of kidney failure in patients with CKD and increased albuminuria.

Introduction

An estimated 840 million people around the world have chronic kidney disease (CKD).[2] CKD is associated with a high risk of kidney failure, cardiovascular complications, and a reduced quality of life.[3,4] For a long time, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) were the only proven effective drugs to slow CKD progression. Since 2019, sodium-glucose co-transporter-2 inhibitors (SGLT2is) and endothelin receptor antagonists (ERAs) have emerged as new therapies to slow progressive kidney function loss and reduce the risk of kidney failure in people with CKD.[5-10] SGLT2is also reduce the risk of heart failure in people with and without CKD, possibly owing to their diuretic properties. Despite these advances in pharmacotherapy, the risk of kidney failure persists in many people, even when receiving optimal treatment including SGLT2is. The high risk of adverse kidney and cardiovascular complications is observed in patients with persistently high levels of albuminuria.[11]

Activation of the endothelin A receptor by endothelin 1 contributes to the pathophysiology of progressive kidney function loss through a variety of mechanisms, including vasoconstriction, podocyte damage, inflammation, and fibrosis.[12] Selective inhibition of the endothelin A receptor has been shown to be kidney-protective in animal models and randomised clinical trials.[13] However, these clinical trials have also demonstrated that ERAs cause fluid retention and oedema, which can lead to heart failure in some patients with CKD.[14] As SGLT2is exert diuretic effects, there is a rationale to combine ERA treatment with SGLT2is to further reduce albuminuria and

possibly augment kidney protection while simultaneously mitigating ERA-induced fluid retention.

Zibotentan is the most potent and selective endothelin A receptor antagonist developed to date.[15] Zibotentan was previously assessed as a treatment option for prostate cancer and is now under investigation as a treatment for CKD.[16] The combination of zibotentan and the SGLT2i dapagliflozin represents a potential therapeutic option for the treatment of CKD, as both drug classes offer kidney protection through different and potentially complementary mechanisms. The ZENITH-CKD trial was undertaken to characterise the effects of the combination of zibotentan and dapagliflozin versus dapagliflozin alone on albuminuria and fluid retention to select the appropriate zibotentan dose for further investigation in clinical outcome trials. We report here the results of the ZENITH-CKD trial.

Methods

Trial design

The ZENITH-CKD trial was a randomised, double-blind, active-controlled multicentre clinical Phase IIb trial. Details regarding the rationale, design, and baseline characteristics have been recently published.[17] The trial was sponsored by AstraZeneca and conducted at 170 clinical practice sites in 18 countries from April 2021 through January 2023. The trial was registered at ClinicalTrials.gov (NCT04724837). Under the original study design, participants who met the eligibility criteria were randomized to

either Part A or Part B. In part A of the study, participants were randomised to placebo, dapagliflozin 10 mg/day, zibotentan 5 mg/day, and zibotentan 5 mg/dapagliflozin 10 mg/day. In part B of the study, two additional treatment arms were added: zibotentan 1·5 mg/dapagliflozin 10 mg/day and zibotentan 0·25 mg/dapagliflozin 10 mg/day.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, consistent with the International Conference on Harmonisation Guideline for Good Clinical Practice and applicable regulatory requirements, and all participants provided written informed consent prior to enrolment. The study protocol and informed consent documents were approved by local Independent Ethics Committees.

Participants

Adults with CKD defined as having an estimated glomerular filtration rate (eGFR) ≥20 mL/min/1·73m^2 and a urinary albumin-to-creatinine ratio (UACR) between 150 and 5000 mg/g were eligible for participation. Participants had no current or prior (within 1 month of enrolment) treatment with an SGLT2i or any fixed-dose combination with an SGLT2i. All participants were required to be receiving a stable dose of an ACE inhibitor or an ARB for at least 4 weeks before screening. However, participants with documented ACE inhibitor or ARB intolerance were allowed to participate. Key exclusion criteria included autosomal dominant or autosomal recessive polycystic kidney disease, acute coronary syndrome events within 3 months before screening, type 1 diabetes, unstable heart failure requiring hospitalisation, or B-type natriuretic peptide (BNP) ≥200 pg/mL or N-terminal pro BNP ≥600 pg/mL (BNP ≥ 400 pg/mL or NT

proBNP ≥ 1200 pg/mL, respectively, if associated with atrial fibrillation). The full inclusion and exclusion criteria are shown in the supplementary section 1.

Randomisation and masking

Investigators used an Interactive Voice or Web Response System to determine treatment assignment. Participants and all study personnel (except the Independent Data Monitoring Committee) were masked to treatment allocation. To ensure blinding to dapagliflozin and zibotentan treatment, daily dosing for all participants consisted of two dose units, one dapagliflozin tablet, containing dapagliflozin 10 mg or placebo (only in part A of the study), and one capsule containing zibotentan 5 mg (only in part A of the study), zibotentan 1·5 mg, zibotentan 0·25 mg, or placebo. Part A was designed to measure the effect of each single agent of zibotentan and dapagliflozin, and their combination versus placebo. Part B, the main efficacy analysis presented in this article, was designed to measure the effect of various doses of zibotentan in combination with dapagliflozin 10 mg versus dapagliflozin 10 mg/placebo. The rationale for the 1·5 mg and 0·25 mg zibotentan dose selection was based on an exposure UACR-response model and included optimization of exposure increases due to renal impairment and to maximise identifiability of the dose-response curve as described previously.[17] Following an ad hoc safety DMC review and evolving treatment guidelines, randomisation to the zibotentan 5 mg/dapagliflozin 10 mg, zibotentan 5 mg and placebo groups was closed. Study medication was packaged identically, with uniform tablet appearance, labelling, and administration schedule. At randomisation, participants were stratified by diabetes status and baseline eGFR (≤45 versus >45 mL/min/1·73 m^2). Randomisation in each

stratum was monitored to ensure that the subgroup of participants with CKD and without diabetes was a minimum of 30% and a maximum of 50% of the total number of randomised participants.

Trial procedures

Following randomisation, in-person study visits took place at 1, 3, 6, 9, 12 and 14 weeks. At week 12, all participants discontinued randomised study medication and proceeded to a post-treatment 2-week wash-out period to assess off-study drug effects. At each study visit, vital signs were recorded, blood and urine samples were sent taken for laboratory assessment, and information on adverse events, concomitant therapies, and study drug adherence was collected. When participants developed fluid retention (defined as a >3% increase in body weight [at least 2.5% must be from total body water] from baseline, or a BNP increase >100% from randomisation and BNP >200 pg/mL without atrial fibrillation [AF], or a BNP increase >100% from baseline and BNP >400 pg/mL with AF), study medication was discontinued, but participants were to continue study visits as per protocol. Investigators were encouraged to keep the dose of the ACE inhibitor or ARB stable for each patient throughout the study. Management of blood pressure, lipids, glucose, and the use of other essential therapies was left to investigator discretion, in accordance with best current clinical practice guidelines.

Study objectives and outcomes

The primary objective was to assess the effect on UACR of zibotentan 1·5 mg in combination with dapagliflozin 10 mg versus dapagliflozin/placebo 10 mg. The primary efficacy endpoint was the change from baseline in log-transformed UACR at 12 weeks.

Secondary endpoints included the change from baseline to week 12 in log-transformed UACR for the zibotentan 0·25 mg/dapagliflozin versus dapagliflozin/placebo comparison, the change from baseline to week 12 in systolic and diastolic blood pressure, the change from baseline in eGFR at weeks 1, 12, and 14, and from week 1 to week 12 in the zibotentan 1·5 mg/dapagliflozin and zibotentan 0·25 mg/dapagliflozin groups versus dapagliflozin/placebo.

Exploratory outcomes included change in body weight and changes in total body water, extracellular water, and intracellular water volumes assessed using bioimpedance spectroscopy (ImpediMed SOZO Body Composition Analyser) at all visits from after randomisation.

Safety was assessed by collecting investigator-reported adverse events, vital signs, physical examination findings, electrocardiograms, clinical laboratory parameters, and other events of special interest such as changes in fluid-related measures (body weight and BNP). Fluid retention, as defined above, was an event of special interest. Adverse events were collected throughout the double-blind treatment period and 2-week follow-up period.

Statistical analysis

The analytical approach and power calculations have been previously published. [17] Enrolment of 150 participants in the zibotentan 1·5 mg/dapagliflozin and dapagliflozin/placebo groups (300 participants in total) provided approximately 80% statistical power to detect a dapagliflozin-corrected reduction in UACR of ≥25% using a one-sided type 1 error of 5%, assuming a 10% drop-out rate and a standard deviation (SD) of 1·0 in change from baseline in the natural log of UACR. For dose–response modelling, a sample size of 150 evaluable participants in the zibotentan 1·5 mg/dapagliflozin and dapagliflozin/placebo groups, and 77 participants in the zibotentan 0·25 mg/dapagliflozin group, provided at least 78% power across multiple dose–response models to detect dose–response significance. This assumes a one-sided type I error of 5% and a maximum UACR reduction of 25% for zibotentan 1·5 mg/dapagliflozin compared to dapagliflozin/placebo.

The primary efficacy analysis includes the participants who were randomised and received any study intervention. Participants were assessed according to the treatment assigned at randomisation. The analysis of change from baseline in UACR to week 12 was based on the natural log-transformed UACR values with values back-transformed onto the original scale to give the percentage mean change from baseline to week 12. The model, mixed model repeated measures, included the fixed categorical effects of the stratification factors, study protocol version, treatment, visit, and treatment-by-visit interaction, plus the continuous covariates of baseline log(UACR) and baseline

log(UACR)-by-visit interaction. An unstructured covariance structure was used for the within-participant errors. Estimates of the geometric mean and the conversion to percentage for change from baseline in UACR for each treatment group were computed under the mixed model (with 90% confidence intervals [CIs]). The geometric mean ratio were calculated between both the zibotentan 1·5 mg/dapagliflozin and zibotentan 0·25 mg/dapagliflozin versus dapagliflozin/placebo groups (with 90% CI and p-value for a test of no treatment effect). As in previous dose-finding studies in participants with CKD, we did not impute missing values, but we analysed all longitudinal UACR values from scheduled visits during the treatment period under the assumption of missingness at random. A similar mixed model for repeated measures approach was used for the secondary endpoint of change from baseline in UACR to week 12 for zibotentan 0·25 mg/dapagliflozin 10 mg versus dapagliflozin alone. Changes in systolic and diastolic blood pressure and eGFR were analysed using the same mixed model for repeated measures. In this model we replaced baseline log UACR with baseline systolic or diastolic blood pressure, or eGFR and replaced log UACR with systolic or diastolic blood pressure, or eGFR in the interaction term with visit. eGFR stratification factor was replaced by eGFR baseline covariate when this covariate was added.

All safety analyses were conducted on the safety population, defined as all participants who were randomised and received any study intervention. We summarised safety outcomes by treatment group, based on the actual treatment they received. SAS version 9·4 (SAS Institute) was used for all analyses.

The descriptive analysis set summarises data from part A and discontinued arms, these participants are not included in the analysis.

Role of the funding source

The ZENITH-CKD clinical trial was funded by AstraZeneca. AstraZeneca contributed to study design, data collection, and statistical analysis. Authors Min Lin, Emma Wijkmark, Glenn Carlson, Anne-Kristina Mercier, Magnus Åstrand, Sebastian Ueckert, Peter J. Greasley and Phil Ambery are employees of the study sponsor and participated in the writing, reviewing, and approval of the manuscript. Authors H.J.L.H., A.K. and D.W. had full access to all data in the study. All authors reviewed and approved the final version and were responsible for the decision to submit for publication.

Results

A total of 1492 participants were assessed for eligibility between April 28 2021 and January 17 2023. Following an ad hoc safety DMC review, a protocol amendment was implemented and randomisation to the zibotentan 5 mg/dapagliflozin and zibotentan 5 mg groups was closed due to increased rates of fluid retention (figure 1). In addition, because evolving guidelines established SGLT2is as the standard of care for the treatment of CKD, randomisation to the placebo group of the trial was also closed. As a result, dapagliflozin/placebo became the comparator to assess the efficacy and safety of zibotentan 1·5 mg/dapagliflozin and zibotentan 0·25 mg/dapagliflozin. There were not sufficient numbers of participants in each arm in the descriptive analysis set to determine whether there were significant differences with respect to the UACR lowering

efficacy of assigned treatments in part A. The fluid related event data in part A is found in the appendix. For main analysis, 449 participants were randomized, of whom 447 (99·6%) received treatment. Two Good Clinical Practice breaches potentially affecting data integrity involving two sites were identified. All 17 patients affected by these issues have been removed from the analyses. The randomization ratio remained unchanged. All participants included in this report had post-randomization UACR values available and could be included in the analysis. At week 12, 148 participants had missing UACR values. Overall, 177 (39·6 %) in the dapagliflozin/placebo group, 179 (40·0%) in the zibotentan 1·5 mg/dapagliflozin group, and 91 (20·4%) in the zibotentan 0·25 mg/dapagliflozin group. A total of 381 participants (84·9%) completed the study. The most frequent reasons for not completing the study were classified as other (n=21 [30·9%]) or participant decision (n=20 [29·4%]). Of 447 participants, 331 (74·0%) participants completed treatment. Among the 112 participants who discontinued treatment, the most frequent reasons for discontinuation of the study treatment were adverse events (n=36 [32·1%]) or “protocol-specified withdrawal criterion met" (27 [24·1%]). The mean compliance to study treatments, as assessed by pill count, was 100.5% (SD 44.4).

Baseline characteristics were well balanced among treatment groups (table 1). The mean age was 62·8 years (SD 12·1), 138 (30·9%) participants were female, and 305 (68·2%%) were White. Median UACR was 565·5 mg/g (25^{th} to 75^{th} Percentile 243.0, 1212.6) and mean eGFR was 46·7 mL/min/1·73m^2 (SD 22·4).

Figure 2 shows the UACR changes over time. In the dapagliflozin/placebo group, the percentage mean change from baseline in UACR remained stable over time. At week 12, the percentage mean change from baseline was –28.3% (90% CI –37·8 to –17.4). Participants randomised to zibotentan 1·5 mg/dapagliflozin had a median UACR of 566·8 mg/g (25^{th}, 75^{th} percentile 235.6, 1202.7) at baseline. At week 12, the percentage mean change from baseline was –52.5% (90% CI –59.0 to –44.9) corresponding to a difference in percentage mean change versus dapagliflozin/placebo of –33·7% (90% CI –42·5 to –23·5; p<0.001). The median UACR in the zibotentan 0·25 mg/dapagliflozin 10 mg group at baseline was 526·7 mg/g (25^{th}, 75^{th} percentile 212.1, 1287.0). At week 12, the difference in percentage mean change from baseline in UACR was –47.7% (–55.7, –38.2) corresponding to a percentage mean change versus dapagliflozin/placebo of –27·0% (90% CI –38·4 to –13·6; p=0.002). The reduction in UACR in the zibotentan/dapagliflozin groups compared to the dapagliflozin/placebo group was observed at week 3 and was consistently greater throughout the active treatment period (figure 2). Two weeks after the discontinuation of zibotentan/dapagliflozin or dapagliflozin/placebo, UACR levels returned to the baseline values and the mean percentage change from baseline was comparable among treatment groups. The effect of zibotentan/dapagliflozin compared with dapagliflozin/placebo in reducing UACR was consistent across subgroups defined by baseline type 2 diabetes and eGFR level (supplementary appendix figure 1).

At baseline, the mean systolic and diastolic blood pressure values were 137·6 mmHg/79·9 mmHg in the dapagliflozin/placebo group, 136·4 mmHg/78·9 mmHg

in the zibotentan 1·5 mg/dapagliflozin group, and 136·5 mmHg/79·6 mmHg in the zibotentan 0·25 mg/dapagliflozin group. Compared with dapagliflozin alone, a larger systolic and diastolic blood pressure decrease was observed in both zibotentan/dapagliflozin combination groups. The corresponding differences in systolic and diastolic blood pressure versus dapagliflozin/placebo at week 12 were -7.6 mmHg (90% CI -10.3, -4.9)/-5.4 mmHg (90% CI -7.1, -3.7) for the zibotentan 1·5 mg/dapagliflozin group and -3.6 mmHg (90% CI -6.8, -0.5) / -3.0 (90% CI -5.0, -1.0) for the zibotentan 0·25 mg/dapagliflozin group (figure 3 A and B). There was no correlation in the zibotentan/dapagliflozin groups and dapagliflozin/placebo group between the percent change in UACR and change in systolic blood pressure from baseline to week 12 (supplementary figure 3).

Baseline mean eGFR was 45·2 mL/min/1·73m^2 for the dapagliflozin/placebo group, 47·4 mL/min/1·73m^2 for the zibotentan 1·5 mg/dapagliflozin group, and 48·4 mL/min/1·73m^2 for the zibotentan 0·25 mg/dapagliflozin group. An acute reduction in eGFR was observed in all treatment groups at week 1, with the largest nominal decrease in the zibotentan 1·5 mg/dapagliflozin group: the mean difference versus dapagliflozin/placebo in change from baseline in eGFR at week 1 was -0.8 mL/min/1.73m^2 (90% CI -2.1, 0.5) and at week 12 -1.1 mL/min/1.73m^2 (90% CI -2.5, 0.3; figure 3 C). The corresponding difference in the zibotentan 0·25 mg/dapagliflozin group at week 1 was 1.1 mL/min/1.73m^2 (90%CI -0.5, 2.6) and at week 12 (-1.2 mL/min/1.73m^2 [90%CI -2.8, 0.5]). Two weeks after the discontinuation of dapagliflozin/placebo and zibotentan 0·25 mg/dapagliflozin, eGFR returned to the

baseline values. In the zibotentan 1·5 mg/dapagliflozin group, eGFR remained stable during wash-out.

The mean bodyweight at baseline was 85·5 kg in the dapagliflozin/placebo group, 85·9 kg in the zibotentan 1·5 mg/dapagliflozin group, and 83·8 kg in the zibotentan 0·25 mg/dapagliflozin group. The mean (SD) body weight change from baseline at week 12 was -0·85 (SD 2.7) kg in the zibotentan 1·5 mg/dapagliflozin group, -0·79 (SD 2.0) kg in the zibotentan 0·25 mg/dapagliflozin group and -1.19 (SD 2.6) kg in dapagliflozin/placebo group (figure 4A). Two weeks after the discontinuation of randomised study medication, mean body weight increased in the dapagliflozin/placebo group and decreased in both zibotentan/dapagliflozin groups. At week 12, an increase in mean percent change from baseline in extracellular fluid of 1.5% (SD 6.87) was observed in the zibotentan 1·5 mg/dapagliflozin group. Extracellular fluid showed little change from baseline in the zibotentan 0·25 mg/dapagliflozin group (-0.8% [SD 6.49]) and it decreased in the dapagliflozin group (-1.8% [SD 5.42]) (Figure 4B–D). Similar patterns were observed for total body water and intracellular fluid. Larger reductions from baseline in LDL-cholesterol and HbA1c were observed in the zibotentan/dapagliflozin groups compared to dapagliflozin/placebo. The full list of laboratory assessments are shown in supplementary appendix table 1.

The number of participants with adverse events are shown in Table 2, analysed for the safety analysis set. During the 12-week treatment period, the proportion of participants who experienced the pre-specified fluid retention endpoint was 7.9% (14/177) in the

dapagliflozin/placebo group, 18.4% (33/179) in the zibotentan 1·5 mg/dapagliflozin group, and 8·8% (8/91) in the zibotentan 0·25 mg/dapagliflozin group (figure 5A). The proportion of overall participants with fluid-related events was 12.3%. Six cases of heart failure were reported: four in the zibotentan 1·5 mg/dapagliflozin group, and two in the zibotentan 0·25 mg/dapagliflozin group. Three of these were reported as serious adverse events (two cases in the zibotentan 1·5 mg/dapagliflozin group, and one case in the zibotentan 0·25 mg/dapagliflozin group). Two of the six cases were associated with a clinically significant rise in BNP to above 200 pg/mL. There was one death in the dapagliflozin/placebo group. Median BNP at baseline in the dapagliflozin/placebo, zibotentan 1·5 mg/dapagliflozin, and zibotentan 0·25 mg/dapagliflozin groups were 36.0 ng/L[25th, 75th percentile 19.0, 69.0], 35.0 ng/L [25th, 75th percentile 20.0, 62.0], and 37.0 ng/L [25th, 75th percentile 17.0, 80.0]. Compared with dapagliflozin/placebo, the difference in percentage mean change from baseline in BNP at week 12 was 3.1% (90% CI -8.7, 16.5) in the zibotentan 1·5 mg/dapagliflozin group and -3.4% (90% CI -16.4, 11.7) in the zibotentan 0·25 mg/dapagliflozin group (figure 5B). During the 12 weeks treatment period, 11 (6·1%), 6 (6·6%), and 8 (4·5%) in the zibotentan 1·5 mg/dapagliflozin, zibotentan 0·25 mg/dapagliflozin, and dapagliflozin/placebo group initiated a diuretic. Hematocrit, serum sodium, serum potassium, or liver function tests were not clinically significant different compared to dapagliflozin/placebo (hereafter referred to as dapagliflozin alone; figure 5C).

Discussion

In the ZENITH-CKD trial, we found that a low-dose zibotentan 0·25 mg per day in combination with dapagliflozin 10 mg per day decreased UACR without major side effects in patients with CKD who were using standard-of-care treatment, including ACE inhibitors or ARBs. Both zibotentan doses in combination with dapagliflozin 10 mg also reduced blood pressure, LDL-cholesterol and HbA1c. Although a fixed-dose combination of zibotentan 1·5 mg/dapagliflozin 10 mg had a slightly larger UACR-lowering effect compared with zibotentan 0·25 mg/dapagliflozin 10 mg, it also elicited more fluid retention.

Clinical practice guidelines for the treatment of people with CKD with or without type 2 diabetes recommend renin-angiotensin-aldosterone system inhibitors and SGLT2is to slow the progression of kidney disease. To ensure that the efficacy and safety of zibotentan could be assessed as adjunct to the current standard of care, all participants were treatment-naïve to SGLT2is and received dapagliflozin 10 mg/day during the trial. A small retrospective post-hoc analysis from a large kidney outcome trial with the ERA atrasentan suggested that when atrasentan was initiated in combination with a SGLT2i, a further reduction in albuminuria of approximately 25% compared to atrasentan monotherapy may be expected, supporting a rationale to test the albuminuria-lowering efficacy of both drug classes in a prospectively designed clinical trial.[18] The 25%–30% additional reduction in albuminuria observed in the current study with zibotentan in combination with dapagliflozin supports this finding in a robust and rigorous efficacy

comparison. This magnitude of effect is clinically relevant and is likely to translate into a favourable effect on clinical kidney endpoints.[19]

Although ERAs may confer profound kidney-protective effects, their clinical development has been hampered due to the occurrence of heart failure as a result of fluid retention, in particular in individuals with CKD who may benefit most from these agents.[20] More selective antagonists of the endothelin A receptor versus those of endothelin B receptors are associated with less fluid retention when used in low doses. Zibotentan exhibits a more than 1000-fold higher selectivity for the endothelin A versus B receptor compared to other ERAs and as such represents a promising agent for the effective and safe use for the treatment of CKD.[15] Concomitant treatment with diuretics is another therapeutic approach to mitigate ERA-induced fluid retention as previously suggested. Natriuretic versus osmotic diuretic mechanisms may have different effects on fluid retention, and for this reason a diuretic approach may not be as effective when compared to SGLT2 inhibition. Specifically, it has been shown that SGLT2is affect intravascular and extravascular volume differentially compared to traditional diuretics.[21] It has been suggested that not only can ETAs cause some increased water and sodium reabsorption in the kidney, but may also increase vascular leak by decreasing venous constriction, thereby contributing to a potential increased extravascular volume. The preferential reduction in extravascular volume by SGLT2is may therefore provide better fluid mitigation of ETA-mediated fluid retention while not being limited by systemic sodium levels. Since dapagliflozin exerts osmotic diuretic properties, a fixed-dose combination of zibotentan with dapagliflozin is a rational strategy to offset fluid retention

and oedema in susceptible individuals, as shown in a small retrospective study.[22] Furthermore, an experimental rat study demonstrated that zibotentan dose-dependently increased fluid retention. This effect disappeared when zibotentan was combined with dapagliflozin.[23] Body weight change is an often used proxy for ERA induced fluid retention. In the current study, body weight increased rapidly upon initiation of zibotentan 1·5 mg/dapagliflozin 10 mg but subsequently decreased during the remaining treatment period which may be explained by the dapagliflozin induced reduction in fat mass due to enhanced glycosuria. Body weight increases and fluid retention were more frequently observed with zibotentan 5 mg monotherapy or in combination with dapagliflozin which led to the early discontinuation of these treatment arms. The finding that the fluid retention induced by zibotentan 5 mg monotherapy was partially prevented by co-administration of dapagliflozin agrees with previous experimental work. Although fluid retention was partially mitigated by dapagliflozin 10 mg at the 5 mg zibotentan dose, the percentage of participants experiencing a 3% or greater increase in total body water was not considered consistent with adequate risk reduction for fluid-related heart failure events, suggesting that a strategy with lower zibotentan doses in combination with dapagliflozin should be pursued.

The reduction in systolic blood pressure observed with zibotentan is clinically relevant and may contribute to potential long-term protective effects, especially since hypertension is common in people with CKD and is associated with progressive kidney function loss and cardiovascular complications. These results confirm and extend previous studies examining the blood pressure-lowering effects of ERAs. High doses of

the ERA aprocitentan reduced blood pressure in individuals with treatment-resistant hypertension, of whom 55% had CKD.[24] Similarly, the ERA darusentan reduced blood pressure in people with treatment-resistant hypertension when recorded with conventional readings or with 24-hour ambulatory blood pressure monitoring.[25] However, the beneficial effect of aprocitentan and darusentan occurred at the expense of more fluid retention and heart failure, especially in CKD patients, suggesting that effective diuretic therapy is needed for safe use in clinical practice. Other cardiovascular risk markers also showed improvements in the zibotentan/dapagliflozin treatment groups. Specifically, the clinically significant mean reduction in LDL cholesterol and improvement in Hba1c compared to dapagliflozin alone support use of the combination in CKD patients already optimised on standard of care for cardiometabolic risk factors.

The albuminuria-lowering effect of zibotentan is unlikely to be attributed to the reduction in blood pressure since blood pressure changes during 12 weeks of zibotentan and dapagliflozin treatment did not correlate with albuminuria changes. Instead, the albuminuria-lowering effects of zibotentan are more likely mediated by direct inhibition of the pathophysiological action of endothelin 1 and may involve improvements in endothelial function, reduction in glomerular hyperfiltration, and protection of podocytes.

The narrow therapeutic window of ERAs warrants carefully designed dose-finding studies to select the optimal dose with maximal albuminuria-lowering effect and minimal fluid retention. Previous dose-finding studies with other ERAs have suggested that the dose–response curve for albuminuria lowering dissociates from the dose–response

curve for fluid retention, allowing for dose-selection sufficient albuminuria-lowering effect and at the same time minimal fluid retention.[26] In the current study, zibotentan 1·5 mg resulted in a modest additional albuminuria-lowering effect compared to zibotentan 0·25 mg but resulted in higher rates of fluid retention, suggesting that the lowest dose of zibotentan used in our study may be optimal for future trials. However, since zibotentan is cleared and eliminated by the kidneys, and exposure (in terms of AUC) increases with renal impairment, higher doses may be required in people with less severe kidney disease to ensure sufficient efficacy.[27]

The safety and tolerability of low-dose zibotentan in combination with dapagliflozin is likely to be acceptable in clinical practice. The number of heart failure-related adverse events or serious adverse events was low in the current study. One heart failure event was related to urological surgery, which is known to provide challenges with respect to fluid management.[28] No heart failure events resulted in death. Overall, the small number of heart failure events could be managed with escalation of diuretic therapy, suggesting an acceptable safety and tolerability profile for the treatment of CKD. No cases of liver dysfunction fulfilling potential Hy's law were reported during the study and small reductions in transaminases and alkaline phosphatase were seen in participants treated with combination therapy. This is consistent with other data suggesting that endothelin antagonists reduce hepatic insulin resistance. The mechanism for this is unknown but may relate to the effects of endothelin receptor blockade on portal pressure. Data from a post-hoc analysis of the SONAR study have shown similar positive effects on liver biochemistry with the ERA atrasentan in participants with type 2 diabetes and CKD.[29]

The results of trials of zibotentan and dapagliflozin in portal hypertension liver cirrhosis are awaited.

While this study has some strengths, including the large sample size, the randomised active-controlled design, the use of bio-impedance spectroscopy to delineate changes in body fluids, and the low drop-out rate, there are also limitations. The primary outcome was a surrogate outcome assessed during a short follow-up of 12 weeks. We were therefore not able to draw conclusions about the longer-term effects of combined zibotentan and dapagliflozin treatment on eGFR decline or clinical kidney endpoints. The study was also not powered to detect differences in fluid retention or heart failure, and heart failure events were not adjudicated by an independent endpoint committee. A future phase 3 clinical trial will provide more comprehensive data about the longer-term efficacy and safety of zibotentan and dapagliflozin on clinical kidney outcomes. In addition, due to the selection of patients based on stringent inclusion and exclusion criteria, as well as other factors that influence clinical trial participation, the results cannot be generalised to patients who do not share the characteristics of the enrolled study population.

In conclusion, combined treatment with low-dose zibotentan and dapagliflozin yielded a robust and significant reduction in albuminuria, with a potentially acceptable safety profile when compared to placebo combined with dapagliflozin alone. These findings support future trials with this treatment combination to further reduce kidney function decline in high-risk patients with CKD and elevated albuminuria.

Author contributions

HJLH, AKM, PG, and PA designed the study and were involved in data collection, data interpretation. GC, AK, and DCW were involved in data collection and interpretation of the results. SU and MA were involved in the interpretation of the results. ML and EW contributed with statistical analysis and interpretation of the results. HJLH and PA wrote the first draft of the publication. All other authors contributed with critical revisions for important intellectual content.

Declaration of interests

H.J.L.H. reports grant funding and honoraria for consultancy as a member of the steering committee of the DAPA-CKD trial paid to their institution from AstraZeneca; research grants paid to his employer from AstraZeneca, Boehringer Ingelheim, Janssen, and Novo Nordisk for clinical trials; consulting fees, paid to his employer, from AstraZeneca, Bayer, Boehringer Ingelheim, Chinook, CSL Behring, Dimerix, Eli-Lilly Gilead, Janssen, Novartis, NovoNordisk and Travere Therapeutics; honoraria for lectures from AstraZeneca, Bayer, and Novo Nordisk.

A.K. reports honoraria for lectures from AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Bayer, Mitsubishi Tanabe, Eli Lilly, Sumitomo Dainippon Pharma, Daiichi-Sankyo, and Ono Pharmaceutical.

D.C.W. has an ongoing contract with AstraZeneca and has received fees for consultancy work and/or speaker engagements from Astellas, Amgen, Bayer, Boehringer Ingelheim, Eledon, Galderma, GSK, Gilead, Janssen, Mundipharma, MSD, Merck, Pharmacosmos, ProKidney, Tricida, Vifor and Zydus.

M.L., E.W., G.C., A-K. M., M.Å., S.U., P.J.G., and P.A. are employees and stockholders of AstraZeneca.

Data sharing

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform . AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/ourmember/astrazeneca

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Figure legends

Figure 1: Participant flow and disposition. Discontinued treatment groups indicated by grey boxes. Participants included in the main efficacy analysis presented in this article indicated by white boxes. Participants randomised to dapagliflozin 10 mg/day during Part A were not shown.

Figure 2: Panel A: Bar graph of the percentage mean change in UACR from baseline to week 12 in the dapagliflozin/placebo 10 mg, zibotentan 1·5 mg/dapagliflozin 10 mg and zibotentan 0·25 mg/dapagliflozin 10 mg groups. Panel B: UACR trajectory over time in the three treatment groups. Vertical bars indicate the 90% CIs of the mean at given time points. UACR=urinary albumin-to-creatinine ratio. CI=confidence interval.

Figure 3: Panel A and B: Mean change from baseline in SBP and DBP changes in the three treatment groups. Panel C: Mean eGFR change from baseline over time in the three treatment groups. eGFR=estimated glomerular filtration rate. Vertical bars indicate the 90% CIs of the mean at given time points. CI=confidence interval.

Figure 4: Panel A: Mean body weight change from baseline over time. Panel B, C and D: Mean percent change in extracellular fluid, intracellular fluid and total body water, from baseline up to Week 12. Vertical bars indicate the 90% CIs of the mean at given time points. CI=confidence interval.

Figure 5: Panel A: Kaplan–Meier curve of fluid retention. Panel B: Percentage mean change in BNP from baseline over time . Panel C: Mean hematocrit change from baseline over time. BNP=B-type natriuretic peptide. Vertical bars indicate the 90% CIs of the mean at given time points. CI=confidence interval.

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743 **Figure 1**

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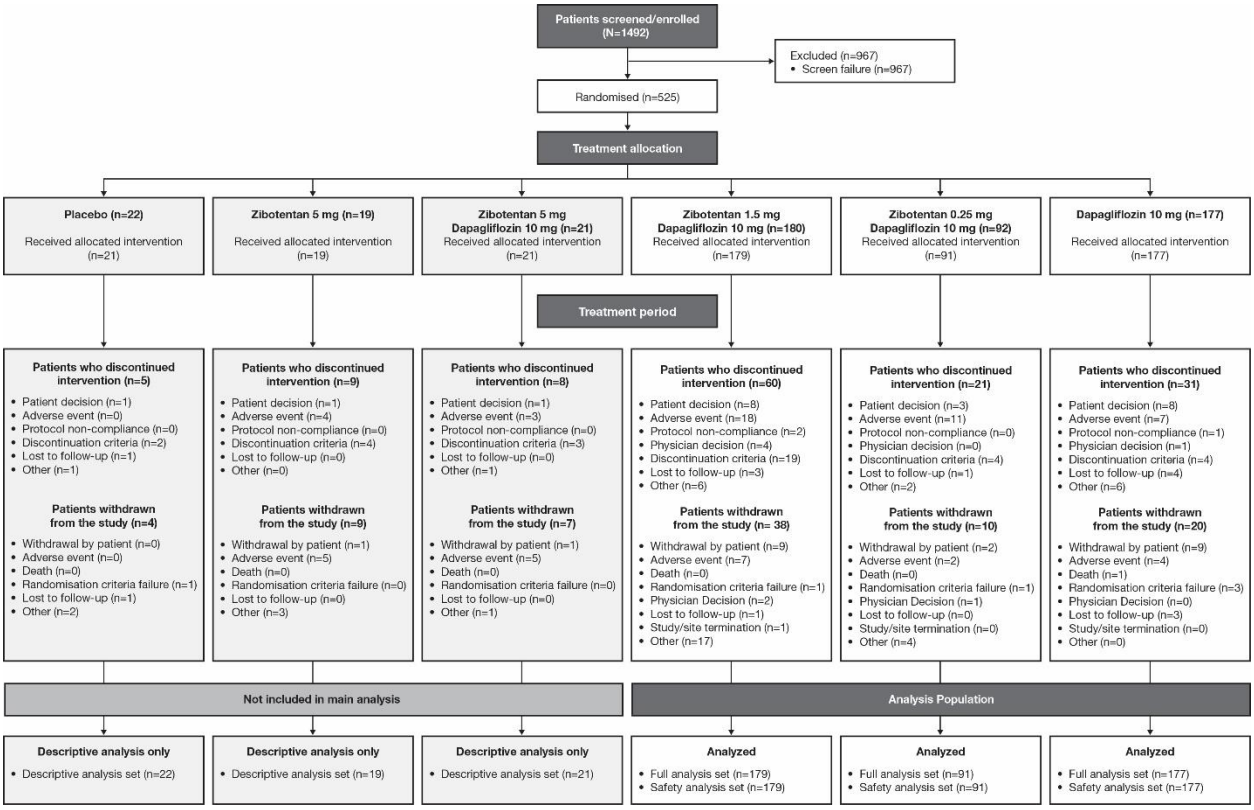

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748 **Figure 2**

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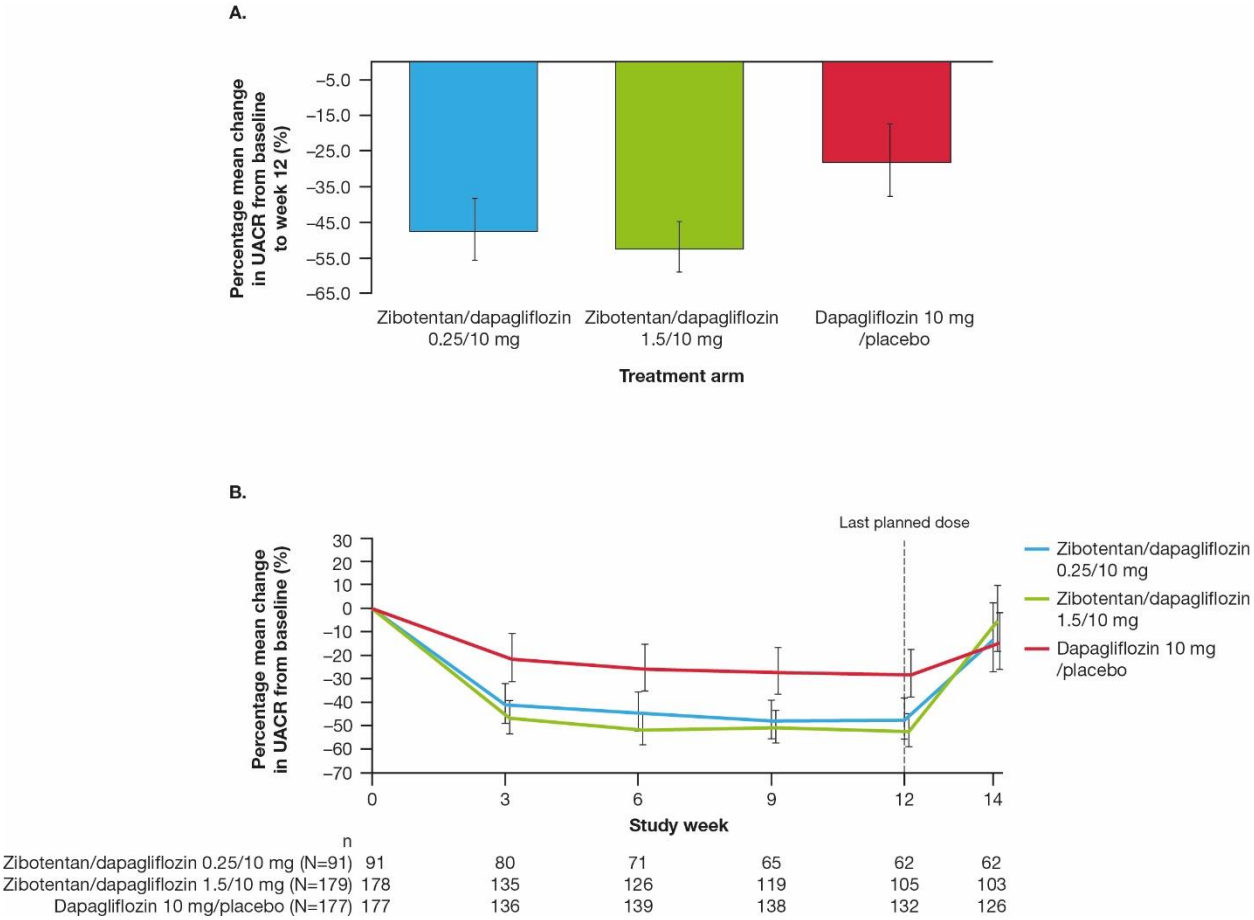

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Zibotentan/dapagliflozin 0.25/10 mg (N=91)	91	80	71	65	62	62
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	178	135	126	119	105	103
Dapagliflozin 10 mg/placebo (N=177)	177	136	139	138	132	126

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752 **Figure 3**

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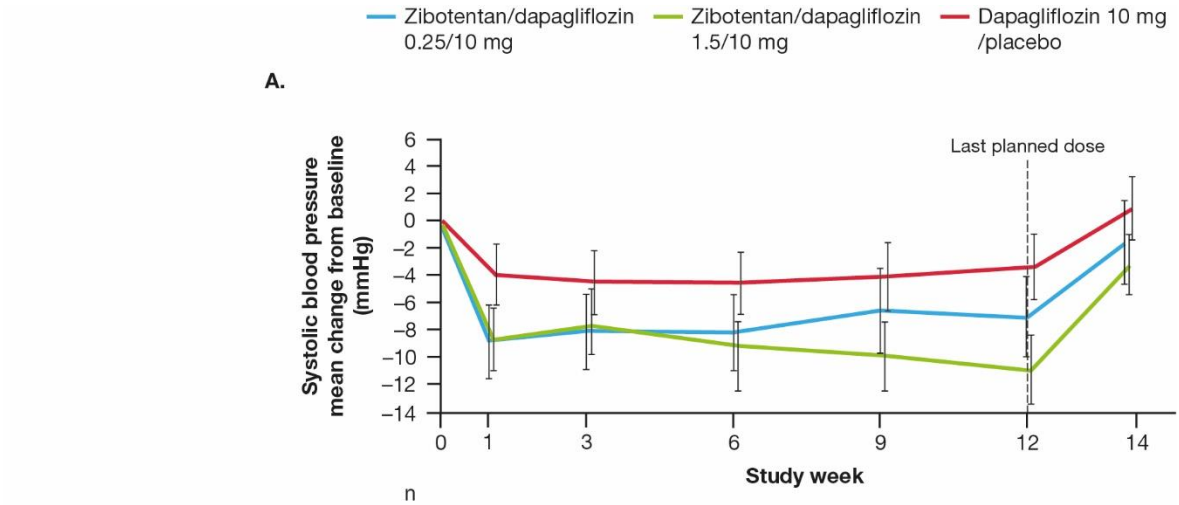

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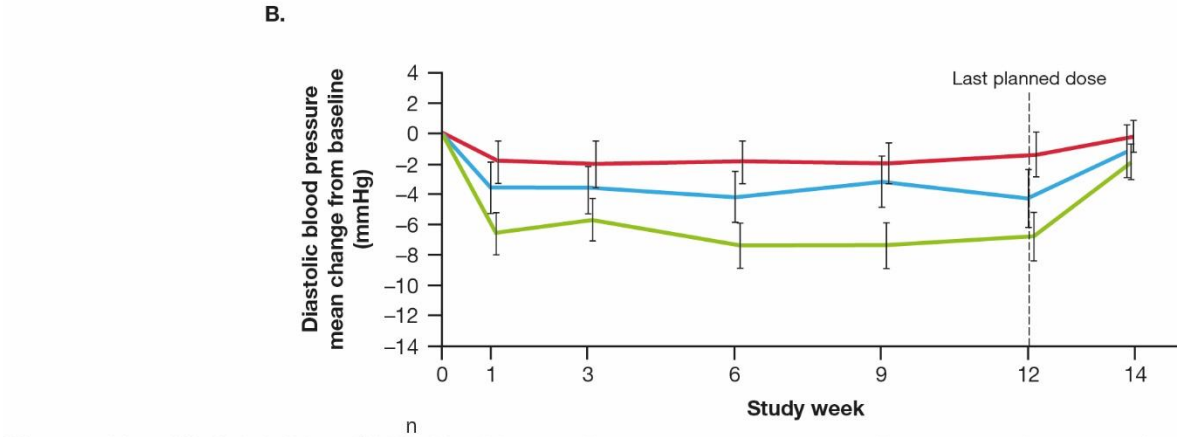
Zibotentan/dapagliflozin 0.25/10 mg (N=91)	91	84	83	75	68	65	74
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	179	154	143	133	124	108	141
Dapagliflozin 10 mg/placebo (N=177)	177	155	155	152	146	137	152



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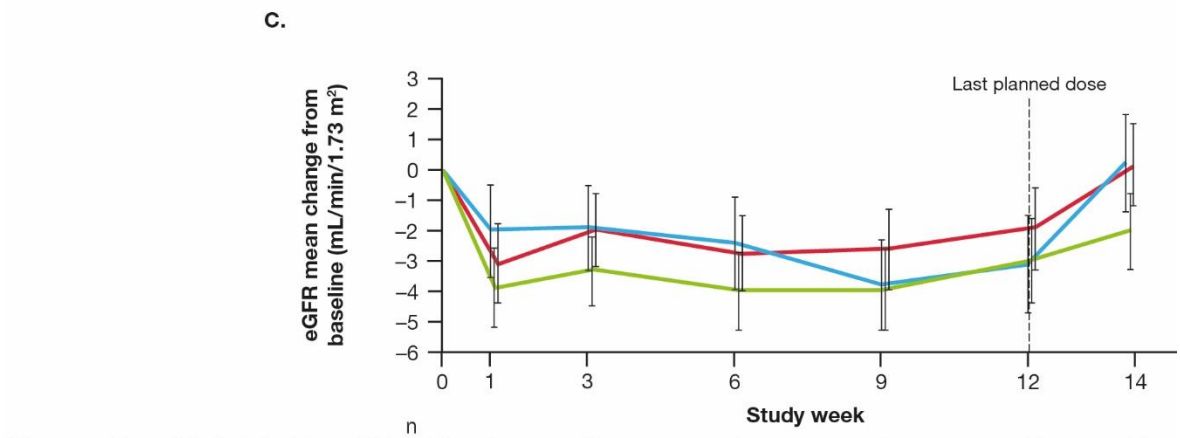
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	179	154	143	133	124	108	141
Dapagliflozin 10 mg/placebo (N=177)	177	155	155	152	146	137	152



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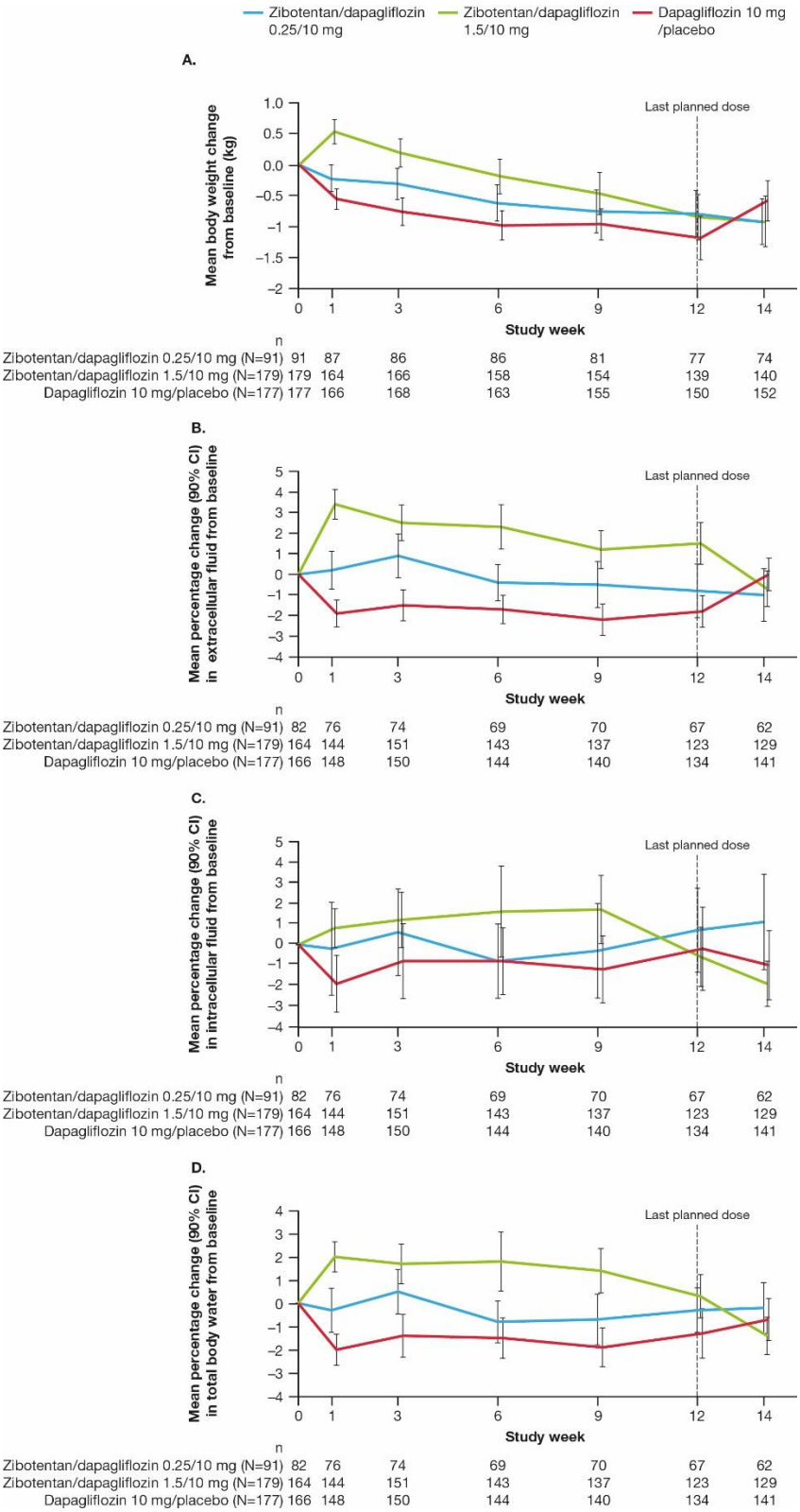
Zibotentan/dapagliflozin 0.25/10 mg (N=91)	91	83	81	72	68	64	63
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	179	152	143	128	123	108	105
Dapagliflozin 10 mg/placebo (N=177)	177	151	152	149	142	135	131

Figure 4


Zibotentan/dapagliflozin 0.25/10 mg
Zibotentan/dapagliflozin 1.5/10 mg
Dapagliflozin 10 mg /placebo
A.
Mean body weight change from baseline (kg)
Last planned dose
Study week
n
Zibotentan/dapagliflozin 0.25/10 mg (N=91) 91 87 86 86 81 77 74
Zibotentan/dapagliflozin 1.5/10 mg (N=179) 179 164 166 158 154 139 140
Dapagliflozin 10 mg/placebo (N=177) 177 166 168 163 155 150 152
B.
Mean percentage change (90% CI) in extracellular fluid from baseline
Last planned dose
Study week
n
Zibotentan/dapagliflozin 0.25/10 mg (N=91) 82 76 74 69 70 67 62
Zibotentan/dapagliflozin 1.5/10 mg (N=179) 164 144 151 143 137 123 129
Dapagliflozin 10 mg/placebo (N=177) 166 148 150 144 140 134 141
C.
Mean percentage change (90% CI) in intracellular fluid from baseline
Last planned dose
Study week
n
Zibotentan/dapagliflozin 0.25/10 mg (N=91) 82 76 74 69 70 67 62
Zibotentan/dapagliflozin 1.5/10 mg (N=179) 164 144 151 143 137 123 129
Dapagliflozin 10 mg/placebo (N=177) 166 148 150 144 140 134 141
D.
Mean percentage change (90% CI) in total body water from baseline
Last planned dose
Study week
n
Zibotentan/dapagliflozin 0.25/10 mg (N=91) 82 76 74 69 70 67 62
Zibotentan/dapagliflozin 1.5/10 mg (N=179) 164 144 151 143 137 123 129
Dapagliflozin 10 mg/placebo (N=177) 166 148 150 144 140 134 141

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761 **Figure 5**

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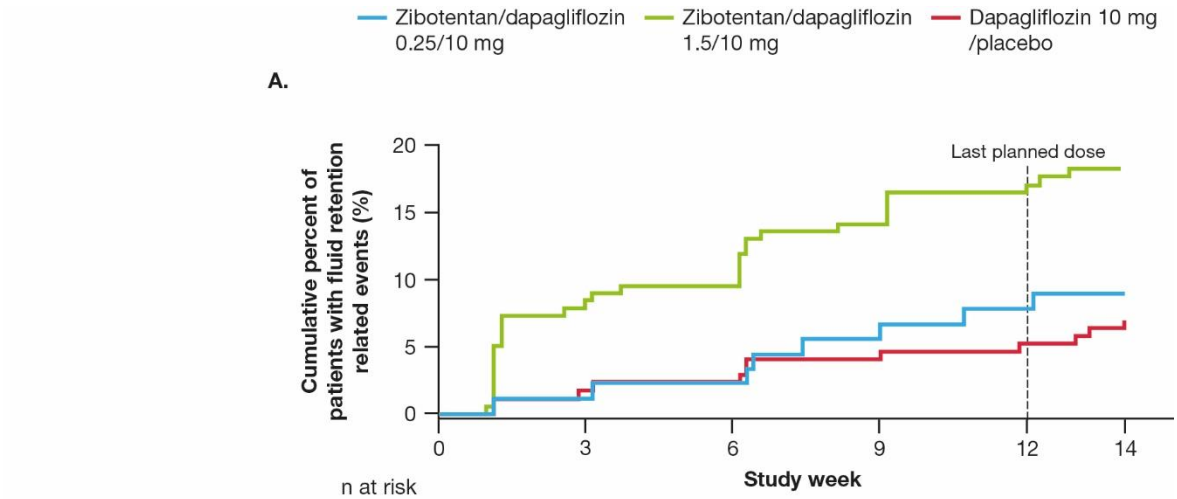
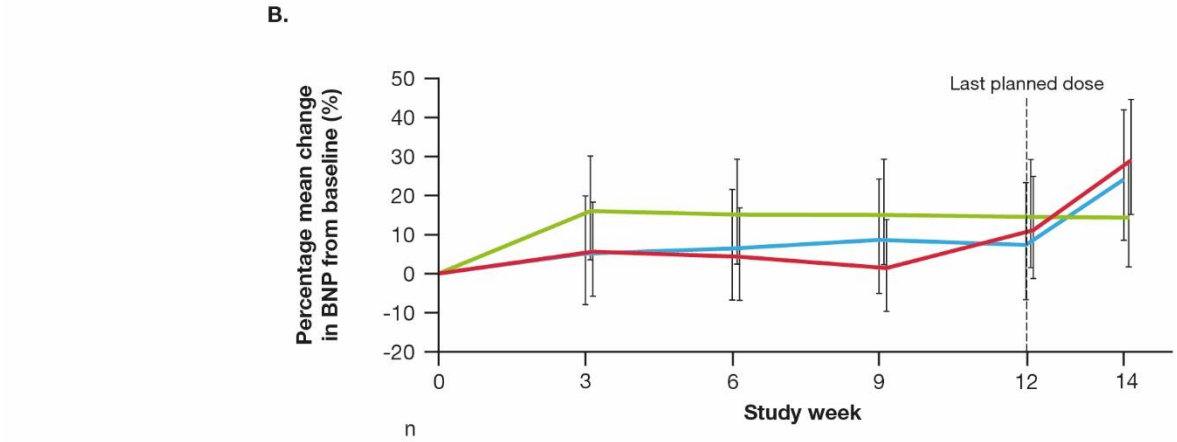
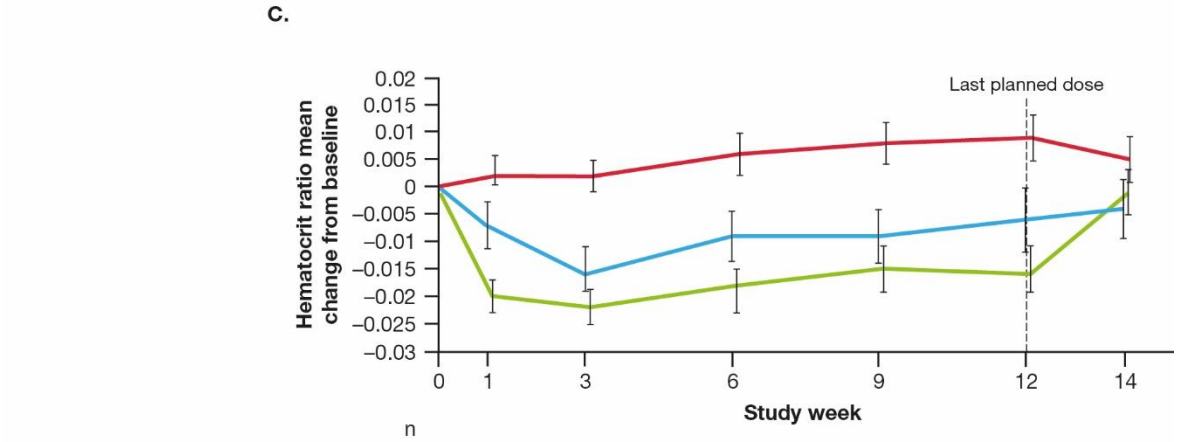


n at risk						
Zibotentan/dapagliflozin 0.25/10 mg (N=91)	91	90	89	84	81	80
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	179	163	156	147	143	139
Dapagliflozin 10 mg/placebo (N=177)	177	170	168	163	160	157



n						
Zibotentan/dapagliflozin 0.25/10 mg (N=91)	91	84	84	79	76	73
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	177	164	146	148	135	137
Dapagliflozin 10 mg/placebo (N=177)	177	161	161	147	146	147



n							
Zibotentan/dapagliflozin 0.25/10 mg (N=91)	90	82	81	81	78	72	70
Zibotentan/dapagliflozin 1.5/10 mg (N=179)	179	144	150	131	141	131	130
Dapagliflozin 10 mg/placebo (N=177)	176	148	157	151	133	139	146

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Tables

Table 1: Demographic and clinical characteristics of the participants at baseline

	Placebo/ dapagliflozin 10 mg N=177	Zibotentan 0·25 mg/ dapagliflozin 10 mg N=91	Zibotentan 1·5 mg/ dapagliflozin 10 mg N=179
Age – year (SD)	63·6 (11·60)	61·3 (12·72)	62·7 (12·33)
Female sex – no. (%)	55 (31·1)	28 (30·8)	55 (30·7)
Race – no. (%)			
White	125 (70·6)	56 (61·5)	124 (69·3)
Black or African American	22 (12·4)	7 (7·7)	17 (9·5)
Asian	26 (14·7)	18 (19·8)	26 (14·5)
Other	4 (2·3)	10 (11·0)	10 (5·6)
Weight – kg (SD)	85·5 (18·20)	83·8 (16·48)	85·9 (16·90)
Body mass index – kg/m^2 (SD)	30 ·2 (5·41)	29·6 (5·01)	30.1 (4·99)
Current nicotine user – no. (%)	25 (14·1)	12 (13·2)	22 (12·3)
Blood pressure – mmHg (SD)			
Systolic	137·6 (17·59)	136·5 (17·76)	136·4 (16·13)
Diastolic	79 ·9 (9·78)	79.6 (10·51)	78.9 (9·37)
eGFR – mL/min/1·73 m^2 (SD)	45·2 (20·71)	48·4 (23·49)	47·4 (23·38)
eGFR ≥60 mL/min/1·73 m^2 – no. (%)	32 (18·1)	22 (24·2)	45 (25·1)
eGFR 45 to <60 mL/min/1·73 m^2 – no. (%)	41 (23·2)	19 (20·9)	27 (15·1)
eGFR 30 to <45 mL/min/1·73 m^2 – no. (%)	62 (35·0)	28 (30·8)	61 (34·1)
eGFR <30 mL/min/1·73 m^2 – no. (%)	42 (23·7)	22 (24·2)	46 (25·7)
Haemoglobin – g/L (SD)	132·0 (16·69)	131·7 (16·46)	130·3 (16·18)
Serum potassium – mmol/L (SD)	4 ·60 (0·455)	4·64 (0·481)	4·64 (0·520)
Median UACR (Q1–Q3)	577·0 (279·5, 1150·6)	526·7 (212·1, 1287·0)	566·8 (235·6, 1202·7)
UACR >1000 mg/g – no. (%)	58 (32·8)	32 (35·2)	55 (30·7)
Type 2 diabetes – no. (%)	105 (59·3)	52 (57·1)	104 (58·1)
CKD aetiology – no. (%)			
Cystic kidney disease	1 (0·6)	0 (0)	3 (1·7)
Type 2 diabetes and CKD	93 (52·8)	44 (48·4)	88(49·2)
Ischaemic/Hypertensive nephropathy	32 (18·2)	20 (22·0)	30 (16·8)
Chronic glomerulonephritis	20 (11·4)	10 (11·0)	25 (14·0)
IgA nephropathy	7 (4·0)	4 (4·4)	8 (4·5)

Others	13 (7·4)	6 (6·6)	17 (9·5)
Unknown	13 (7·4)	11 (12·1)	19 (10·6)
Other	17 (9·7)	6 (6·6)	14 (7·8)
Family history of premature cardiovascular disease – no. (%)	38 (21·5))	12 (13·2)	38 (21·2)
Heart failure – no. (%)	17 (9·6)	3 (3·3)	11 (6·1)
Prior medication – no. (%)			
ACE inhibitor	56 (31·6)	33 (36·3)	58 (32·4)
ARB	98 (55·4)	48 (52·7)	96 (53·6)
Diuretic	75 (42·4)	36 (39·6)	61 (34·1)
Calcium Channel Blocker	91 (51·4)	47 (51·6)	88 (49·2)
β-blocker	56 (31·6)	41 (45·1)	67 (37·4)
Statin	125 (70·6)	60 (65·9)	130 (72·6)

ACE=angiotensin-converting enzyme. ARB=angiotensin receptor blocker. CKD=chronic kidney disease. eGFR=estimated glomerular filtration rate. IgA=Immunoglobulin A. SD=standard deviation. UACR=urinary albumin-to-creatinine ratio.

769 ***Table 2:*** **Number of participants with adverse events (safety analysis set)***

	Placebo/ dapagliflozin 10 mg N=177	**Zibotentan 0·25 mg/ dapagliflozin 10 mg N=91**	**Zibotentan 1·5 mg/ dapagliflozin 10 mg N=179**
	No. of patients (%)	No. of patients (%)	No. of patients (%)
Any adverse event	66 (37·3)	45 (49·5)	85 (47·5)
Adverse events leading to drug discontinuation‡	7 (4·0)	11 (12·1)	22 (12·3)
BNP increase	2 (2.2)	6 (3.4)	1 (0.6)
Fluid retention	1 (1.1)	4 (2.2)	1 (0.6)
Periperal oedema	1 (1.1)	3 (1.7)	0 (0)
Hypotension	(1.1)	1 (0.6)	1 (0.6)
Serious adverse events	4 (2·3)	2 (2·2)	9 (5·0)
Adverse events of clinical interest			
Headache	2 (1.1)	6 (6·6)	8 (4·5)
Metabolic acidosis	2 (1·1)	4 (4·4)	7 (3·9)
BNP increase	1 (0·6)	2 (2·2)	9 (5·0)
Hypertension	1 (0·6)	5 (5·5)	0 (0)
Fluid retention	1 (0·6)	1 (1·1)	5 (2·8)
Peripheral oedema	1 (0·6)	4 (4·4)	7 (3·9)
Deaths	1 (0·6)	0	0

770 *All (serious) adverse events were reported by participating investigators and collected
771 without further adjudication
772 ‡Only adverse events leading to drug discontinuation that occurred in more than two
773 participants are reported
774 One case reported as an adverse event in the 0·25 mg arm was classified as an
775 adverse event based on a follow-up ECHO showing a reduction in ejection fraction from
776 52% to 28%. Their baseline electrocardiogram was left bundle branch block; a within-
777 study silent myocardial infarction can't be excluded as the cause of reduction in ejection
778 fraction. BNP=B-type natriuretic peptide. SD=standard deviation.