Precision Pharmacotherapy With Zibotentan in Microvascular Angina: a randomized, placebo-controlled, cross-over trial.

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Precision Pharmacotherapy With Zibotentan in Microvascular Angina: A Randomized, Placebo-Controlled, Crossover Trial

Brief title: The PRIZE trial

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Abstract

Microvascular angina, linked to endothelin system dysregulation, was the focus of this double-blind, placebo-controlled, randomized, sequential crossover trial (NCT04097314). The trial compared zibotentan, an oral endothelin A receptor selective antagonist, with placebo in 118 patients with microvascular angina. Over 12 weeks, participants received either 10 mg daily zibotentan or placebo, with the primary outcome treadmill exercise duration. The study found no significant difference in exercise duration with zibotentan (-4.26 seconds; 95% CI: -19.60 to 11.06; P=0.5871). However, zibotentan increased plasma big endothelin-1, endothelin-1, and global myocardial blood flow, while reducing hemoglobin, diastolic, and systolic blood pressure (all $p<0.001$). Adverse events were more common during the zibotentan period (60.2%) compared to placebo (14.4%, $p<0.001$). In conclusion, daily administration of 10 mg zibotentan for 12 weeks did not enhance exercise duration and was commonly associated with adverse effects related to fluid retention. Further trials exploring lower zibotentan doses in combination with agents to mitigate fluid retention, and longer treatment durations, are warranted.

Introduction

Microvascular angina is a chronic condition characterized by abnormal myocardial blood flow leading to ischemic symptoms, and impairments in exercise capacity and health-related quality of life.[1,2] This condition more commonly affects women, and there are no evidence-based, disease-modifying therapies.[3,4]

Endothelin-1, a peptide secreted by endothelial cells, is a highly potent constrictor of the human coronary arterioles.[5,6] Dysregulation of the endothelin system is implicated in the pathogenesis of microvascular angina.[7,8] Microvascular angina is associated with elevated circulating concentrations of endothelin-1, and prolonged exposure to 'excess' endothelin causes vasoconstriction and vascular remodelling.[7,9] Endothelin-1 mediates enhanced vasoconstriction in the peripheral arterioles of participants with microvascular angina compared to control subjects.[10]

The chronic elevation of circulating endothelin-1 in microvascular angina may be influenced by genetic factors. rs9349379 is a common non-coding single nucleotide polymorphism (SNP) of the protein-coding phosphatase and actin regulator 1 (*PHACTR1*) gene on chromosome 6.[11] This SNP regulates expression of the endothelin 1 (*EDN1*) gene in human vascular cells and the minor G allele of this SNP (population prevalence ~36%) is associated with increased circulating concentrations of endothelin-1,[11] including in individuals with ischemic heart disease.[12] We found that the prevalence of the rs9349379 SNP was higher in patients with microvascular angina than in age- and sex-matched controls.[8] Patients with the rs9349379 G allele had higher serum endothelin-1 and over double the odds of coronary microvascular dysfunction. Additionally, the patients were more likely to have impaired myocardial blood flow and reduced exercise tolerance.[8]

Zibotentan, the most selective antagonist of the endothelin A receptor with no off-target binding to the endothelin B receptor, was evaluated in oncology trials and did not improve survival.[13–15]

We identified zibotentan as a potential disease-modifying therapy for patients suffering from microvascular angina; however, it has not been used before in this patient group and is currently unlicensed.[14,16] We hypothesized that zibotentan 10 mg daily for 12 weeks in addition to background medical therapy could be an efficacious and safe treatment for patients with microvascular angina.[16] We further hypothesized that the SNP regulator of *EDN1* gene expression, rs9349379 (G allele), could be a novel genomic biomarker for treatment response in this population.

Results

From 28 October 2019 until 28 September 2022, 222 patients were screened at twelve sites in the United Kingdom (Figure 1 and Supplementary Figure S1). Of these, 49 were excluded based on eligibility criteria and 173 participants underwent genotyping. Based on genotype criteria, 129 participants were included, and 44 participants were excluded. The patients and investigators were blinded to the genotype results. At the end of enrolment, 8 participants withdrew while genotype results were pending and a further 3 participants were excluded.

One hundred and eighteen participants (mean (standard deviation, SD) age 64 (9) years, 71 (60.2%) female) with microvascular angina were randomized. 115 of 118 participants fulfilled COVADIS criteria for probable (64/115 (55.7%)) and definite (51/118 (44.3%)) microvascular angina and 32 (27.1%) had concomitant vasospastic angina. Overall, 109 (92.4%) participants were prescribed one or more medications for angina and 112 (94.9%) participants were prescribed anti-platelet or lipid lowering medication for prevention of atherosclerotic cardiovascular events. Seventy-five (64%) participants had a history of hospitalization for chest pain.

Of 118 randomized participants, 22 (18.6%) were AA, 65 (55.1%) were AG, 31 (26.3%) were GG allele combinations for the rs9349379 SNP, and 96 (81.4%) had either AG or GG genotype, respectively.

During period 1, 59 participants were assigned to zibotentan, and 59 participants were assigned to receive placebo. In period 2, 50 participants progressed to placebo and 54 participants progressed to zibotentan. At the end of the trial, 25 (21.2%) of 118 participants had withdrawn, including 9 (7.6%) during treatment with placebo and 16 (13.6%) during treatment with zibotentan. No participant was lost to follow-up.

Of 118 participants who were randomized, 117 participants had an exercise test at baseline, 103 participants had an exercise test at baseline and at least one exercise test during follow up after either placebo or zibotentan and were included in the primary analysis, and 89 participants had complete data. The baseline characteristics are summarized in Table 1 and Supplementary Table 1. Medical therapy is described in Supplementary Table 2.

Exercise test findings and patient-reported outcome measures are described in Table 2 and Supplementary Table 3. The mean (standard deviation, SD) total exercise time at baseline was 303 (133) seconds (n=117 with at least one exercise test post-randomization), including 279 (114) seconds in 70 females and 338 (152) seconds in 47 males. Fifty-nine (50%) participants had exercise-limiting angina. The median (interquartile range) Seattle Angina Questionnaire-7 item (SAQ-7) summary score was 60 (46, 75), consistent with fair (moderate) health status.

Outcomes

The primary outcome, the within-individual difference in exercise duration following treatment for 12 weeks with zibotentan 10 mg daily versus placebo, was not improved by zibotentan

(between-treatment difference, -4.26 seconds; 95% confidence interval (CI) -19.60 to 11.06; P=0.5871) (Table 2). There were no interactions for the effect of zibotentan on the primary outcome with baseline characteristics including age (0.7942), sex (p=0.9968), body mass index (p=0.6867), rs9349379 (G allele) genotype (p=0.4554), estimated glomerular filtration rate (p=0.6098), systolic blood pressure (p=0.4539), or a history of vasospastic angina (p=0.058).

Secondary outcomes are presented in Table 2 and Supplementary Tables S1-S3. Compared to placebo, zibotentan, 10 mg daily for 12 weeks, did not improve secondary outcome measures derived from exercise testing or patient-reported outcome measures of angina burden, health-related quality of life, illness perception, psychological wellbeing or treatment satisfaction for medication (Table 2).

Adherence with trial medication

Adherence with trial medication, defined as consumption ≥80% of expected for the relevant period (treatment run-in, Period 1, Period 2), was achieved in 73 (81.1%) and 95 (97.9%) of the participants on zibotentan and placebo, respectively. A change in trial medication dosing occurred in 50 (42.4%) and 14 (11.9%) participants on zibotentan and placebo, respectively, including 22 (18.6%) and 8 (6.8%) participants who terminated treatment (p=0.0111, chi-squared test).

Fifty-one participants completed both treatment periods without any changes to the dosing of the trial medication. In this subgroup, exercise time did not differ after zibotentan versus placebo.

Safety

Zibotentan was associated with changes in hematology, liver function, lipid profile and glycated hemoglobin, but not cardiac biomarkers (Supplementary Table S4). Seventy-one (60.2%) and 17 (14.4%) participants experienced an adverse event with zibotentan or placebo, respectively

(p<0.0001) (Table 3). Most of the adverse events with zibotentan involved headache (40/118 (33.9%) vs. 7/118 (5.9%); p<0.0001), nasal congestion (29/118 (24.6%) vs. 4/118 (3.4%); p<0.0001), peripheral edema (13/118 (11.0%) vs. 1/118 (0.8%); p=0.0024), and breathlessness (6/118 (5.1%) vs. 0; p=0.0387), likely reflecting endothelin B receptor activation in response to increased circulating concentrations of endothelin-1. Adverse events were unrelated to genotype (AA vs. AG-GG 14/22 (63.64%) vs. 63/96 (65.62%); p=1.000).

Five serious adverse events occurred in 4 participants on placebo and 7 serious adverse events occurred in 7 participants on zibotentan. No unblinding occurred. Four suspected unexpected serious adverse reactions occurred, 1 on placebo and 3 on zibotentan.

Where data were available (n=34), 16 (47.1%) participants were confirmed as developing COVID-19 infection post-randomization, including 10 (29.4%) participants with COVID-19 occurring while on zibotentan and 4 (11.8%) participants with COVID-19 occurring when on placebo. Two (5.9%) participants had COVID-19 infection after the end of receiving the study medication. None of the participants reported COVID-19 with both treatments.

Hemodynamics and biomarkers

The effects of zibotentan on biomarkers are shown in Supplementary Table S5. Compared to treatment with placebo, zibotentan reduced diastolic blood pressure (mmHg) (-6.19 (-8.41, -3.97); p<0.001) and systolic BP (mmHg) (-5.49 (-8.49, -2.50; p<0.001) but not heart rate (effect estimate -0.20 (-2.24, 1.84), p=0.8506). Zibotentan increased circulating plasma concentrations of big endothelin-1 (pmol/L) (0.16 (0.11, 0.21); p<0.001) and endothelin-1 (pg/ml) 1.17 (0.91, 1.42); p<0.001), amino terminal peptide of type III procollagen (0.53 (0.14, 0.92); p=0.009) and body

weight (kg) (0.44 (-0.01, 0.90); p=0.057) and reduced total cholesterol (mmol/L) (-0.36 (-0.52, -0.21); p<0.001) and triglycerides (mmol/L) (-0.20 (-0.36, -0.04); p=0.0180).

In the trial population, plasma endothelin-1 concentration did not differ by genotype (p=0.1366) (Supplementary Figure S2).

Cardiovascular imaging

In a magnetic resonance imaging (MRI) substudy involving 18 participants, zibotentan increased left ventricular mass and volume and altered myocardial tissue characteristics consistent with water retention (Supplementary Table S6).

Zibotentan increased mean global myocardial blood flow (ml/min/g) at rest (effect estimate (95% CI) 0.14) (0.07, 0.20); n=18; p<0.001) (Figure 2), but not during adenosine hyperemia (n=18; p=0.9192). The subendocardial: subepicardial blood flow ratios at rest and during stress were not different during zibotentan (Supplementary Table S6).

Pharmacokinetics

In this sequential crossover study, zibotentan plasma concentration was measured pre-dose in 111 (94.5%) participants including 97 (97.0%) after placebo and 94 (96.9%) after zibotentan. During the zibotentan period, 81 of 94 participants had a zibotentan observation and 13 did not. The zibotentan plasma concentration was less than the lower limit of quantification in 14 (17.3%) participants, likely reflecting interruption of treatment, and 67 (82.7%) participants had observed values. The median (interquartile range) zibotentan pre-dose plasma concentration in 81 participants was 137.0 [16.5, 426.0] ng/ml (range 1.0 to 1300 ng/ml). Considering adherence, of 14 participants with zibotentan plasma concentrations less than the lower limit of quantification,

3 had missing data, 7 (63.6%) had adherence documented and 4 (36.4%) had lack of adherence documented.

During the placebo period, the zibotentan concentration was less than the lower limit of quantification in 82 (100%) participants and data were not available in 15 participants.

Discussion

We used genotyping to enrich the trial population within individuals with microvascular angina who might have exhibited an enhanced response to adjunctive treatment with zibotentan, an endothelin A receptor antagonist. The population had a moderate burden of angina, and two thirds were female. Target-related adverse effects led to poor tolerability, limiting any therapeutic benefit. Improvements in several cardiometabolic biomarkers occurred during treatment with zibotentan. Changes in hemoglobin with zibotentan indicated hemodilution consequent to fluid retention whilst blood pressure was lowered due to systemic vasodilation. Overall, this study has provided novel insights about zibotentan treatment in a non-oncology population.

Circulating concentrations of endothelin-1 were increased by zibotentan, in contrast to prior studies, perhaps reflecting reduced plasma clearance of endothelin-1.[13] However, an increase in big endothelin-1 also suggests increased expression of the endothelin gene, perhaps through an auto-regulatory feedback mechanism, an indirect effect of zibotentan, or increased signaling through endothelin B receptors. The ratio of ET-1: big ET-1 was unchanged, implying the clearance of ET-1 via ET-B receptors was not reduced. The activation of endothelin B receptors on endothelial cells leads to the release of nitric oxide and prostacyclin, both of which are vasodilators.[17] Observed target effects included reductions in systolic and diastolic blood pressure, hemoglobin and some biomarkers (reflecting altered fluid homeostasis and hemodilution) and an

increase in resting myocardial blood flow. Fluid accumulation with zibotentan may explain some of the biomarker changes and improvements in liver blood flow may be relevant. The reduction in alanine transaminase may reflect improved liver function, hemodilution, or both.

On the other hand, 60% of participants experienced an adverse event with zibotentan, whereas only 14% of participants experienced an adverse event with placebo. The most common adverse events with zibotentan were headache, nasal congestion, peripheral oedema and breathlessness, consistent with the class of drugs and reflecting endothelin B receptor activation in response to increased circulating concentrations of endothelin-1. Patient-reported outcome measures of health-related quality of life, including the Seattle Angina Questionnaire-7, EuroQol-5D-5L, Brief Illness Perception Questionnaire, and the four-item Patient Health Questionnaire-4 for detecting anxiety and depression did not improve, nor did treatment satisfaction for medication. Therefore, the future clinical development of endothelin A receptor antagonist therapy for microvascular angina should target minimizing adverse reactions.

We repurposed zibotentan using the only available oncology dose (10 mg) at the outset of this trial, which is a higher dose than in other recent trials of zibotentan.[18] The median (interquartile range) zibotentan pre-dose plasma concentration in 81 participants was 137.0 [16.5, 426.0] ng/ml (range 1.0 to 1300 ng/ml), which is in line with expectations based on earlier clinical studies characterizing the pharmacokinetics of zibotentan.[19,20]

The MRI substudy has provided mechanistic insights into the effects of the 10-mg dose of zibotentan in this population. The increases in ventricular volumes and myocardial native T1 relaxation time (Supplementary Table S6) revealed by MRI reflect fluid retention. Myocardial edema diminishes oxygen and nutrient delivery to the myocardium and impairs lusitropy (diastolic

function) which may explain why anginal symptoms, exercise capacity and stress myocardial blood flow did not improve.

The 10 mg dose of zibotentan was also associated with systemic hemodynamic effects, including an increase in resting myocardial blood flow that partly reflects a reduction in resting coronary microvascular tone due to endothelin A receptor antagonism. Data from prior studies of non-endothelin vasoactive drugs support a specific effect of endothelin A receptor antagonism by zibotentan on myocardial blood flow. In a MRI study of 58 patients with ischemic heart disease, intravenous treatment with serelaxin, a recombinant form of human relaxin-2 peptide, was associated with similar placebo-corrected reductions in systolic (-13.5 mmHg (P=0.0003)) and diastolic -8.4mmHg (P=0.001) blood pressure but without any changes in circulating endothelin-1 plasma levels or myocardial blood flow during resting and hyperemic conditions.[21] This result contrasts with the effect of zibotentan on resting myocardial blood flow including in the subendocardium (Supplementary Table S6) which is the anatomical location of the subendocardial microvascular plexus.[22] Since the subendocardial: subepicardial ratio of myocardial blood flow at rest did not change with zibotentan, the treatment-related changes in global myocardial blood flow at rest reflect alterations in systemic hemodynamics, fluid homeostasis and coronary microvascular dilatation. Myocardial blood flow did not increase during intravenous adenosine leading to A2 adenosine receptor activation in coronary endothelial and smooth muscle cells.[23] This may also reflect maximal coronary vasodilatation under resting conditions. Myocardial perfusion reserve is defined as myocardial blood flow during stress divided by myocardial blood flow at rest, and the improvement in myocardial blood flow at rest helps explain the reduction in myocardial perfusion reserve.

Treatment with 10 mg of zibotentan daily for 12 weeks did not improve exercise duration, angina symptoms, or health related quality of life. There are several potential explanations. First, maximal myocardial and systemic vasodilation, reflected by reduced blood pressure, may have limited the physiological response to physical exercise. Second, the 12-week treatment duration may have been insufficient to reverse coronary microvascular remodeling. Third, zibotentan was added to background medical therapy. The design of our study contrasts with ORBITA-2, a placebo-controlled clinical trial of the effect of percutaneous coronary intervention (PCI) on angina. In the ORBITA-2 trial, medical therapy for angina was discontinued 2-weeks before randomization and withheld until the last visit.[24] The rationale for this approach was to selectively assess the effect of PCI on anginal symptoms, without the confounding effects of angina drug therapy. Fourth, since most participants experienced an adverse event, the 10-mg dose led to target-related side effects that outweighed any improvement in symptoms. Fifth, there was a statistically significant effect of treatment period (Visit 5 vs. Visit 4) on exercise duration, and rate pressure product was unchanged (Supplementary Table S3), reflecting an increase in achieved exercise by the participants during the randomized trial, independent of the trial medication. This motivational effect of trial participation was adjusted-for in the primary analysis. However, the precision pharmacotherapy strategy did not prove effective since there was no benefit of 10-mg of zibotentan.

Importantly, this study has provided new data on the effects of zibotentan which is an unlicensed endothelin A receptor selective antagonist. Short-term treatment with 10 mg of zibotentan daily lowered blood pressure, glycated hemoglobin and low-density lipoprotein cholesterol. These effects could be beneficial for populations with hypertension and cardiometabolic disease. The coronary microcirculation is located in the subendocardium and impaired myocardial blood flow in the subendocardium is a primary pathological feature of microvascular angina.[1,22] The

improvement in subendocardial blood flow, reflecting a target-related physiological effect, is encouraging. However, this effect did not translate into patient benefits.

At the time of designing this trial, only one dose (10 mg) of zibotentan was available. Therefore, a dose-ranging design was not feasible. Furthermore, the limited shelf-life of the tablets curtailed the treatment period duration of this crossover trial. Since then, new clinical development programs have emerged for zibotentan in a range of conditions and very low dose (e.g. 0.25 mg) and low-dose (e.g. 1.5 mg) preparations of zibotentan are undergoing evaluation including as monotherapy, and in combination with dapagliflozin 10 mg, a sodium-glucose co-transporter 2 (SGLT2) inhibitor. These therapies are being evaluated for the treatment of chronic kidney disease,[18] liver cirrhosis with portal hypertension,[25] and systemic sclerosis.[26] The rationale for use of a lower dose of zibotentan is to achieve the beneficial effects of selective endothelin A receptor antagonism whilst minimizing the adverse effects of endothelin B receptor blockade. Since SGLT2 inhibition leads to osmotic diuresis, combination therapy should reduce adverse effects relating to fluid retention and improve treatment compliance. The elevated levels of endothelin-1 measured in this study raise the possibility of increased endothelin B related activity being indirectly caused by zibotentan. Results of the ZENITH-CKD study have established the proof of principle that lower doses of zibotentan can achieve therapeutic effect and in combination with 10 mg of dapagliflozin in a chronic kidney disease population the fluid retaining effects can be adequately mitigated.[18] This trial provides supporting data for a new clinical trial in microvascular angina using a very low dose of zibotentan in combination with dapagliflozin 10 mg daily.

People with microvascular angina have reduced quality of life and repeatedly use healthcare services. In our study, 60% of the screened population had a history of hospitalization for chest pain, 37% had 3 or more hospitalizations and 44% had 2 or more coronary angiograms. The study

312 provides novel data on ischemic heart disease in women, since 61% of the participants were female.
313 Contemporary experts have highlighted the lack of disease-modifying therapy for this condition;
314 and this trial was identified as holding promise.[4] Based on this unmet patient need, and the results
315 of our trial, we believe a future clinical trial should assess whether lower doses of zibotentan, alone
316 or in combination with dapagliflozin will be better tolerated and whether longer-term treatment
317 will be effective. Since the potential beneficial effects of endothelin A receptor antagonism may
318 be mediated through cardiovascular remodeling, a future trial should involve a longer duration of
319 treatment e.g. 6 – 12 months. The observed blood pressure lowering effect of zibotentan supports
320 further evaluation through clinical trials for resistant hypertension.[27]

321 Our results indicate that myocardial blood flow quantified using cardiovascular MRI may
322 represent a novel biomarker for clinical trials in microvascular angina. In the future, myocardial
323 blood flow quantified by MRI could be used as an eligibility criterion and as surrogate outcome
324 measure of treatment effect.[28] The target population for a future clinical trial of a much lower dose
325 of zibotentan could be focused to individuals with low resting myocardial blood flow and serial
326 MRI could be used to assess for a treatment effect. In the current study, the observed improvement
327 in exercise duration during the randomized trial as compared to during the run-in period reflects
328 the participants' subjective response to participating in the trial. This indicates that the exercise
329 test is a less objective outcome measure than a laboratory or imaging biomarker in this population.

330 In addition, in patients with type 2 diabetes, SGLT2 inhibition improves myocardial blood flow
331 reserve measured using ^{13}N-ammonia PET/CT raising the possibility of a benefit from SGLT2
332 inhibition in the microvascular angina population.[29] This suggests a similar strategy to that used in
333 the ZENITH-CKD trial combining low-dose zibotentan with an SGLT-2 inhibitor may be additive

from the efficacy point of view and mitigate fluid retentive effects of the endothelin A antagonist.[18,30]

Limitations

Fifty (42.2%) participants had a change in zibotentan trial medication and 22 (18.6%) participants permanently discontinued zibotentan treatment. Only one dose of zibotentan (10-mg) was available at this outset of this trial, therefore a dose-ranging study was not possible. The short-term (12-week) treatment duration and lack of a wash-out period between treatment periods was determined by the finite shelf-life of the tablets. Plasma endothelin-1 concentration did not associate with genotype, and this may reflect a lack of statistical power due to the sample size. Data are not available for the enrolled population (n=222) pre-genetic filter. Of note, treatment allocation was not randomized according to genotype therefore this trial was not testing precision pharmacotherapy versus standard care. Hemoglobin but not hematocrit was recorded in the database.

This study was undertaken during the COVID-19 pandemic and study activity was interrupted due to social restrictions (Supplementary Table 9). The social restrictions limiting daily activities may have reduced the severity of anginal symptoms experienced by the participants.

Stress/rest cardiovascular MRI was not specified as an eligibility criterion and enrolment into the MRI substudy was limited by the COVID-19 pandemic.

In conclusion, precision pharmacotherapy involving short term treatment with 10 mg of zibotentan daily did not improve exercise duration and target-related adverse effects were common. Resting myocardial blood flow improved. A future clinical trial should assess whether lower doses of

zibotentan will be better tolerated, potentially in combination with an SGLT2 inhibitor to mitigate fluid retention, and whether longer-term treatment will be effective.

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Author Contributions

CB designed the study and wrote the first draft of the manuscript. RY and AMcC developed the statistical analysis plan and performed the statistical analyses. The co-authors reviewed the manuscript drafts. Each author has individually contributed to either the grant application, the delivery of the study or helped to devise the protocol. All authors have given final approval for the current version to be published. The PRIZE Study Group includes individuals who have

contributed to the study. Individuals who do not fulfil author criteria are named in the Supplement.

Competing Interests Statement

CB is employed by the University of Glasgow which holds consultancy and research agreements with Abbott Vascular, AstraZeneca, Auxilius Pharma, Boehringer Ingelheim, Coroventis, GSK, HeartFlow, Menarini, Novartis, Siemens Healthcare, Somalogic and Valo Health. I.S. receives research grants from Astra Zeneca, Cambridge, UK and Kancera, Solna, Sweden. T.J. Ford: Consultant/speaker/honorarium from Abbott Vascular, Boston Scientific, Boehringer Ingelheim, Biotronik, Bio-Excel, and Novartis. RAL reports advisory board: Janssen Pharmaceuticals, Abbott, Philips, and speaker's honoraria: Abbott, Philips, Medtronic, Servier, Omniprex, Menarini. These companies had no role in the design or conduct of the study, or in the data collection or interpretation. APD holds research grants from AstraZeneca and is a member of scientific advisory boards of Janssen, ENB Therapeutics, and Pharmazz. CB, TJF and AD are named on a pending patent for the use of zibotentan for microvascular angina. The University of Glasgow holds the patent. None of the other authors have any relevant disclosures.

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509 **Table 1.** Clinical characteristics of the randomized trial population (n=118). An expanded version of this
510 table including the screened and trial populations is provided in the Supplement (Table S1).

	All data (n=118)	**Zibotentan → Placebo (n=59)**	**Placebo → Zibotentan (n=59)**
Demographics			
Age ± SD, years	63.5 (9.2)	64.3 (9.4)	62.7 (8.9)
Male sex, n (%)	47 (39.8)	24 (40.7)	23 (39.0)
Female sex, n (%)	71 (60.2)	35 (59.3)	36 (61.0)
Ethnicity, n (%)			
White	113 (95.8)	55 (93.2)	58 (98.3)
Medical history, n (%)			
Hospitalization for chest pain	75 (63.6)	33 (55.9)	42 (71.2)
≥3 Hospitalizations for chest pain	19 (25.3)	8 (24.2)	11 (26.2)
Vasospastic angina	32 (27.1)	16 (27.1)	16 (27.1)
Hypertension	64 (54.2)	33 (55.9)	31 (52.5)
Diabetes, treated	25 (21.2)	13 (22.0)	12 (20.3)
Percutaneous coronary intervention	21 (17.8)	12 (20.3)	9 (15.3)
Myocardial infarction	13 (11.0)	8 (13.6)	5 (8.5)
Atrial fibrillation or flutter	13 (11.0)	6 (10.2)	7 (11.9)
History of two or more coronary angiograms	52 (44.1)	23 (39.0)	29 (49.2)
Presenting characteristics, mean (SD)			
Body mass index, kg/m^2	29.0 (4.5)	29.6 (4.4)	28.3 (4.7)
Heart rate, bpm	71 (11)	71 (12)	71 (10)
Systolic blood pressure, mmHg	135 (18)	134 (17)	136 (18)
Diastolic blood pressure, mmHg	77 (12)	76 (12)	78 (12)
Canadian Cardiovascular Society angina class, n (%)			

I	17 (14.5)	9 (15.3)	8 (13.8)
II	75 (64.1)	41 (69.5)	34 (58.6)
III	24 (20.5)	9 (15.3)	15 (25.9)
IV	0 (0.0)	0 (0.0)	0 (0.0)
Not available	1 (0.9)	0 (0.0)	1 (1.7)
Medication, n (%)			
Angina medication	109 (92.4)	53 (89.8)	56 (94.9)
Preventive medication	112 (94.9)	57 (96.6)	55 (93.2)
Laboratory results at randomization			
Hemoglobin, mean (SD), g/L	139 (13)	138 (13)	140 (13)
Minimum eGFR, ml/min/1.73m^2	72 (13)	71 (14)	73 (13)
HbA1c, mean mmol/mol Hb, %	41.5 (9.6)	42.1 (9.3)	41.0 (10.0)
NT-proBNP, median [IQR], pg/mL	84 [50, 172]	77 [45, 143]	104 [58, 194]

Because of sparse categories, race was dichotomized to white versus non-white. Combined existing drug treatments recorded at screening. Angina medication is defined as a combination of any of beta-blocker, calcium channel blocker, long-acting nitrate, nicorandil, ranolazine and ivabradine. Preventive medication is defined as any aspirin, anti-platelet medication, statin and other lipid lowering drug.

Assumptions applied for measurement limit values to enable full data to be summarized as numeric. Subsequent summaries are given accounting for measurement limits. Some observed values are recorded that exceed the measurement limits of other records.

518 **Table 2.** Primary and secondary efficacy outcomes (zibotentan vs. placebo), intention-to-treat.

	Baseline, n	**Baseline value**	**Zibotentan vs. placebo, n**	**Effect estimate**	**95% CI**	**p-value**
Primary outcome						
Exercise duration, mean (SD), seconds	117	303 (133)	103	-4.26	(-19.60, 11.06)	0.5871
Secondary outcomes						
Exercise testing						
Time to 1 mm ST-depression, seconds*	56	309 (137)	103	1.0698*	(0.66, 1.74) *	0.7855*
Maximum ST-segment deviation, mV	114	-0.4 (1.5)	101	0.29	(-0.08, 0.66)	0.1217
Time to 75% of max age-related heart rate during exercise, seconds*	73	220 (124)	103	0.9591*	(0.63, 1.47) *	0.8472*
Metabolic equivalent (METs), O2/kg/min	117	7.8 (2.4)	103	-0.27	(-0.58, 0.03)	0.0822
DUKE Score	114	1.7 (8.9)	101	-1.78	(-3.59, 0.04)	0.0585
Angina burden, median (IQR)						
Seattle Angina Questionnaire-7 summary score	117	60 (46, 75)	101	-1.87	(-5.20, 1.44)	0.2721
Health status, mean (SD)						
Health-related quality of life EQ-5D-5L score	117	0.83 (0.16)	103	-0.007	(-0.03, 0.02)	0.5925
Patient assessed EQ-5D-5L score	117	70 (20)	103	-2.08	(-5.34, 1.18)	0.2148
Illness perception, median (IQR)						
Brief Illness Perception Questionnaire score	117	40 (30, 46)	102	0.17	(-1.86, 2.22)	0.8691

Anxiety and depression, mean (SD)						
PHQ-4 total score	117	2 (3)	103	0.01	(-0.53, 0.55)	0.9611
Treatment satisfaction questionnaire for medication						
Effectiveness scale	117	63 (19)	102	-1.03	(-4.93, 2.89)	0.6083
Convenience scale	117	84 (16)	102	-0.58	(-3.05, 1.92)	0.6498
Satisfaction scale	117	69 (23)	102	-2.76	(-6.66, 1.14)	0.1693

*Time (s) to 1 mm ST-depression and time (s) to 75% of max age-related heart rate during exercise were analyzed based on survival with no baseline adjustments using a mixed effects cox model with fixed effects of treatment, visit and random effect of participant and hazard ratios are shown rather than effect estimates. Of 118 participants who were randomized, 117 participants had an exercise test at baseline, 103 participants had an exercise test at baseline and at least one exercise test during follow up after either placebo or zibotentan and were included in the primary analysis, and 89 participants had complete data.

Table 3. Participants (n) experiencing adverse events during treatment with zibotentan and placebo.

Adverse event	Participant, n	Placebo		Zibotentan		p-value
		All Events n	Participants n (%)	All Events n	Participants n (%)	
Randomized participants, n (%)	118 (100)		118 (100)		118 (100)	
Any event	77 (65.3)	17	17 (14.4)	71	71 (60.2)	<0.0001
Peripheral edema	14 (11.9)	1	1 (0.8)	13	13 (11.0)	0.0024
Fatigue	4 (3.4)	0	0 (0.0)	4	4 (3.4)	0.1303
Joint swelling	8 (6.8)	1	1 (0.8)	7	7 (5.9)	0.0721
Dizziness	4 (3.4)	0	0 (0.0)	4	4 (3.4)	0.1303
Headache	43 (36.4)	7	7 (5.9)	46	40 (33.9)	<0.0001
Nasal congestion	33 (28.0)	4	4 (3.4)	32	29 (24.6)	<0.0001
Breathlessness	6 (5.1)	0	0 (0.0)	7	6 (5.1)	0.0387
Withdrawal during treatment	25 (21.2)	-	9 (7.6)	-	16 (13.6)	0.2044
Serious adverse event*	12 (10.2%)	5	4 (3.4)	7	7(5.9)	0.5368
Death	0	-	0	-	0	-

The table lists the number of participants experiencing adverse events and the total of all events. The chi-squared test compares subjects with an event occurring during each treatment, where percentages are given out of all randomized participants irrespective of any post randomization withdrawals. No correction for the non-independence of participants in each treatment group due to crossover is being made and instead groups are being considered as independent of each other. One serious adverse event occurred in the screening period, and another occurred during the placebo run-in period.

Methods

Trial design and oversight

Design

The study was designed as a registry-based randomized trial (Figure 1 and Supplementary Figure 1). The registry population included individuals with microvascular angina who provided written informed consent at visit 1. The trial involved a prospective, multicenter, randomized, double-blind, placebo-controlled, sequential crossover design to assess the effects of zibotentan 10 mg or matched placebo, once daily for 12 weeks.[16] The trial was designed to assess the superiority of the addition of oral zibotentan to guideline-indicated therapy as compared with placebo and guideline-indicated treatment for patients with microvascular angina.[31,32] The trial population included participants who fulfilled eligibility and who then pass through genotype filtering, which involved filtering out some individuals with the AA alleles of the rs9349379 SNP, and who were finally randomized at visit 3.

Clinical information, patient reported outcome measures (PROMS), and a blood test were acquired at enrolment (visit 1) and again at the end of the medical optimization period (visit 2), after a 3-week placebo run-in (visit 3, baseline), and at the end of treatment period 1 (visit 4) and treatment period 2 (visit 5, end of trial). A genomic blood test was obtained at visit 1. An exercise tolerance test was obtained on four occasions including visits 1, 3, 4 and 5. An optional imaging study involved cardiovascular MRI at visits 3, 4 and 5.

Oversight

The trial was co-sponsored by NHS Greater Glasgow & Clyde and the University of Glasgow and funded by the Medical Research Council (MR/S018905/1) of the UK Research and Innovation (UKRI).

The trial conduct was overseen by a Trial Steering Committee and an Independent Data and Monitoring Committee. The Trial Steering Committee included an independent chairperson, two independent physicians, the chief investigator, a representative from the sponsor and a patient representative. This committee provided overall supervision of the trial to ensure that it was conducted in accordance with the principles of Good Clinical Practice and the relevant regulations. Decisions about continuation or termination of the trial or substantial amendments to the protocol were the responsibility of the Trial Steering Committee who advised the sponsor.

An Independent Data Monitoring Committee included two independent medical experts and an independent biostatistician. They received unblinded reports of trial safety data and progress. This committee could recommend to the Trial Steering Committee and the sponsor that the trial should stop in the event of concerns about patient safety.

Since the trial involved a crossover design and was not designed to assess between-group differences in clinical endpoints, a Clinical Event Committee was not required.

The trial was undertaken in compliance with the approved protocol and the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928), Good Clinical Practice (GCP) guidelines, the Sponsor's (Standard Operating Procedures (SOPs), and other regulatory requirements, as amended.

AstraZeneca provided the investigational medicinal product (IMP) through the Open Innovation program [AstraZeneca Open Innovation Internet]. AstraZeneca reviewed and approved the protocol. AstraZeneca had no role in the study design and were not involved in the preparation, drafting or editing of the manuscript. AstraZeneca conducted a factual accuracy check of this manuscript, but any decisions to incorporate comments were made solely at the discretion of the authors. All the authors reviewed and approved the manuscript and they assume full responsibility for the accuracy and completeness of the data and for the fidelity of the trial to the protocol (Supplement).

Setting

The study involved twelve hospitals in the United Kingdom (Supplementary Table S7): Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde Health Board (Glasgow); Glenfield Hospital, University Hospitals of Leicester NHS Trust (Leicester), Oxford University Hospitals NHS Foundation Trust and Division of Cardiovascular Medicine at the University of Oxford, John Radcliffe Hospital (Oxford); Royal Papworth Hospital NHS Foundation Trust (Cambridge); Blackpool Victoria Hospital, Blackpool Teaching Hospitals NHS Foundation Trust (Blackpool); Royal Free London NHS Foundation Trust (London); Leeds General Hospital, Leeds Teaching Hospitals NHS Trust (Leeds); Guy's and St Thomas' NHS Foundation Trust (London); Hammersmith Hospital, Imperial College Healthcare NHS Trust Hospitals Foundation NHS Trust (London); Royal Devon University Healthcare NHS Foundation Trust (Exeter); Newcastle Hospitals NHS Foundation Trust (Newcastle); and the Basildon University Hospital, Mid and South Essex NHS Foundation Trust (Basildon).

Participant identification

Patients who had an established diagnosis of microvascular angina were prospectively screened in secondary care. Patients were identified from clinical databases, clinics and clinical procedure lists. The clinical pathways included (1) out-patient clinics; (2) diagnostic stress tests, e.g. stress perfusion cardiovascular MRI, stress echocardiography, stress nuclear imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) or an exercise ECG leading to a diagnosis of microvascular angina; (3) invasive or computed tomography (CT) coronary angiography.

Informed consent

Written informed consent was an eligibility criterion and consent was required before any study assessments were undertaken. The informed consent form covered enrolment into the registry, the genetic screening test for eligibility, the screening period, the run-in-period and the randomized trial. Additionally, participants were invited to provide optional consent for follow-up using linkage of electronic health records in the longer term. Ongoing consent was confirmed during each study visit. Should consent be withdrawn, then the participant was withdrawn from the study without affecting the individual's standard of care.

Eligibility criteria

The inclusion criteria were: (1) age ≥18 years; (2) microvascular angina; (3) able to comply with study procedures; and (4) written informed consent. Microvascular angina was described by the Coronary Vasomotion Disorders International Study (COVADIS) group criteria (Supplementary Table S8).[33] Participants in this trial had to fulfil criteria (1) and (2). Probable microvascular angina

was defined as having 3 of the 4 COVADIS criteria and definite microvascular angina requires all 4 criteria.

The exclusion criteria were: (1) exercise tolerance >540 seconds in men and >430 seconds in women (i.e. actual exercise duration (s) achieved on the Bruce protocol commensurate with predicted), or, lack of anginal symptoms and/or ST-segment depression (0.1 mV) limiting exercise; (2) non-cardiovascular exercise-limiting problem e.g. morbid (or severe) obesity (body mass index (BMI) ≥40.0 kg/m2); (3) genotype not available; (4) women who are pregnant, breast-feeding or of child-bearing potential (WoCBP) without a negative pregnancy test and who are unwilling or unable to follow the reproductive restrictions (defined in the Supplement) and use highly effective contraception (defined in the Supplement) for the duration of the trial treatment and 30 days after last dose of trial drug; (5) men who are sexually active with a WoCBP who are unwilling to use condoms or other highly effective methods of contraception for the duration of trial medication and for 14 weeks after the last dose of trial medication; (6) heart failure (New York Heart Association Grade ≥II i.e. mild symptoms and slight limitation during ordinary activity; (7) recent (<6 months) myocardial infarction; (8) a history of epilepsy, other CNS adverse events, neurologic symptoms or signs consistent with spinal cord compression or CNS metastases; (9) moderate or more severe renal impairment (glomerular filtration rate (GFR) < 45 mL/min); (10) liver disease with a Child-Pugh score of A (5-6 points) or higher; and (11) participation in another intervention study involving a drug within the past 90 days or 5 half-lives whichever is longer (co-enrolment in observational studies is permitted).

The eligibility criteria for the cardiovascular MRI study are described in the Supplement.

Genetic enrichment

The chronic elevation of circulating endothelin-1 in microvascular angina may be influenced by genetic factors. A genotype-based selection for the AA, AG and GG alleles of the rs9349379 SNP endothelin-1 gene enhancer was undertaken to achieve a G-allele frequency of at least 50% for the rs9349379 SNP in the study population. Participants and investigators were blinded to genotype.

Participants who were eligible on clinical grounds underwent PHACTR1 genotyping for rs9349379. A whole blood sample (EDTA, 4.0 ml; bar-code identifier) for genotyping was obtained at visit 1 and shipped from the site in a Royal Mail Safebox™ to the Genetics Laboratory, Queen Elizabeth University Hospital in NHS Greater Glasgow and Clyde Health Board. Genomic DNA was extracted and initially stored at 4°C until testing was completed. A Sanger sequencing approach, using the forward primer "F_GTGCAATTCTCCAAGGCTCC" and the reverse primer "R_TTTAAAACTCAGCTCGTGGAAAA", was used to sequence part of intron 3 of the *PHACTR1* gene to determine the genotype of the rs9349379 SNP. When the participant's genotype was established, the DNA sample was then archived at -20°C. Genotype results were prospectively entered into the electronic case report form in the database managed by the Robertson Centre for Biostatistics (clinical trials unit).

A predefined genotype selection algorithm was applied by the lead statistician (A.M.) in the clinical trials unit. The sampling rates of AA and AG patients were set before the start of the trial, based on expected allele frequencies. Participants with the GG genotype continued to the run-in period, whereas only a proportion of those with the AA and AG genotypes were invited to proceed. This approach boosted the relative frequency of the G genotypes in the randomized trial population, with the objective of achieving at least 50% G allele frequency. The enrichment process was balanced against the rate of recruitment into the trial, and if the recruitment fell behind timelines,

then the sampling rates could be modified to increase the number of randomized participants, at the expense of having a lower than 50% G allele frequency. The genotype distribution was prospectively monitored by the Trial Steering Committee and the Independent Data Monitoring Committee.

If a consented patient was found to be ineligible for the run-in/treatment period of the randomized trial, they remained in the study population, including consent for long term follow up using electronic health record (HER) linkage.

Research schedule

The protocol included five visits. The research procedures involved prospective collection of clinical data and a time-course of investigations.

Visit 1 - Medical optimization

The first visit involved a clinical assessment to confirm eligibility, PROMS, a blood test (including for genomic biomarkers and pharmacodynamics), and an exercise tolerance test.

Since microvascular angina is a chronic condition, most patients were already established on maintenance drug therapy. However, we anticipated that in some cases, cardiovascular risk factors, including blood pressure and lipids, may not have been optimally controlled. The healthcare staff assessed whether the wellbeing of the study participant could be improved through standard of care measures in line with practice guidelines.[31] Modifiable cardiovascular risk factors, including blood glucose, glycated hemoglobin, lipids, blood pressure and body weight were assessed, and optimization measures were implemented according to a standard operating procedure involving pharmacological and non-pharmacological measures.[31] The optimization period was limited to 6 weeks. If angina drug therapy was changed, then a period of 4 weeks was required before

proceeding into the treatment run-in period. When the angina medication, including the drug type and dose, remained stable for 4 weeks and the participant's symptoms were stable in the opinion of the investigator, then the participant could proceed to the next treatment run-in period starting from visit 2. Following optimization, the angina therapy remained the same following entry into the treatment run-in period (Visit 2) and thereafter.

Visit 2 - treatment run-in

The second visit occurred 6 weeks after enrolment and involved a clinical assessment, PROMS, a pregnancy test for women of child-bearing potential, a blood test, and dispensing of trial medicine.

Participants entered a three-week run-in period from visit 2 to visit 3. Participants received a once daily single blind placebo medication. The purpose of this run-in period was to give the participants experience of taking investigational medication. Since assessments of adherence with investigational medication and safety were objectives of the trial, a run-in period with zibotentan was not included since individuals who might be intolerant of zibotentan could have withdrawn before proceeding into period 1. The trial was designed to provide representative data on the experience of the participants when receiving the trial medication.

Visit 3

The third visit represented the baseline for the randomized clinical trial. Participants who were selected based on genotype criteria proceeded to visit 3. During this visit, clinical information, PROMS, a blood test, and exercise tolerance test were performed.

Adherence with trial medication

Adherence with trial medication during the run-in and subsequent visits was documented. Adherence with trial medication (defined as >80%) was assessed by (1) participant-reported

adherence with therapy, calculated by the number of tablets taken during the current treatment period compared with the number expected to have been taken (accounting for any clinician advised dose reductions documented in the Medication Termination/Interruption/Dose Frequency Log), and (2) a tablet count based on the return of any remaining tablets at the end of the treatment period, and (3) the date and time of the last dose prior to the visit.

Randomization

Randomization occurred during visit 3, after completion of a single-blind placebo run-in.

Eligibility criteria were reassessed before randomization and only participants in whom eligibility had been re-confirmed and who were adherent with the trial medication during the run-in period with were eligible for randomization.

Treatment period

Eligible and consenting patients were randomized with equal probability to the two groups reflecting the sequential order of zibotentan or placebo in Period 1 and Period 2, respectively: Group 1 = zibotentan in Period 1 then placebo in Period 2; Group 2 = placebo in Period 1 then zibotentan in Period 2. The randomization was minimized with respect to a concomitant history of vasospastic angina, study site, genotype, and sex in blocks of size 10. Specifically, each participant was randomized to receive zibotentan 10 mg daily for 12 weeks and then placebo for 12 weeks, or placebo for 12 weeks followed by zibotentan 10 mg daily for 12 weeks.

Blinding

The trial had a double-blind design. Specifically, the trial participants, carers, investigators, and sponsor were blinded to the treatment allocation. Outcome assessments were undertaken by staff who were also blinded.

Breaking of the study blinding in an emergency was only to be performed where knowledge of the treatment was essential for patient care. Any emergency unblinding would occur via a telephone Interactive Voice Response System (IVRS). Unblinding the treatment allocation may be required when reporting suspected unexpected serious adverse reactions (SUSARs) to the regulatory authorities. This was performed by the sponsor pharmacovigilance office without unblinding the investigators or the participants.

Visits 4 and 5

The fourth and fifth (final) visits occurred at the end of the first and second treatment periods. The assessments that were undertaken during visit 3 were repeated during visits 4 and 5.

During the first treatment period, participants were assigned in random order to take either 10 mg of zibotentan daily or matched placebo for 12 weeks and then following Visit 4, the trial medication was switched to placebo or 10 mg of zibotentan daily for 12 weeks.

Exercise tolerance test

Rationale

Exercise testing using the Bruce protocol is a standard of care in clinical cardiology and evidence-based for assessing functional capacity, susceptibility to effort-related anginal symptoms and myocardial ischemia in patients with stable angina.[34] Treadmill exercise time (s) is a reproducible outcome measure, although the severity of myocardial ischemia may attenuate during repeated testing with an approximately 10% test-retest variability.[35–40] In a study of repeated exercise testing in older women the intra-class correlation coefficient of exercise duration was 0.88.[37] In a clinical trial involving 33 patients with microvascular angina, there was 100% compliance with serial exercise tests (n=4 per subject).[41] In developing the design of the trial, participant feedback during

Patient and Public Involvement (PPI) meetings supported the use of exercise tests based on safety and tolerability. Treadmill exercise testing is also endorsed by regulators, such as the Federal Drug Administration, for assessing the efficacy of angina medications.

Exercise test protocol

The full Bruce protocol for maximal exercise testing was used according to published standards from the American Heart Association (AHA) Scientific Statement.[34] The Bruce Protocol involves 3-minute periods of incremental levels of exercise undertaken on a treadmill at a walking pace.[42] A non-cardiac reason, e.g. arthritis, that limits exercise duration to less than predicted was an exclusion criterion. The same exercise test equipment was used during repeated visits for each participant.

Detailed information on the exercise test protocol is provided in the Supplement. Prior to the exercise test taking place, site staff advised participants to abstain from taking their angina medication for 24-hour hours before the study visit and be fasting be fasting for 3 hours. The electrocardiograph settings included ST-amplitude measurements at the J-point and at J + 80 milliseconds for assessing change during exercise.

A target minimum increase in heart rate of 85% of the age-predicted maximum heart rate was recommended. The participant's assessment of the intensity of physical activity was rated using the Borg Scale for Rating Perceived Exertion. The response was recorded at the point when the exercise test ended. The absolute and relative criteria for stopping an exercise test were predefined (Supplement).

Participant responses were recorded by the attending staff, namely, (1) perceived exertion, (2) angina (other criteria are listed in the AHA Scientific Statement). A Borg Scale stopping criterion of ≥13 (somewhat hard) out of 20 was adopted. A Borg Scale of >15 represents achievement of NMED-A129750A

768 the anaerobic threshold. The four-level Angina Scale for Exercise Tolerance Testing was used to
769 rate and report anginal symptoms during exercise. A widely established stopping criterion for
770 anginal symptoms is level 2 of 4 (some pain, moderately severe and definitely uncomfortable but
771 still tolerable). These scales were displayed to staff and participants to standard-set the stopping
772 criteria for the sites. The scales were displayed in front of the treadmill to standard-set the stopping
773 criteria for the sites. The Bruce protocol involves graded exercise testing using a treadmill. The
774 protocol involves stages each of 3-minutes duration. Stage 1 begins at a walking pace (1.7 miles
775 per hour) with a 10% gradient. After 3 minutes, Stage 2 begins with an increase in walking speed
776 to 2.5 miles per hour at a gradient of 12%. After 6 minutes, Stage 3 begins with the ramp speed
777 increasing to 3.4 miles per hour with a steeper gradient of 14%. Stage 4, beginning at 12 minutes,
778 involves a ramp speed of 4.2 miles per hour and a gradient of 16%.

779 Staff completed a report form for each exercise test. The information included the treadmill model,
780 the speed (mph) and slope (gradient) of the treadmill at the start and end of the test, total exercise
781 time, heart rate and blood pressure at the start and end of the test, an indication if the test was
782 stopped earlier than anticipated (age and sex-predicted exercise duration) and if so, then the reason
783 for stopping, including chest tightness, breathlessness, fatigue, dizziness, palpitations and non-
784 cardiac symptoms (e.g. leg pain). The Angina Scale for Exercise Tolerance Testing, Angina Index
785 and the Borg Scale for Rating of Perceived Exertion were also documented (Supplement). The
786 electrocardiograms (ECGs) were acquired at rest with the participant standing and then again at 1-
787 minute intervals during exercise and after the end of exercise at 1-minute intervals for 3 minutes
788 until the end of the test. They were de-identified and transferred securely to the University of
789 Glasgow Electrocardiology Core Laboratory at Glasgow Royal Infirmary for visual review and

measurement checking. The ECG features were predefined according to contemporary criteria.[34] The ECG review form is provided in the Supplement.

A basic ECG interpretation, e.g. normal, LBBB, ischemic ST-T changes, as well as a rhythm interpretation, were made. Each ECG was assessed by two reviewers acting together. Selected measurements, e.g. change in ST amplitude at J + 80 msecs were transferred to a spreadsheet for statistical analysis, with particular attention being paid to serial ST-T changes in the sequentially acquired ECGs. An automated interpretation of the ECG was occasionally available but was not required. Hence, the ECG variables were based on a combination of automated ECG measurements, and changes over exercise, including predefined features determined by expert core laboratory staff (P.M., J.K.) review.[34]

Blood samples

To investigate the safety of zibotentan and the effects on cardiovascular, inflammation and metabolic pathways, and circulating concentrations of zibotentan, blood samples were collected at enrolment (visit 1) and at all subsequent visits (2-5). Specifically, blood samples were collected at enrolment (visit 1), the end of the medical optimization period (visit 2, weeks 0 – 6), baseline (visit 3, week 7 – 9, end of the treatment run-in), and the end of period 1 (visit 4, week 10 – 22) and period 2 (visit 5, week 23 – 34). Blood samples collected into EDTA (for biomarkers) were handled according to a sample handling manual which was provided to all sites. The blood samples were centrifuged locally and the plasma was separated and frozen at -80ºC within 2 hours of sampling. Residual samples were transferred to the NHS Glasgow Biorepository for storage at the end of the study.

Blood samples for safety analyses

Since limited information is available on the safety of zibotentan in non-oncology populations, blood samples were collected at each of the visits to enable real time local laboratory analysis throughout the study. The analyses were undertaken in United Kingdom Accreditation Service (UKAS) accredited laboratories at the sites. The tests included hematology (hemoglobin (Hb), white cell count, platelet count), renal function (potassium, glucose, urea, creatinine, and glomerular filtration rate (eGFR) estimated using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation,[43] liver function (alanine transaminase, aspartate transaminase, alkaline phosphatase, albumin, bilirubin), lipid profile (total cholesterol, high-density lipoprotein, low-density lipoprotein cholesterol, very-low density lipoprotein cholesterol, cholesterol/high density lipoprotein ratio, triglycerides), glycated hemoglobin and N-terminal (NT)-pro hormone brain natriuretic peptide (NT-proBNP) or brain natriuretic peptide).

Pharmacodynamics

In order to research the mechanisms of any potential benefit of oral zibotentan, the within-subject treatment-related changes in the circulating concentrations of cardiac injury (NT-proBNP, troponin I), inflammation (C-reactive protein, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion protein 1 (VCAM-1) and interleukin-6 (IL-6)), metabolism (glucose, total cholesterol, high-density lipoprotein, triglyceride, uric acid), endothelial activation (mid regional pro-adrenomedullin (MR-proADM), collagen turnover (amino terminal peptide of type III procollagen), fluid homeostasis (copeptin), renal function (cystatin C, serum creatinine, eGFR),[43] and their changes over time, were investigated. The measurements were undertaken in a central laboratory in the University of Glasgow, blinded to the other clinical data.

EDTA plasma samples (and aprotinin-treated plasma) for research analyses were stored at -80ºC in the Glasgow Biorepository until batch analysis at the end of the study. The biochemical analyses were performed in the GlasBRU Laboratory, British Heart Foundation Glasgow Cardiovascular Research Centre in the University of Glasgow. EDTA plasma samples were stored to analyze high-sensitivity cardiac troponin I and NT-proBNP on first thaw. Troponin I (ng/ml) and NT-proBNP (pg/ml) were measured in blood samples collected at Visit 1 and Visit 2. NT-proBNP (pg/ml) was measured to provide a biochemical measurement of left ventricular remodeling (within-subject change in NT-proBNP at follow-up from baseline) and troponin I to provide a biochemical measurement of myocardial necrosis.[44]

For measurement of both and high sensitivity cardiac troponin I (i1000SR ARCHITECT, Abbott Diagnostics, UK) and NT-proBNP (e411, Roche Diagnostics, UK), the laboratory used an automated method calibrated and quality controlled using the manufacturers reagents. The laboratory also participated in the National External Quality Assurance Scheme (NEQAS) for these assays.

Glucose, cystatin-C, C-reactive protein, uric acid and lipids including total cholesterol, HDL-cholesterol and triglycerides (c311, Roche Diagnostics, UK) as well as copeptin and MR-proADM (B·R·A·H·M·S Kryptor, Themofisher Scientific, UK) were measured using automated methods using the manufacturers calibrators and quality control materials. ICAM-1 VCAM-1 and IL-6 (Ella Protein Simple, Bio-Techne, UK), P3NP (ELISA, Cisbio Assays, France),endothelin-1 (Quantikine ELISA, Bio-Techne, UK), and big endothelin-1 (Biomedica Immunoassays, Austria) were measured by immunoassays using the manufacturers calibrators and quality controls. All assays were conducted in EDTA plasma, apart from big endothelin-1 and endothelin-1, which was conducted in aprotinin protease inhibitor treated plasma.

Pharmacokinetics

Blood samples were obtained at visits 3, 4 and 5 to measure steady-state plasma concentrations of zibotentan. The blood test was scheduled at a single time-point before dose, i.e. a trough, pre-dose blood sample. The trial medication was withheld on the day of the visit until the blood sample was obtained.

Zibotentan (ng/mL) was measured in plasma lithium heparin using liquid chromatography with tandem mass spectrometry (York Bioanalytical Solutions Limited). Validation of the assay in human plasma was undertaken using calibration standards and quality control samples. Long term stability of plasma samples stored at -20°C was assessed. For a nominal zibotentan of 1.50 ng/mL and 400 ng/mL in 6 human plasma samples stored at -20°C for 10 months, the mean (ng/mL), precision coefficient of variation (%) and difference from nominal (%) were 1.44, (4.8), (-4.0) and 396, (8.5), (-1.0), respectively.

Cardiovascular magnetic resonance imaging

Overview

Myocardial perfusion is commonly impaired in patients with microvascular angina and cardiovascular MRI provides a quantitative measure of myocardial blood flow. The rational for undertaking the MRI study was to determine whether, compared with placebo, treatment with zibotentan improves myocardial blood flow.

Participants underwent MRI on the same scanner using an identical imaging protocol at each visit. Adenosine stress perfusion MRI was scheduled for 3 occasions (Visits 3, 4 and 5). The rationale for undertaking MRI at these time-points was to assess myocardial blood flow at baseline and again following treatment with zibotentan or placebo for 12 weeks. Since undertaking stress

perfusion cardiovascular MRI on three occasions may not be feasible for some participants, the MRI protocol was optional. Social restrictions during the COVID-19 pandemic limited access to the MRI protocol (Supplementary Table S10).

Cardiovascular MRI was undertaken at five sites including the University of Glasgow Imaging Centre of Excellence, Queen Elizabeth University Hospital, the Royal Free Hospital, London (1.5 Tesla, Siemens), the Royal Papworth Hospital, Cambridge (1.5T Siemens), the University of Oxford Centre for Clinical Magnetic Resonance Research (3.0T, Siemens) and Leeds General Infirmary (Supplementary Table S9).

Cardiovascular MRI acquisition

The participants were scanned using a clinical research-dedicated MRI scanner (MAGNETOM, Siemens Healthineers, Erlangen, Germany) at each site (Supplementary Table S9). Typically, two 18-channel surface coils were placed anteriorly and a 32-channel spine coil was placed posteriorly.

The MRI protocol included:

- standard localizers - three orthogonal 'white blood' sequences (axial, sagittal and coronal) and long axis cine imaging (vertical long axis, horizontal long axis and 3 chamber view) to identify the left ventricular outflow tract (LVOT). The localizer acquisitions were conducted according to the site's best practice,
- cine imaging for cardiac dimensions and function including 4- and 3-chamber long axes
- T1-mapping (modified look-locker inversion recovery sequence (MOLLI) 3-level, base, mid, distal),

- adenosine stress imaging of myocardial blood flow; intravenous gadobutrol (Gadovist®, Bayer; 1.0 mmol/ml solution for injection) contrast media administration at a dose of 0.05 mmol/kg at 4 ml/s using an automated pump injection system,
- cine imaging of the left ventricular short axis stack,
- rest imaging of myocardial blood flow; intravenous gadobutrol (Gadovist®, Bayer; 1.0 mmol/ml solution for injection) contrast media administration at a dose of 0.05 mmol/kg at 4 ml/s, then a top-up intravenous dose of 0.05 mmol/kg through the pump injector at 4 ml/s; total dose 0.15 mmol/kg
- late gadolinium enhancement imaging,
- post-contrast T1 mapping (MOLLI).

Balanced steady state free precession sequences were used to acquire ventricular cine imaging in three long axis planes, followed by a short axis stack from the apex to the atrio-ventricular ring, each with 30 phases. Images were obtained using retrospective electrocardiogram-gating at end-expiration. Typical scan parameters at 3.0 Tesla were: voxel size 2.0 x 2.0 x 8.0 mm; repetition time (TR)/ echo time (TE), actual TR = 30 ms (35 ms maximum) /1.12 ms; flip angle 55o, matrix 192 x 192 pixels; slice thickness 8 mm, with 2 mm gap.

Three left ventricular short axis (basal, mid and apical) and orthogonal long axis T1 motion-corrected, optimized, MOLLI recovery sequences before contrast media administration and then again 15 minutes after contrast administration using the following typical parameters at 3.0 Tesla: FOV 360 x 306 mm, slice thickness 8.0 mm, voxel size: 1.9 x 1.9 x 8.0 mm, TR 341 ms, TE 1.01 ms, flip angle 35 degrees, minimum T1 100 ms, inversion-time (TI) increment 80ms, bandwidth

1085Hertz/pixel. The T1 mapping protocols used 5s(3s)3s and 4s(1s)3s(1s)2s sampling, pre-contrast and post-contrast, respectively

Late gadolinium enhancement images including three long axis acquisitions and a short axis stack were acquired 15 minutes after intravenous injection of 0.15 mmol/kg of gadobutrol (Gadovist®, Bayer) contrast media administration using segmented phase-sensitive inversion recovery turbo fast low-angle shot. A full left ventricular stack, aligned to the T1 maps (and cines), and including at least one long axis view (vertical long axis, horizontal long axis or 3 chamber view) was acquired. Phase-sensitive inversion recovery MRI techniques reduce variability relating to myocardial nulling which is required for late gadolinium enhancement imaging of infarct vs. unaffected myocardium. If a phase-sensitive protocol was not used, then a MOLLI time scout was performed prior to using an inversion recovery turbo gradient echo sequence. Phase swaps were performed where appropriate to rule out artefact. Typical imaging parameters at 3.0 Tesla were: matrix = 192 x 111, flip angle = 14°, TE =1.05 ms, bandwidth =1085 Hz/pixel, echo spacing = 2.1 ms and trigger pulse = 1 ms. The voxel size was 1.9 x 1.9 x 7 mm^3. Inversion times were individually adjusted to optimize nulling of visually normal myocardium (typical values, 250 to 350 ms).

In the event of inadequate breath-holding during late enhancement imaging, then a single shot technique or MOCO phase-sensitive inversion recover late gadolinium enhancement technique was used.

Typical late enhancement imaging parameters: Matrix 192 x 256 pixels; flip angle 25o; TE 3.36 ms; bandwidth 130 Hz/pixel; echo spacing 8.7ms and trigger pulse 2. The voxel size was 1.8 x 1.3 x 8 mm. Inversion times were individually adjusted to optimize nulling of apparently normal myocardium (typical values, 200 to 300 ms).

Myocardial perfusion imaging

The pulse sequence acquisition was selected according to the field strength of the MRI scanner. If perfusion imaging was acquired at 1.5 Tesla, then a SSFP pulse sequence was used. If imaging was acquired at a 3.0 Tesla, then a fast low-angle shot (FLASH) pulse sequence was used. The perfusion method consisted of a dual sequence approach. The first pulse sequence acquisition involved a low resolution acquisition to estimate the arterial input function (AIF) from the dynamic signal intensity change in the left ventricular blood pool. The second pulse sequence acquisition was undertaken for higher resolution imaging of signal intensity changes in the left ventricular myocardium. Typically, linear order base to apex short axis scans were prescribed using a long axis cine in a systolic phase. The perfusion images were acquired more in systole. In this way, acquisition of the left ventricular outflow tract was avoided. Vasodilator stress was achieved by intravenous infusion of adenosine at a dose of 140 μg/kg/min for 4 min (increased to 210 μg/kg/min for a further 2 minutes in the absence of symptoms or an increase in heart rate of <10 beats per minute). At peak stress, a gadolinium-based contrast agent (Gadovist®, Bayer Healthcare) was injected using an automated pump injector at 4 ml/s at a dose of 0.05 mmol/kg followed by rest first-pass myocardial perfusion imaging (Gadovist® (Bayer Healthcare) injected at 4 ml/s at a dose of 0.05 mmol/kg,) at least 10 minutes later.

Typical first-pass imaging parameters for a saturation recovery with an inversion pulse sequence: myocardial slice parameters - T1 105 ms for SSFP at 1.5T, 110 ms for FLASH at 3.0T; TR/TE = 142/1.04 for 1.5T SSFP; TR/TE = 146/1.0 for 3.0T FLASH; acquisition window 5000 ms; one concatenation; 3 short axis slices. If three slices could not be acquired within the R-R cycle then 2 concatenations were used. A minimum of 60 measurements was acquired, increasing to 90 measurement if the cardiac output was low. Imaging was initiated and then, after 8 heart beats, the

intravenous gadolinium contrast media bolus was administered. If 2 concatenations were used, then 45 measurements were acquired and the gadolinium bolus was administered after 16 heartbeats.

Considering practical steps, the participants were invited to abstain from caffeine-containing beverages or foodstuffs for 24 hours and vasoactive medications for 48 hours prior to the MRI examination. At the start of the MRI scan, heart rate and blood pressure were automatically acquired at rest and again during the adenosine infusion (140 μ/kg/min). Heart rate and blood pressure were acquired at 2-minute intervals. If no symptoms occurred and the heart rate increase was <10% (or systolic blood pressure decreases <10mmHg), then the adenosine infusion rate was increased to 170 mcg/kg/min. If after a further 2-minutes no symptoms had occurred and the heart rate increase was <10% (or systolic blood pressure decrease <10 mmHg), then the adenosine infusion rate was increased to 210 μ/kg/min for a further 2-minutes, and then the gadolinium bolus was administered. The patient was advised to breathe normally and shallow during the pump discharge and perfusion imaging acquisition.

Cardiovascular MRI analysis

The MRI scans were de-identified, archived as .dat files and uploaded to the electronic database. A image analyst (A.M.) with 3 years of MRI experience, blind to treatment assignment, analyzed all the MRI data which were subsequently reviewed by C.B. (with >20 years of MRI experience) who was also blinded. At the sites, the cardiovascular MRI scans were reviewed according to local standards of care.

Reference ranges

Contemporary, local reference ranges were derived using the 3T MRI scanner (MAGNETOM Prisma, Siemens Healthineers, Erlangen, Germany) by A.M. and C.B. as part of standard quality assurance in the University of Glasgow Clinical Imaging Research Facility. The scans were analyzed using dedicated software (cvi42 software for Cardiovascular MRI, version 5.10, Circle Cardiovascular, Canada) to derive mean, upper, and lower reference ranges. This software package was also used for the cardiovascular MRI analyses of the trial participants.

Ventricular function

The imaging analyses were performed utilizing dedicated cardiovascular MRI software (cvi42 software (version 5.10, Circle Cardiovascular, Canada)). Routinely reported measures of left ventricular and right ventricular function were carried out according to guidelines of the Society of Cardiovascular Magnetic Resonance. Ventricular endocardial and epicardial contours were manually drawn at end-diastole and end-systole, which was deemed to be the phase with the smallest blood pool cavity. Papillary muscles were excluded from myocardial mass and included in volumes. Global left ventricular strain (circumferential, longitudinal, and radial) and global right ventricular strain (longitudinal) were derived using the software's tissue tracking module to determine peak values for each parameter. Atrial areas were manually drawn on 4-chamber horizontal long axis views at atrial diastole (defined with respect to mitral valve closure).

Parametric mapping

Motion corrected T1 scans were analyzed using dedicated software (cvi42 software (version 5.10, Circle Cardiovascular, Canada). The individual images were reviewed to ensure that motion correction was successful. Parametric maps were generated and goodness-of-fit (R^2) was reviewed.

1006 Myocardial segments with artefact that impaired diagnostic quality and/or measurement accuracy,
1007 including pixels/segments with R^2<0.99, were excluded from analysis.

1008 Epi- and endocardial borders were manually drawn and care was taken to include only myocardial
1009 tissue with a 10% epi- and endocardial offset applied to avoid partial volume effects. The right
1010 ventricular insertion points were used to segment the myocardium as per the American Heart
1011 Association's 16 segment left ventricular model53. For blood pool pre- and post-contrast T1
1012 regions-of-interest were drawn within the left ventricular cavity on the 3 short axis maps, with care
1013 taken to avoid artifact and papillary muscles.

1014 Hematocrit values were acquired the day of the visit.

1015 *Late gadolinium enhancement imaging*
1016 The archive of late gadolinium enhancement images for each participant was initially qualitatively
1017 reviewed for image quality and artefacts. The imaging set included the short axis stack and three
1018 or more orthogonal long axis views.

1019 The location of any late gadolinium enhancement was defined as sub-endocardial, mid-wall, sup-
1020 epicardial, or pericardial. Myocardial hyperenhancement in the basal septum was reviewed and if
1021 compatible with a septal perforator artery, this feature was excluded from the late gadolinium
1022 enhancement analyses. Hyperenhancement at right ventricular insertion points may be observed
1023 in individuals without cardiac disease. Therefore, this feature was not defined as pathological.

1024 The full width at half maximum (FWHM) technique was used to evaluate myocardial late
1025 gadolinium enhancement imaging. This method is reported to be highly reproducible,[45,46] and less
1026 conducive to 'over-reporting' the extent of late gadolinium enhancement when compared with
1027 other methods.[46,47] The FWHM technique is described as the optimal semi-automated

quantification method in risk-stratifying participants with suspected myocarditis, demonstrating the strongest association with major adverse cardiac events.[46] Late gadolinium enhancement was quantified as the percentage of left ventricular mass.

Automated quantitative perfusion mapping was performed using the method described by Kellman et al, including the Gadgetron framework.[48] The method involves a dual sequence approach for myocardial perfusion acquisition and arterial input function acquisition simultaneously, allowing for quantification of myocardial blood flow (ml/min/g) for each pixel of myocardium. The software allows for automated endocardial and epicardial contouring and segmentation using the American Heart Association 16- and 32- segment model. Automated endocardial and epicardial sub-segmentation is achieved by offsetting the epicardial border to 50%. The global myocardial blood flow is automatically calculated by the average of all the pixels and is measured at stress and rest. Global myocardial perfusion reserve (MPR) is the ratio of stress to rest myocardial blood flow. MPR can also be calculated specifically for the subendocardial layer (MPRENDO) (calculated by stress MBF_{ENDO}/ rest MBF_{ENDO}). Myocardial blood flow estimated using this method correlates with invasive measures of microvascular dysfunction and cardiovascular prognosis.[28,49]

Automated contouring was reviewed and quality-checked by the imaging cardiologists (A.M., C.B.). A quality assurance review was also undertaken (P.K.). If errors were noted, automated contouring was removed and replaced by manual contours.

Primary outcome

The primary outcome was treadmill exercise duration (seconds) using the Bruce protocol. The primary analysis estimated the mean within-participant difference in exercise duration following treatment with zibotentan versus placebo.

Secondary outcomes

The secondary outcomes included exercise test parameters, health status questionnaires, safety (frequency and severity of severe adverse events (SAEs) and adverse events), feasibility (withdrawal rate), and biomarkers of efficacy (pharmacokinetics, pharmacodynamics).

Exercise testing

Time to 1 mm ST-depression, seconds; Maximum ST-segment deviation, mV; Time to 75% of max age-related heart rate during exercise, seconds; Metabolic equivalent (METs), O2/kg/min; DUKE Score.[50]

Angina burden

The Seattle Angina Questionnaire-7 (SAQ-7) is a validated, disease-specific questionnaire that quantifies limitations caused by angina, the frequency of angina, treatment satisfaction, and subjective perception of quality of life.[51] Each component score is converted and collated to give a total score out of 100, where a higher score indicates better function. SAQ scores are independently associated with mortality, hospitalization, and resource use and useful as an outcome measure in clinical trials.[52–56] The SAQ is also a sensitive instrument in patients with microvascular angina.[57]

Health-related quality of life

Self-reported health status was assessed using the generic EuroQol (EQ)-5D-5L score and the patient assessed EQ-5D-5L score.[58]

Illness perception

Self-reported illness perception was assessed using the Brief Illness Perception Questionnaire score.[59]

Anxiety and depression

Anxiety and depression were assessed using the PHQ-4 scores.[60]

Treatment satisfaction questionnaire for medication

The Treatment Satisfaction Questionnaire (TSQM-9) provides information regarding medication side effects, effectiveness, convenience and overall satisfaction.[61]

The questionnaires were completed by participants at enrolment (visit 1) and 28–60 days after the last episode of hospital care (visit 2), blind to the other research data. The SAQ-7 is patient-reported measure of the burden of angina and it is established as an outcome measure in clinical trials.[56] Self-reported health status was assessed using the generic EuroQOL EQ-5D-5L questionnaire,[58] and the Brief Illness Perception Questionnaire (Brief-IPQ).[59] The Patient Health Questionnaire-4 (PHQ–4) was utilized to assess anxiety and depressive disorders.[60]

Exploratory outcome

A custom-developed questionnaire for symptoms and quality of life was completed at visits 1, 2, 3, 4 and 5. The responses in relation to treatment were assessed as an exploratory outcome. Participants will be invited to complete this diary each time symptoms occurred during the study.

Statistics

The statistical analyses were pre-defined in a Statistical Analysis Plan. Treatment effects on the primary, and continuous secondary outcomes, at the end of each period were analyzed using linear mixed effects models with fixed effects of baseline value, treatment, treatment period, and random effect of subject. Secondary outcomes of time to event data were analyzed using mixed effects cox model with fixed effects of treatment, visit and random effect of subject.

The analyses were undertaken intention-to-treat and are reported by treatment and period. Continuous variables are summarized by mean, standard deviation (SD), or Q1, median, and Q3. Categorical variables are summarized by N (%). No adjustments have been made for missing data or for multiple comparisons, and missing data are reported. Significance tests with 2-sided p-values are accompanied by confidence intervals for estimated effect sizes and measures of association. The widths of the confidence intervals have not been adjusted for multiplicity. A p-value of 0.05 was taken as statistically significant.

Sample size calculation

The primary outcome was the treadmill exercise time (seconds). A 30-second difference in exercise duration was considered clinically significant.[62] The standard deviation of the difference between two exercise test measurements was assumed to be 85 seconds.[63] To achieve 80% power to detect a mean difference of 30 seconds between treatments in a 2×2 crossover design and a level of significance of 0.05 (alpha error) required complete data in 65 participants. A minimum of 100 participants was intended to be randomized to allow for data quality issues and loss to follow-up. Considering the medical optimization period (visits 1 – 2) and the treatment run-in period (visits 2 – 3), a withdrawal rate of up to 30% was projected (n=42 participant), meaning 144 participants

were intended to start the treatment run-in period in order that 100 participants would enter into the randomized trial.

Pre-specified subgroup analyses were intended for sex, a history of vasospastic angina, genotype subgroups, tertiles of age, BMI, eGFR and systolic blood pressure.

Trial management and timelines

The trial was conducted in line with the current Guidelines for Good Clinical Practice in Clinical Trials. A Trial Management Group included those individuals responsible for the day-to-day management of the trial including the chief investigator, project manager and representatives from the sponsor and scientific laboratories. The roles of this group included facilitating the progress of the study, ensuring that the protocol was adhered to and taking appropriate action to safeguard participants and the quality of the study itself. Decisions about continuation or termination of the study or substantial amendments to the protocol were the responsibility of the sponsor. The Trial Management Group met at weekly intervals from May 2020 to October 2021.

COVID-19

Coronavirus disease 2019 (COVID-19) was recognized as a pandemic by the World Health Organization (WHO) on 11 March 2020. The timelines for healthcare restrictions in the National Health Service are described in Supplementary Table S10. In response to national guidance, recruitment to this study was suspended by the sponsor on March 16, 2020. The suspension was lifted on June 10, 2020 and the sponsor provided a guideline for mitigation measures in line with recommendations provided by the United Kingdom government.

Ethics

The study was approved by the UK National Research Ethics Service (Reference 19/NE/0110).

Registration

The ClinicalTrials.gov identifier is NCT04097314.

Data Availability Statement

Data requests will be considered by the Steering Group which includes representatives of the Sponsor, the University of Glasgow, senior investigators independent of the research team, and the chief investigator. The Steering Group will take account of the scientific rationale, ethics, logistics, and resource implications. Data access requests should be initially submitted by email to the Chief Investigator (Colin Berry, corresponding author). The source data includes the deidentified numerical data used for the statistical analyses and deidentified imaging scans (MRI) and ECGs. Data access will be provided through the secure analytical platform of the Robertson Centre for Biostatistics. This secure platform enables access to deidentified data for analytical purposes, without the possibility of removing the data from the server. Requests for transfer of deidentified data (including source imaging scans) will be considered by the Steering Group and if approved, a collaboration agreement would be expected. The Steering Group will consider any cost implications and cost recovery would be expected on a not-for-profit basis.

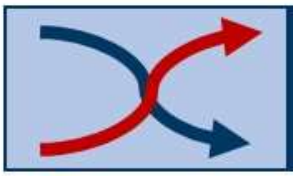
Code Availability Statement

The statistical code will be available online in Github on publication of the manuscript:

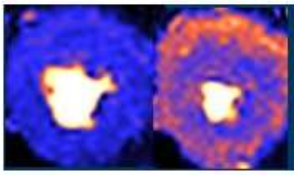
https://github.com/RobertsonCentre/PRIZE

CENTRAL ILLUSTRATION The PRIZE clinical trial.

222 patients with microvascular angina were screened in 12 hospitals in the United Kingdom.
118 participants with eligibility criteria after rs9349379 genotype filtering were randomized.



Prospective, randomized, double-blind, placebo-controlled, sequential crossover trial, assessing the effects of zibotentan, an endothelin A receptor selective antagonist, 10 mg daily or placebo for 12 weeks.



18 participants took part in an intravenous adenosine stress / rest myocardial perfusion MRI study with quantitative pixel mapping of myocardial blood flow.

PRIMARY OUTCOME

Compared to placebo, zibotentan treatment did not improve exercise duration.

SECONDARY OUTCOMES

Zibotentan treatment did not improve patient reported outcome measures of health-related quality of life or treatment satisfaction with medication.

Zibotentan treatment increased plasma big endothelin-1 and endothelin-1, and decreased blood pressure and plasma cholesterol, triglycerides and glycated hemoglobin.

Zibotentan treatment increased myocardial blood flow (ml/min/g) at rest in all segments but not during intravenous adenosine infusion.

Target-related adverse effects led to poor tolerability, limiting therapeutic benefit. Very low doses (0.25 mg, 1.5 mg) of zibotentan merit investigation.

Figure Legends

Figure 1. Flow diagram of the registry-based randomized trial.

Clinical information, patient reported outcome measures (PROMS), and a blood test were acquired at enrolment (visit 1), at the end of the medical optimization period (visit 2), after a 3-week placebo run-in (visit 3, baseline), at the end of treatment period 1 (visit 4) and treatment period 2 (visit 5, end of trial). A genomic blood test was obtained at visit 1. An exercise tolerance test was obtained on four occasions including visits 1, 3, 4 and 5. An optional imaging study involved cardiovascular MRI at visits 3, 4 and 5.

The registry population included individuals with microvascular angina who provided written informed consent at visit 1. The trial population included participants who fulfilled eligibility and genotype criteria and who were randomized at visit 3.

Figure 2. Effect of zibotentan on mean myocardial blood flow (ml/min/g), (A) global, n=14, and (B) subendocardium, n=14.

Figure 1. Flow diagram.

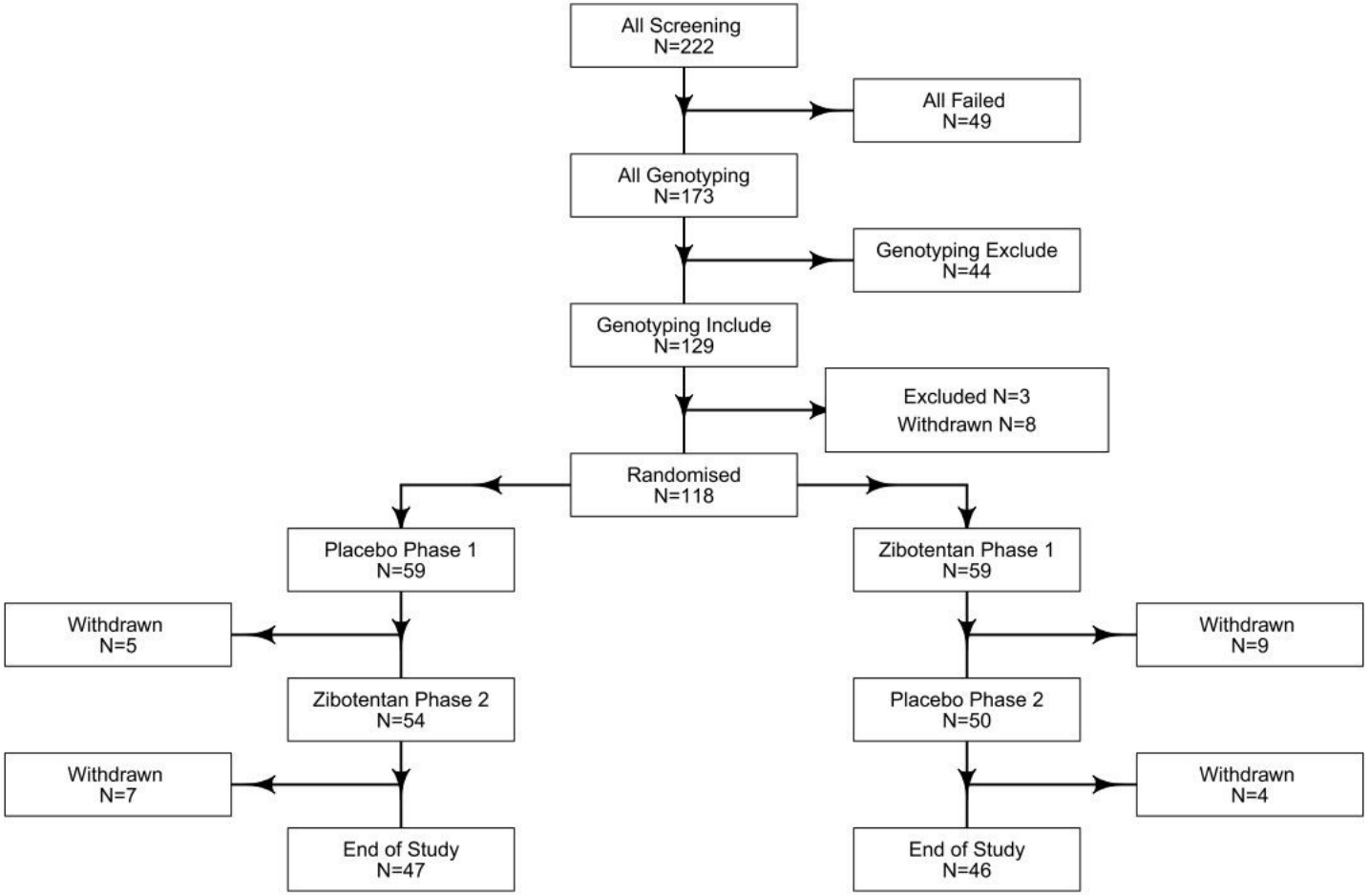
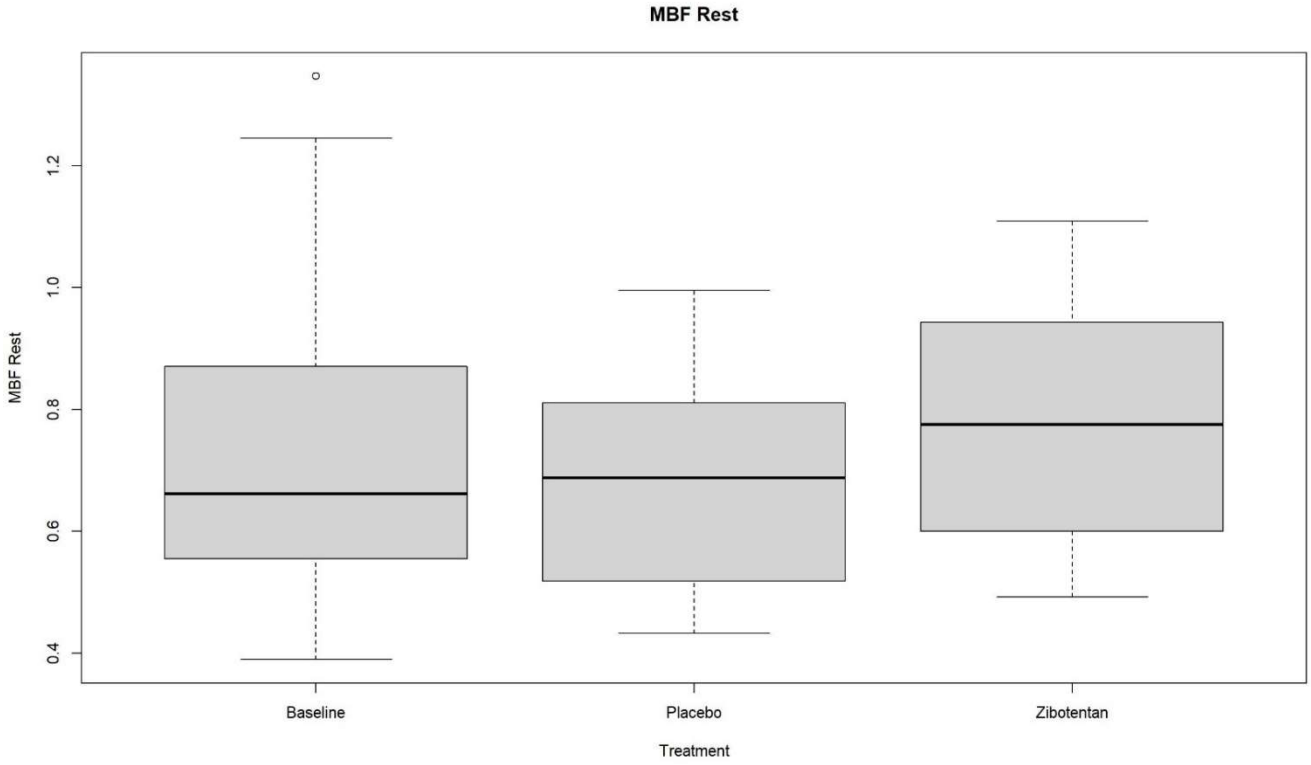
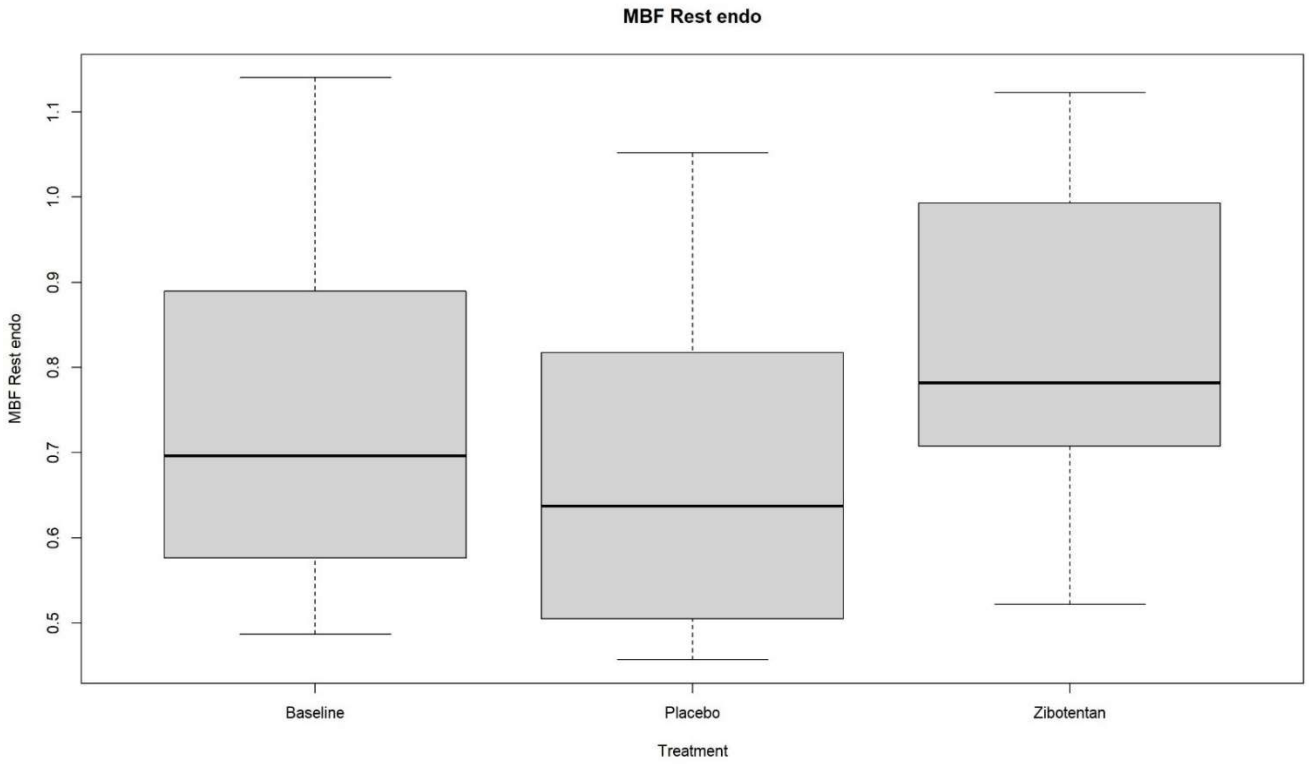


Figure 2. Myocardial blood flow at rest – (A) global, and (B) subendocardium measured by MRI.





References

1. Panting, J. R. *et al.* Abnormal subendocardial perfusion in cardiac syndrome X detected by cardiovascular magnetic resonance imaging. *N Engl J Med* **346**, 1948–1953 (2002).
2. Ford, T. J., Corcoran, D. & Berry, C. Stable coronary syndromes: pathophysiology, diagnostic advances and therapeutic need. *Heart* **104**, 284–292 (2018).
3. Samuels, B. A. *et al.* Comprehensive Management of ANOCA, Part 1-Definition, Patient Population, and Diagnosis: JACC State-of-the-Art Review. *J Am Coll Cardiol* **82**, 1245–1263 (2023).
4. Smilowitz, N. R. *et al.* Comprehensive Management of ANOCA, Part 2-Program Development, Treatment, and Research Initiatives: JACC State-of-the-Art Review. *J Am Coll Cardiol* **82**, 1264–1279 (2023).
5. Yanagisawa, M., Kurihara, H., Kimura, S., Goto, K. & Masaki, T. A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca2+ channels. *J Hypertens Suppl* **6**, S188-191 (1988).
6. Davenport, A. P., Kuc, R. E., Maguire, J. J. & Harland, S. P. ETA receptors predominate in the human vasculature and mediate constriction. *J Cardiovasc Pharmacol* **26 Suppl 3**, S265-267 (1995).
7. Kaski, J. C. *et al.* Concentration of circulating plasma endothelin in patients with angina and normal coronary angiograms. *Br Heart J* **74**, 620–624 (1995).
8. Ford, T. J. *et al.* Genetic dysregulation of endothelin-1 is implicated in coronary microvascular dysfunction. *Eur Heart J* **41**, 3239–3252 (2020).
9. Cox, I. D. *et al.* Elevated endothelin concentrations are associated with reduced coronary vasomotor responses in patients with chest pain and normal coronary arteriograms. *J Am Coll Cardiol* **34**, 455–460 (1999).

10. Ford, T. J. *et al.* Systemic microvascular dysfunction in microvascular and vasospastic angina. *Eur Heart J* **39**, 4086–4097 (2018).
11. *rs9349379 RefSNP Report - dbSNP - NCBI*. https://www.ncbi.nlm.nih.gov/snp/rs9349379.
12. Gupta, R. M. *et al.* A Genetic Variant Associated with Five Vascular Diseases Is a Distal Regulator of Endothelin-1 Gene Expression. *Cell* **170**, 522-533.e15 (2017).
13. Morris, C. D. *et al.* Specific inhibition of the endothelin A receptor with ZD4054: clinical and pre-clinical evidence. *Br J Cancer* **92**, 2148–2152 (2005).
14. Zibotentan (ZD4054). https://openinnovation.astrazeneca.com/home/preclinical-research/preclinical-molecules/zibotentan.html.
15. Fizazi, K. *et al.* Phase III, randomized, placebo-controlled study of docetaxel in combination with zibotentan in patients with metastatic castration-resistant prostate cancer. *J Clin Oncol* **31**, 1740–1747 (2013).
16. Morrow, A. J. *et al.* Rationale and design of the Medical Research Council's Precision Medicine with Zibotentan in Microvascular Angina (PRIZE) trial. *Am Heart J* **229**, 70–80 (2020).
17. de Nucci, G. *et al.* Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci U S A* **85**, 9797–9800 (1988).
18. Heerspink, H. J. L. *et al.* Zibotentan in combination with dapagliflozin compared with dapagliflozin in patients with chronic kidney disease (ZENITH-CKD): a multicentre, randomised, active-controlled, phase 2b, clinical trial. *Lancet* **402**, 2004–2017 (2023).
19. Mercier, A.-K. *et al.* Pharmacokinetics and Tolerability of Zibotentan in Patients with Concurrent Moderate Renal and Moderate Hepatic Impairment. *Clin Pharmacokinet* **62**, 1713–1724 (2023).

20. Tomkinson, H. *et al.* Pharmacokinetics and tolerability of zibotentan (ZD4054) in subjects with hepatic or renal impairment: two open-label comparative studies. *BMC Clin Pharmacol* **11**, 3 (2011).
21. Corcoran, D. *et al.* Vascular effects of serelaxin in patients with stable coronary artery disease: a randomized placebo-controlled trial. *Cardiovasc Res* **117**, 320–329 (2021).
22. Fulton, W. F. THE DYNAMIC FACTOR IN ENLARGEMENT OF CORONARY ARTERIAL ANASTOMOSES, AND PARADOXICAL CHANGES IN THE SUBENDOCARDIAL PLEXUS. *Br Heart J* **26**, 39–50 (1964).
23. Layland, J., Carrick, D., Lee, M., Oldroyd, K. & Berry, C. Adenosine: physiology, pharmacology, and clinical applications. *JACC Cardiovasc Interv* **7**, 581–591 (2014).
24. Rajkumar, C. A. *et al.* A Placebo-Controlled Trial of Percutaneous Coronary Intervention for Stable Angina. *New England Journal of Medicine* **0**, null (2023).
25. AstraZeneca. *A Two Part Phase IIa/b Multicentre, Randomised, Double-Blind, Placebo-Controlled, Parallel Group Dose-ranging Study to Assess Efficacy, Safety, and Tolerability of the Combination of Zibotentan and Dapagliflozin, and Dapagliflozin Monotherapy Versus Placebo in Participants With Cirrhosis With Features of Portal Hypertension*. https://clinicaltrials.gov/study/NCT05516498 (2023).
26. Stern, E. P. *et al.* Zibotentan in systemic sclerosis-associated chronic kidney disease: a phase II randomised placebo-controlled trial. *Arthritis Res Ther* **24**, 130 (2022).
27. Abraham, G. R. & Davenport, A. P. From ABCD to E for endothelin in resistant hypertension. *Cell* **186**, 240–242 (2023).
28. Knott, K. D. *et al.* The Prognostic Significance of Quantitative Myocardial Perfusion: An Artificial Intelligence-Based Approach Using Perfusion Mapping. *Circulation* **141**, 1282–1291 (2020).

29. Capece, U. *et al.* Dapagliflozin-Induced Myocardial Flow Reserve Improvement is not Associated with HDL Ability to Stimulate Endothelial Nitric Oxide Production. *Diabetes Ther* (2023) doi:10.1007/s13300-023-01491-5.
30. Abraham, G. R. *et al.* Current and future strategies for targeting the endothelin pathway in cardiovascular disease. *Nat Cardiovasc Res* **2**, 972–990 (2023).
31. Knuuti, J. *et al.* 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J* **41**, 407–477 (2020).
32. Kunadian, V. *et al.* An EAPCI Expert Consensus Document on Ischaemia with Non-Obstructive Coronary Arteries in Collaboration with European Society of Cardiology Working Group on Coronary Pathophysiology & Microcirculation Endorsed by Coronary Vasomotor Disorders International Study Group. *Eur Heart J* **41**, 3504–3520 (2020).
33. Ong, P. *et al.* International standardization of diagnostic criteria for microvascular angina. *Int J Cardiol* **250**, 16–20 (2018).
34. Fletcher, G. F. *et al.* Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. *Circulation* **104**, 1694–1740 (2001).
35. Dagenais, G. R., Pitt, B. & Ross, R. S. Exercise tolerance in patients with angina pectoris. Daily variation and effects of erythrityl tetranitrate, propranolol and alprenolol. *Am J Cardiol* **28**, 10–16 (1971).
36. Benhorin, J. *et al.* Ischemic threshold during two exercise testing protocols and during ambulatory electrocardiographic monitoring. *J Am Coll Cardiol* **22**, 671–677 (1993).
37. Fielding, R. A., Frontera, W. R., Hughes, V. A., Fisher, E. C. & Evans, W. J. The reproducibility of the Bruce protocol exercise test for the determination of aerobic capacity in older women. *Med Sci Sports Exerc* **29**, 1109–1113 (1997).

38. Maybaum, S., Ilan, M., Mogilevsky, J. & Tzivoni, D. Improvement in ischemic parameters during repeated exercise testing: a possible model for myocardial preconditioning. *Am J Cardiol* **78**, 1087–1091 (1996).
39. Tzivoni, D. & Maybaum, S. Attenuation of severity of myocardial ischemia during repeated daily ischemic episodes. *J Am Coll Cardiol* **30**, 119–124 (1997).
40. Nordrehaug, J. E., Danielsen, R., Stangeland, L., Rosland, G. A. & Vik-Mo, H. Respiratory gas exchange during treadmill exercise testing: reproducibility and comparison of different exercise protocols. *Scand J Clin Lab Invest* **51**, 655–658 (1991).
41. Jadhav, S. *et al.* Effects of metformin on microvascular function and exercise tolerance in women with angina and normal coronary arteries: a randomized, double-blind, placebo-controlled study. *J Am Coll Cardiol* **48**, 956–963 (2006).
42. Bruce, R. A., Kusumi, F. & Hosmer, D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J* **85**, 546–562 (1973).
43. Levey, A. S. *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* **150**, 604–612 (2009).
44. Welsh, P. *et al.* N-terminal pro-B-type natriuretic peptide and the prediction of primary cardiovascular events: results from 15-year follow-up of WOSCOPS. *Eur Heart J* **34**, 443–450 (2013).
45. Flett, A. S. *et al.* Evaluation of techniques for the quantification of myocardial scar of differing etiology using cardiac magnetic resonance. *JACC Cardiovasc Imaging* **4**, 150–156 (2011).
46. Gräni, C. *et al.* Comparison of myocardial fibrosis quantification methods by cardiovascular magnetic resonance imaging for risk stratification of patients with suspected myocarditis. *J Cardiovasc Magn Reson* **21**, 14 (2019).

47. Khan, J. N. *et al.* Comparison of semi-automated methods to quantify infarct size and area at risk by cardiovascular magnetic resonance imaging at 1.5T and 3.0T field strengths. *BMC Res Notes* **8**, 52 (2015).
48. Kellman, P. *et al.* Myocardial perfusion cardiovascular magnetic resonance: optimized dual sequence and reconstruction for quantification. *J Cardiovasc Magn Reson* **19**, 43 (2017).
49. Kotecha, T. *et al.* Automated Pixel-Wise Quantitative Myocardial Perfusion Mapping by CMR to Detect Obstructive Coronary Artery Disease and Coronary Microvascular Dysfunction: Validation Against Invasive Coronary Physiology. *JACC Cardiovasc Imaging* **12**, 1958–1969 (2019).
50. Mark, D. B. *et al.* Prognostic value of a treadmill exercise score in outpatients with suspected coronary artery disease. *N Engl J Med* **325**, 849–853 (1991).
51. Spertus, J. A. *et al.* Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. *J Am Coll Cardiol* **25**, 333–341 (1995).
52. Arnold, S. V. *et al.* Effects of ranolazine on disease-specific health status and quality of life among patients with acute coronary syndromes: results from the MERLIN-TIMI 36 randomized trial. *Circ Cardiovasc Qual Outcomes* **1**, 107–115 (2008).
53. Mozaffarian, D., Bryson, C. L., Spertus, J. A., McDonell, M. B. & Fihn, S. D. Anginal symptoms consistently predict total mortality among outpatients with coronary artery disease. *Am Heart J* **146**, 1015–1022 (2003).
54. Arnold, S. V. *et al.* Economic impact of angina after an acute coronary syndrome: insights from the MERLIN-TIMI 36 trial. *Circ Cardiovasc Qual Outcomes* **2**, 344–353 (2009).
55. Spertus, J. A. *et al.* Health-Status Outcomes with Invasive or Conservative Care in Coronary Disease. *N Engl J Med* **382**, 1408–1419 (2020).

56. Thomas, M., Jones, P. G., Arnold, S. V. & Spertus, J. A. Interpretation of the Seattle Angina Questionnaire as an Outcome Measure in Clinical Trials and Clinical Care: A Review. *JAMA Cardiol* **6**, 593–599 (2021).
57. Ford, T. J. *et al.* Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. *J Am Coll Cardiol* **72**, 2841–2855 (2018).
58. EQ-5D-5L – EQ-5D. https://euroqol.org/eq-5d-instruments/eq-5d-5l-about/.
59. Broadbent, E., Petrie, K. J., Main, J. & Weinman, J. The brief illness perception questionnaire. *J Psychosom Res* **60**, 631–637 (2006).
60. Kroenke, K., Spitzer, R. L., Williams, J. B. W. & Löwe, B. An ultra-brief screening scale for anxiety and depression: the PHQ-4. *Psychosomatics* **50**, 613–621 (2009).
61. Bharmal, M. *et al.* Validation of an abbreviated Treatment Satisfaction Questionnaire for Medication (TSQM-9) among patients on antihypertensive medications. *Health Qual Life Outcomes* **7**, 36 (2009).
62. Al-Lamee, R. *et al.* Percutaneous coronary intervention in stable angina (ORBITA): a double-blind, randomised controlled trial. *Lancet* **391**, 31–40 (2018).
63. Stone, P. H. *et al.* Comparison of propranolol, diltiazem, and nifedipine in the treatment of ambulatory ischemia in patients with stable angina. Differential effects on ambulatory ischemia, exercise performance, and anginal symptoms. The ASIS Study Group. *Circulation* **82**, 1962–1972 (1990).

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