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Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial

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In heart failure with preserved ejection fraction (HFpEF), the occurrence of myocardial fibrosis is associated with adverse outcome. Whether pirfenidone, an oral antifibrotic agent without hemodynamic effect, is efficacious and safe for the treatment of HFpEF is unknown. In this double-blind, phase 2 trial (NCT02932566), we enrolled patients with heart failure, an ejection fraction of 45% or higher and elevated levels of natriuretic peptides. Eligible patients underwent cardiovascular magnetic resonance and those with evidence of myocardial fibrosis, defined as a myocardial extracellular volume of 27% or greater, were randomly assigned to receive pirfenidone or placebo for 52 weeks. Forty-seven patients were randomized to each of the pirfenidone and placebo groups. The primary outcome was change in myocardial extracellular volume, from baseline to 52 weeks. In comparison to placebo, pirfenidone reduced myocardial extracellular volume (between-group difference, −1.21%; 95% confidence interval, −2.12 to −0.31; $P = 0.009$), meeting the predefined primary outcome. Twelve patients (26%) in the pirfenidone group and 14 patients (30%) in the placebo group experienced one or more serious adverse events. The most common adverse events in the pirfenidone group were nausea, insomnia and rash. In conclusion, among patients with HFpEF and myocardial fibrosis, administration of pirfenidone for 52 weeks reduced myocardial fibrosis. The favorable effects of pirfenidone in patients with HFpEF will need to be confirmed in future trials.

Heart failure with preserved ejection fraction (HFpEF) is common and is associated with high morbidity and mortality[1]. HFpEF involves a diverse range of pathophysiological mechanisms, and this heterogeneity may have contributed to the neutral findings of some phase 3 trials that have considered HFpEF as a single entity and taken a one-size-fits-all approach to its treatment[2]. By contrast, trials that have targeted specific biological mechanisms, such as the Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy (ATTR-ACT) trial, and the Rivaroxaban with or without Aspirin in Patients with Heart Failure and Chronic Coronary or Peripheral Artery Disease (COMPASS) trial, have shown benefit[3,4]. Predictive enrichment trial design means selecting patients who are more likely to respond to a given therapy on the basis of a biological mechanism or specific disease pathway[5,6].

Preclinical studies have identified an important pathophysiological role for myocardial fibrosis in heart failure[7–9] and, in patients with HFpEF, myocardial fibrosis, measured using cardiovascular magnetic resonance, is associated with death and hospitalization for heart failure[10]. Pirfenidone is an oral, small-molecule, antifibrotic agent without hemodynamic effect, that is approved for patients with idiopathic pulmonary fibrosis[11]. In preclinical models, pirfenidone is associated with regression of myocardial fibrosis[12–20]. In a novel approach to heart failure that involves specifically targeting the extracellular matrix, we identified patients with HFpEF and myocardial fibrosis, and tested whether pirfenidone would result in regression of myocardial fibrosis.

Results

Patients. From 7 March 2017 to 19 December 2018, 601 patients were screened at six sites in the United Kingdom. Of these, 136 had a baseline assessment. Twenty-nine patients were excluded for reasons of ineligibility, and 13 further patients were found to have extracellular volume (ECV) < 27% (median ECV 24.7%, interquartile range (IQR) 24.5–24.9), that is, below the threshold for entry. Ninety-four patients were randomly assigned to receive pirfenidone or placebo (Fig. 1). At the end of the trial, 12 patients had withdrawn from the study and two had died. No patient was lost to follow-up. A total of 80 patients were included in the final efficacy analysis. Baseline characteristics were similar between treatment groups (Table 1 and Supplementary Table 3). The mean age of patients was 78 years, and 46% were female. Nearly all patients had New York Heart Association functional class II or III symptoms (95%), mean left ventricular ejection fraction was 64% and median N-terminal pro-brain natriuretic peptide (NT-proBNP) was 1,104 pg ml^{-1}. Mean myocardial ECV was 30.1%.

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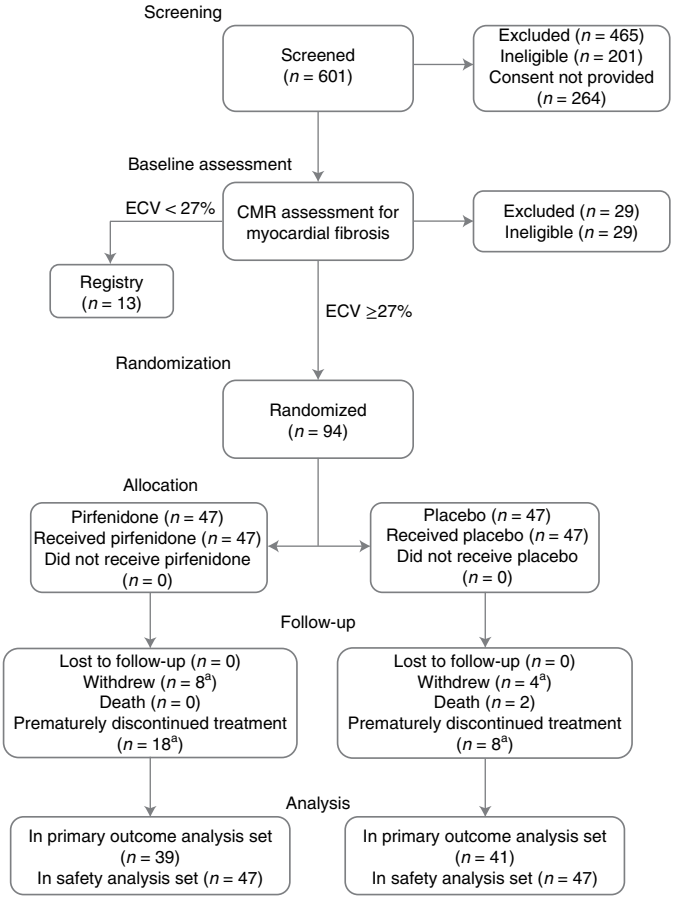

Fig. 1 | Screening, randomization and follow-up. [a]The number of patients indicated as prematurely discontinuing treatment includes all patients who withdrew from the trial. CMR, cardiovascular magnetic resonance.

Outcomes. The primary outcome, change in myocardial ECV from baseline to 52 weeks, was significant, with a greater reduction in those assigned to pirfenidone rather than placebo (between-group difference, −1.21%; 95% confidence interval (CI), −2.12 to −0.31; $P=0.009$) (Table 2 and Fig. 2). The sensitivity analysis, which used multiple imputation to adjust for missing primary outcome values, yielded similar results (between-group difference, −1.14%; 95% CI, −2.04 to −0.25; $P=0.01$). The causal analysis demonstrated that, for each additional 100 capsules of pirfenidone taken (that is, 11 days of treatment at the target dose), there was a mean reduction in myocardial ECV at 52 weeks of 0.06% (95% CI, −0.10 to −0.01; $P=0.01$).

Secondary outcomes are presented in Table 3 and Supplementary Table 4. Pirfenidone was associated with a reduction in log NT-proBNP compared to placebo ($P=0.02$), with the effect seen by week 13 (the reduction in median NT-proBNP from baseline to week 13 with pirfenidone was 415 ng l^{-1} versus 326 ng l^{-1} with placebo; Supplementary Table 5). Pirfenidone was associated with a small increase in left ventricular ejection fraction (between-group difference, 2.16%; 95% CI unadjusted for multiplicity, 0.51 to 3.81). Pirfenidone was associated with a reduction in left ventricular mass (between-group difference, −7.00 g; unadjusted 95% CI, −12.7 to −1.29) and maximal wall thickness (between-group difference, −0.06 cm; unadjusted 95% CI, −0.12 to −0.01) but there was no significant change in left ventricular mass indexed for body surface area. There were no differences in left ventricular diastolic function, atrial size and function, or right ventricular size and function.

Table 1 | Selected characteristics of the patients at baseline

Characteristics	Pirfenidone (n = 47)	Placebo (n = 47)
Age, yr	78 (72–82)	81 (76–83)
Female sex, n (%)	22 (47)	21 (45)
White race, n (%)[a]	45 (96)	43 (92)
Hypertension, n (%)	39 (83)	40 (85)
Diabetes, n (%)	16 (34)	12 (26)
Atrial fibrillation/flutter, n (%)[b]	22 (47)	24 (51)
Hospitalization for heart failure in past six months, n (%)	8 (17)	7 (15)
Systolic blood pressure, mmHg	134 (123–148)	139 (125–145)
Body mass index, kg m^{-2}	31 (27–34)	29 (26–33)
NYHA functional class, n (%)		
I	0 (0)	5 (11)
II	26 (55)	19 (40)
III	21 (45)	23 (49)
IV	0 (0)	0 (0)
eGFR, ml min^{-1} per 1.73 m^2	58 (46–76)	53 (38–65)
Median NT-proBNP (pg ml^{-1}), median (IQR)	975 (445–2,064)	1,372 (626–2,817)
Left ventricular ejection fraction, %	67 (60–70)	65 (55–69)
Left ventricular mass index, g m^{-2}	62 (54–71)	66 (53–73)
Extracellular volume, %	28.9 (27.6–31.0)	30.4 (28.3–32.2)
Global longitudinal strain, %[c]	−15.7 (−19.0 to −12.1)	−16.2 (−18.3 to −14.0)
E/A ratio[d]	1.0 (0.8–1.3)	1.1 (0.8–1.4)
Lateral e′, cm s^{-1}	10.3 (8.7–12.6)	10.1 (8.5–10.9)
Septal e′, cm s^{-1}	7.4 (6.3–9.6)	6.2 (5.4–7.2)
Average E/e′[c]	10.4 (9.1–13.6)	12.8 (10.3–15.5)
Left atrial volume index, ml m^{-2}	68 (56–83)	69 (58–85)
Left atrial strain S (reservoir), %[c]	18.3 (10.8–24.6)	15.6 (9.2–20.4)
Right ventricular ejection fraction, %	53 (48–59)	51 (43–57)
PCr:ATP ratio[e]	1.2 (1.0–1.4)	1.1 (0.9–1.4)
6-minute walk test, m	286 (160–349)	262 (173–328)
KCCQ overall summary score (0–100)[f]	50.7 (38.9–72.6)	55.9 (39.1–70.8)

Values are mean ± s.d. unless otherwise indicated. eGFR, estimated glomerular filtration rate; NYHA, New York Heart Association. [a]Race was patient-reported. [b]Patients in atrial fibrillation or atrial flutter on baseline electrocardiogram. [c]Owing to technical factors, the following imaging measurements were unobtainable at baseline: global longitudinal strain (one patient in the pirfenidone group), average E/e′ (one patient in the placebo group), left atrial strain S (reservoir) (one patient in the pirfenidone group and one in the placebo group). [d]E/A ratio was not measured in patients in atrial fibrillation (22 in the pirfenidone group and 24 in the placebo group). [e]The ratio of phosphocreatine (PCr) to adenosine triphosphate (ATP) concentrations, corrected for blood and partial saturation of PCr and ATP. [f]Values for the Kansas City Cardiomyopathy Questionnaire scores range from 0 to 100, with higher scores indicating fewer symptoms and physical limitations associated with heart failure.

Patients in the pirfenidone group showed a small increase in 6-min walk distance at 52 weeks, whereas those in the placebo group showed a decrease, but the difference was not significant (between-group difference, 15.54 m; unadjusted 95% CI, −9.55 to 40.63). Pirfenidone was associated with improvements in 8 out of 10 KCCQ scores, including clinically important improvements[21] in

Table 2 | Primary outcome

	Pirfenidone			Placebo				
	Baseline ($n=47$)	52 weeks ($n=39$)	Δ from baseline to 52 weeks	Baseline ($n=47$)	52 weeks ($n=41$)	Δ from baseline to 52 weeks	Between-group difference (95% CI)[a]	*P* value
Myocardial ECV (%)	29.5 ± 2.5	28.6 ± 2.7	−0.7 ± 1.4	30.7 ± 2.9	31.1 ± 3.8	0.5 ± 2.4	−1.21 (−2.12 to −0.31)	0.009

Data are mean ± s.d. [a]Analysis of covariance (ANCOVA), two-sided, adjusted for baseline myocardial ECV, sex and treatment group; $F=7.11$, $P=0.009$.

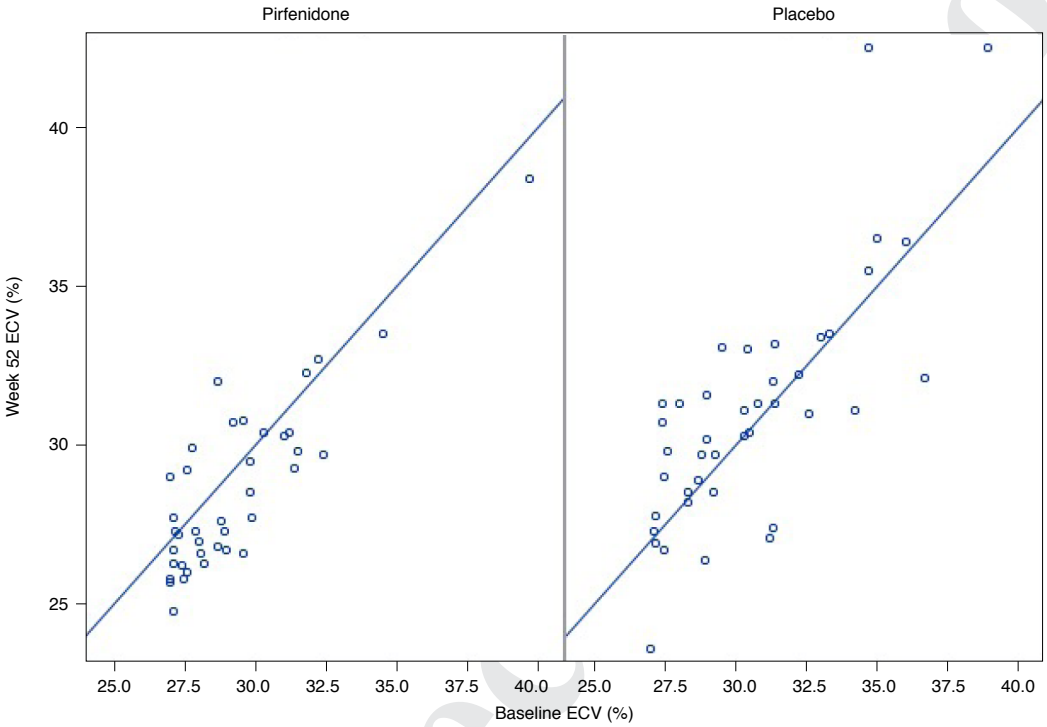

Fig. 2 | Myocardial ECV. Myocardial ECV measured at baseline and week 52 by treatment group.

all three summary scores, but the differences were not statistically significant.

Safety. Following randomization, 18 patients (38%) in the pirfenidone group and six patients (13%) in the placebo group prematurely discontinued treatment for reasons other than death, predominantly due to adverse events (14 in the pirfenidone group and three in the placebo group). Twelve patients (26%) in the pirfenidone group experienced one or more serious adverse event compared to 14 patients (30%), including two deaths, in the placebo group (Supplementary Table 6). Four of the 12 (33%) participants who withdrew from the study experienced a serious adverse event, compared to 20 of the 80 (25%) participants who completed the study. The most frequent adverse events are detailed in Table 4 (Supplementary Table 7 provides a complete list). Treatment-emergent changes in safety outcomes are summarized in Supplementary Table 8. The number of cardiac adverse events did not differ between groups.

Sub-study. Sixty randomized patients (30 per treatment group) and eight non-randomized patients underwent ^{31}P magnetic resonance spectroscopy. At baseline, there was a modest inverse correlation between myocardial ECV and phosphocreatine (PCr) to adenosine triphosphate (ATP) ratio ($r=-0.26$; $P=0.03$). There was no difference in change in PCr:ATP ratio between treatment groups (Supplementary Tables 10 and 11 provide further details).

Discussion

Among patients with HFpEF and myocardial fibrosis, treatment with pirfenidone for 52 weeks reduced myocardial fibrosis and log NT-proBNP. Pirfenidone was associated with a side-effect profile consistent with that reported in previous studies in idiopathic pulmonary fibrosis. There was no excess of cardiac adverse events.

The historical disconnect between phase 2 and 3 drug trials in heart failure means that most phase 3 trials are neutral, despite often promising phase 2 results[22,23]. In the PIROUETTE trial, we aimed to connect a prognostically important disease mechanism (myocardial fibrosis) with drug mechanism of action (antifibrotic), select patients with evidence of this disease mechanism for entry and use a primary outcome measure tailored to the mechanism of action (myocardial ECV). In designing the study in this way, we believe that the results more reliably inform the decision as to whether or not to progress to phase 3.

Targeting the extracellular matrix is a novel approach to heart failure. Preclinical studies have indicated that the extracellular matrix may have a primary role in the development of heart failure[7–9]. Myocardial fibrosis regression has been observed previously

Table 3 | Secondary outcome measures

Secondary outcome	Pirfenidone			Placebo			
	Baseline ($n=47$)	52 weeks ($n=39$)	Δ from baseline to 52 weeks	Baseline ($n=47$)	52 weeks ($n=41$)	Δ from baseline to 52 weeks	ANCOVA model between-group difference (95% CI)
Left ventricle							
LVEDVi, ml m^{-2}	59 (50-75)	59 (50-72)	−2 (−7-8)	60 (51-76)	59 (51-66)	−3 (−9-5)	0.47 (−3.22-4.16)
LVESVi, ml m^{-2} [a]	19 (14-27)	19 (15-24)	0 (−3-2)	21 (15-32)	19 (16-28)	−1 (−4-2)	−1.43 (−3.16-0.30)
LVEF, %[a]	67 (60-70)	68 (64-71)	1 (−1-2)	65 (55-69)	65 (56-69)	0 (−2-1)	2.16 (0.51-3.81)
LV mass index, g m^{-2}	62 (54-71)	60 (52-70)	−1 (−6-2)	66 (53-73)	63 (55-75)	0 (-3-4)	−2.48 (−5.47-0.50)
Native T1, ms	1,050 (1,032-1,071)	1,032 (1,018-1,043)	−15 (−30-3)	1,056 (1,031-1,073)	1,058 (1,020-1,089)	3 (−15-19)	−24.3 (−39.1 to −9.49)
Absolute myocardial ECM volume, ml	33.5 (27.9-41.9)	29.0 (24.8-37.2)	−2 (−5.4 to −0.2)	32.7 (28.7-46.3)	36.5 (28.7-44.2)	0.8 (−1.3-2.3)	−3.06 (−4.96 to −1.16)
Absolute myocardial cell volume, ml	82.3 (66.2-97.7)	73.4 (64.7-93.1)	−2.9 (−11.1-2.1)	78.2 (66.6-98.7)	77.2 (68.1-93.3)	−0.4 (−4.8-3.5)	−3.41 (−7.28-0.47)
E/A ratio	1.0 (0.8-1.3)	0.8 (0.7-1.1)	−0.1 (−0.3-0.1)	1.1 (0.8-1.4)	0.8 (0.6-1.0)	−0.1 (−0.3-0.0)	0.10 (−0.09-0.30)
Lateral e', cm s^{-1}	10.3 (8.7-12.6)	8.5 (7.0-10.7)	−1.3 (−2.9-0.1)	10.1 (8.5-10.9)	9.0 (6.8-10.2)	−0.8 (−2.7-0.2)	−0.16 (−1.18-0.86)
Septal e', cm s^{-1}	7.4 (6.3-9.6)	6.7 (6.0-8.1)	−0.8 (−2.3-0.2)	6.2 (5.4-7.2)	6.5 (5.0-7.7)	0.0 (−0.8-1.3)	0.02 (−0.81-0.84)
Average E/e', cm s^{-1} [b]	10.4 (9.1-13.6)	12.5 (8.9-14.5)	0.9 (−0.5-1.9)	12.8 (10.3-15.5)	13.1 (11.3-15.7)	−0.1 (−1.6-2.3)	0.25 (−1.37-1.86)
GLS, %[c]	−15.7 (−19.0 to −12.1)	−17.7 (−19.5 to −13.0)	−0.4 (−3.5-1.7)	−16.2 (−18.3 to −14.0)	−16.9 (−18.9 to −13.3)	0.1 (−1.1-1.2)	−1.17 (−2.58-0.24)
PCr:ATP ratio (BCPSC)[d]	1.2 (1.0-1.4)	1.3 (1.1-1.6)	0.1 (−0.3-0.4)	1.1 (0.9-1.4)	1.2 (1.0-1.6)	0.0 (−0.2-0.5)	−0.06 (−0.32-0.20)
Right ventricle							
RVEDVi, ml m^{-2}	68 (60-80)	70 (59-80)	2 (−7-10)	67 (57-78)	66 (60-78)	3 (−5-8)	1.27 (−3.22-5.76)
RVEF (%)[a]	53 (48-59)	55 (50-59)	1 (−7-6)	51 (43-57)	50 (44-57)	−1 (−4-5)	1.62 (−1.26-4.50)
PAP, mmHg[e]	34 (22-38)	33 (26-37)	−3 (−5-0)	33 (27-40)	34 (25-43)	01 (−6-7)	−0.44 (−7.07-6.19)
Left atrium							
LA volume, ml	130 (106-159)	127 (98-164)	1 (−10-10)	131 (108-163)	136 (115-161)	6 (−1-13)	−3.24(−11.0-4.55)
LA volume index, ml m^{-2}	68 (56-83)	63 (54-90)	1 (−3-6)	69 (58-85)	72 (58-87)	3 (0-8)	0.64 (−5.15-6.44)
LA strain (reservoir), %[f]	18.3 (10.8-24.6)	21.1 (12.0-28.5)	0.8 (−3.8-3.9)	15.6 (9.2-20.4)	13.9 (8.5-20.2)	0.0 (−2.3-3.3)	0.38 (−2.28-3.04)
LA strain (booster), %	13.4 (8.7-15.3)	14.8 (10.0-18.4)	1.9 (−1.8-4.1)	12.4 (9.7-14.6)	14.9 (10.2-19.4)	2.5 (−1.2-4.3)	−0.45 (−3.34-2.44)
LA strain (conduit), %[d]	11.1 (8.6-13.4)	12.0 (7.5-13.8)	−0.5 (−5.0-2.0)	8.8 (7.4-10.5)	8.5 (6.8-10.4)	−0.4 (−1.9-2.2)	0.56 (−1.08-2.20)
6MWT							
6MWT, m[g]	286 (160-349)	308 (234-360)	1 (−27-27)	262 (173-328)	245 (183-355)	−9 (37-23)	15.54 (−9.55-40.63)
KCCQ							
KCCQ, overall summary score (0-100)	50.7 (38.9-72.6)	63.9 (53.8-76.0)	7.6 (−2.6-20.8)	55.9 (39.1-70.8)	60.4 (36.0-79.2)	1.4 (−5.6-12.9)	6.45 (−0.19-13.09)
KCCQ, clinical summary score (0-100)[h]	52.1 (41.7-69.8)	64.3 (52.1-74.6)	6.3 (−1.6-15.6)	56.8 (41.7-70.3)	61.5 (40.4-76.3)	2.1 (−6.8-9.6)	5.51 (−0.85-11.87)
KCCQ, total symptom score (0-100)	57.3 (39.6-78.1)	70.8 (53.1-82.3)	10.4 (0.0-18.8)	66.7 (49.0-79.2)	64.6 (52.1-81.3)	0.0 (−8.3-14.6)	5.90 (−2.42-14.22)

Values are mean (s.d.) unless stated otherwise. ANCOVA, two-sided, adjusted for baseline value; unadjusted for multiple comparisons, therefore outcomes are considered exploratory. Patients in atrial fibrillation at baseline ($n=46$; 22 in the pirfenidone group and 24 in the placebo group) and at follow-up ($n=40$; 17 in the pirfenidone group and 23 in the placebo group) were unable to have the following parameters measured: A-wave velocity, E/A ratio, left atrial strain A (booster), left atrial strain rate – SR-A. [a]Measurement was unobtainable in one patient at 52 weeks (one in the pirfenidone group). [b]Measurements were unobtainable in one patient at baseline (one in the placebo group) and one patient at 52 weeks (one in the placebo group). [c]Measurement was unobtainable in one patient at baseline (one in the placebo group). [d]Measurements were performed in 60 patients at baseline (30 in the pirfenidone group and 30 in the placebo group) and 50 patients at 52 weeks (25 in the pirfenidone group and 25 in the placebo group). [e]Measurements were unobtainable at baseline ($n=35$; 21 in the pirfenidone group and 14 in the placebo group) and at week 52 ($n=34$; 20 in the pirfenidone group and 14 in the placebo group). [f]Measurements were unobtainable in two patients at baseline (one in the pirfenidone group and one in the placebo group). [g]Measurements were not performed in 10 patients at 52 weeks (five in the pirfenidone group and five in the placebo group). [h]The Kansas City Cardiomyopathy Questionnaire (KCCQ) was completed by all patients at baseline and 52 weeks. If patients answered, 'Limited for other reasons or did not do' for a specified number of responses the scores are set to 'missing value'. 6MWT, 6-min walk test; ECM, extracellular matrix; GLS, global longitudinal strain; LA, left atrial; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; PAP, pulmonary artery systolic pressure; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction.

Table 4 | Adverse events occurring in at least 20% of patients in either treatment group

Adverse event	Pirfenidone ($N=47$)	Placebo ($N=47$)
Any adverse event	46 (98)	46 (98)
Nausea	15 (32)	6 (13)
Insomnia	14 (30)	4 (9)
Rash	13 (28)	7 (15)
Diarrhea	12 (26)	13 (28)
Dyspepsia	12 (26)	4 (9)
Blood urea increased	11 (23)	9 (19)
Lower respiratory tract infection	11 (23)	13 (28)
Lethargy	11 (23)	8 (17)
Decreased appetite	10 (21)	8 (17)
Dizziness	10 (21)	5 (11)
Dyspnea	10 (21)	15 (32)
Hot flush	10 (21)	3 (6)
Blood alkaline phosphatase increased	7 (15)	10 (21)

Data are shown as counts (percentages).

in humans following interventions with hemodynamic effect, both drug and mechanical, but our study differs in that it demonstrates, in humans, the efficacy of an antifibrotic intervention without hemodynamic effect[24–27]. The associated reduction in natriuretic peptide levels provides support for the extracellular matrix having a causal role in heart failure and being an efficacious therapeutic target.

The magnitude of the reduction in myocardial ECV that we have observed with pirfenidone in the current trial would be associated with a 9–28% reduction in a composite of annual rate of hospitalization for heart failure or all-cause mortality in recent longitudinal cohort studies of patients with HFpEF[10,28]. This reduction requires investigation in a prospective trial. Myocardial fibrosis, measured using myocardial ECV, is strongly associated with invasively measured load-independent intrinsic left ventricular myocardial stiffness[29]. It may be that the reduction in log NT-proBNP that we observed with pirfenidone was due to an improvement in left ventricular myocardial stiffness secondary to myocardial fibrosis regression. This requires further investigation. It is unclear why no change was observed in echocardiographic measures of diastolic function. It may be that the structure of the extracellular matrix, such as the degree of collagen crosslinking as well as total fibrotic burden, is important[30]. Alternately, it may be because echocardiographic measurements of diastolic function are load-dependent and have limited accuracy in the context of preserved left ventricular ejection fraction[31–33], are not representative of any specific disease process, and may be less reflective of diastolic function than myocardial ECV itself[29]. We also found no effect of pirfenidone on left atrial volume or function. This may reflect the range of factors, other than atrial fibrosis, that influence left atrial size and function, such as atrial fibrillation (49% of patients had atrial fibrillation) and chronicity of atrial remodeling (the median age was 79 years). Indeed, in a preclinical study using a canine heart failure model, pirfenidone was associated with attenuation of atrial fibrosis, but the left atrial size still increased[16].

This trial has some limitations. The study population was generally older than in other trials in HFpEF, although the condition is associated with older age and older patients are often under-represented. The trial was not powered for the secondary outcomes, so the secondary findings are considered exploratory. There was some baseline imbalance in NT-proBNP, although this was adjusted for in the analysis. Finally, we cannot exclude the systemic antifibrotic effects of pirfenidone impacting some secondary outcomes[34]; however the specificity of the primary outcome measure to ventricular myocardium, and the parallel reduction in NT-proBNP, indicate a direct cardiac effect.

In conclusion, among patients with HFpEF and an increased ECV, a marker of myocardial fibrosis, ECV was reduced by treatment with pirfenidone over 52 weeks. The findings suggest that pirfenidone could have favorable effects in patients with this condition. Further trials are necessary to determine the clinical effectiveness and safety of pirfenidone in HFpEF.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01452-0.

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Methods

Trial design and oversight. The Pirfenidone in Patients with Heart Failure and Preserved Left Ventricular Ejection Fraction (PIROUETTE) trial was a predictive enrichment, randomized, double-blind, placebo-controlled, phase 2 trial. The design of the trial has been described previously[35]. The trial was sponsored by Manchester University NHS Foundation Trust and funded by the United Kingdom National Institute for Health Research. Trial management, independent data management and independent statistical analyses were performed by Liverpool Clinical Trials Centre, a United Kingdom Clinical Research Collaboration fully registered Clinical Trials Unit. The study protocol was approved by a research ethics committee (NHS Health Research Authority, North West—Liverpool Central Research Ethics Committee (16/NW/0717)) and trial conduct was overseen by a trial steering committee and an independent data and safety monitoring committee. The investigational medicinal product was gifted by Roche Products Limited. Roche Products Limited had no role in study design and were not involved in the preparation, drafting or editing of the manuscript. Roche Products Limited 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 assume full responsibility for the accuracy and completeness of the data and for the fidelity of the trial to the protocol (Supplementary Note).

Trial patients. Eligibility requirements included an age of 40 years or older, symptoms and signs of heart failure, an ejection fraction of 45% or higher at baseline, and elevated natriuretic peptides at baseline (with different thresholds depending on the presence of atrial fibrillation or the occurrence of recent hospitalization for heart failure). As part of the predictive enrichment strategy, eligible patients underwent cardiovascular magnetic resonance and those with evidence of myocardial fibrosis, defined as an ECV of 27% or higher, were randomized. Those without myocardial fibrosis were entered into a registry and invited to take part in a sub-study. Detailed eligibility criteria are provided in Supplementary Table 1.

Trial procedures. All patients provided written informed consent. Baseline procedures included cardiovascular magnetic resonance, echocardiography, electrocardiography, 6-min walk test, laboratory tests and completion of the KCCQ. The trial procedures have been described previously[35]. Following eligibility confirmation, participants were randomized in a 1:1 ratio to treatment with either pirfenidone or matching placebo for 52 weeks using block randomization, stratified by sex, with computer-generated randomization allocations and randomly varying block sizes (Pirouette Randomisation System, Version 1.7). Randomization was done using web randomization software accessed using a secure website provided via the clinical trials unit. Treatment was titrated, as tolerated, over a two-week period to a target of three capsules three times per day (target pirfenidone dose 2,403 mg per day), with adjustments permitted if unacceptable side effects occurred. All background medications were continued. Baseline procedures were repeated at the final visit (week 52). The visit schedule including safety monitoring is detailed in the trial protocol, available online.

Trial sub-study. We conducted a sub-study to investigate the relationship between myocardial fibrosis and myocardial energetics, and the impact of pirfenidone. We hypothesized that myocardial fibrosis would be associated with impaired energetics, and regression of fibrosis would be associated with an improvement in energetics. PCr:ATP ratio was measured using ^{31}P magnetic resonance spectroscopy at baseline in a subgroup of patients due to be randomized, and repeated at the final visit (week 52), and at baseline in patients without myocardial fibrosis (ECV less than 27%) but who were otherwise eligible. Patient selection was consecutive until the required number were recruited. The ^{31}P magnetic resonance spectroscopy procedure has been described previously[35].

Trial outcomes. The primary outcome was absolute change in myocardial ECV from baseline to week 52, measured using cardiovascular magnetic resonance (Argus tools, Siemens AG). Secondary outcomes included the following:

1. Absolute change in left ventricular and right ventricular mass, volumes, ejection fraction and tissue characteristics from baseline to week 52, measured using cardiovascular magnetic resonance (CVI42, Circle Cardiovascular Imaging).
2. Absolute change in absolute myocardial extracellular matrix volume from baseline to week 52, measured using cardiovascular magnetic resonance (Argus tools, Siemens AG).
3. Absolute change in myocardial cell volume from baseline to week 52, measured using cardiovascular magnetic resonance (Argus tools, Siemens AG).
4. Absolute change in left ventricular diastolic function, strain, backscatter and torsion from baseline to week 52, measured using echocardiography (Echopacs, GE Vingmed Ultrasound).
5. Absolute change in left atrial and right atrial volume, and left atrial function from baseline to week 52, measured using cardiovascular magnetic resonance and echocardiography (CVI42, Circle Cardiovascular Imaging; Echopacs, GE Vingmed Ultrasound).
6. Absolute change in pulse wave velocity and aortic distensibility from baseline to week 52, measured using cardiovascular magnetic resonance (CVI42, Circle Cardiovascular Imaging).
7. Absolute change in myocardial energetic status (PCr:ATP ratio) from baseline to week 52, measured using ^{31}P magnetic resonance spectroscopy (Java magnetic resonance user interface (jMRUI), Katholieke Universiteit Leuven).
8. Absolute change in NT-proBNP, and high-sensitivity troponin T from baseline to week 13, baseline to week 26 and baseline to week 52.
9. Absolute change in exercise tolerance from baseline to week 52, measured using 6-min walk distance.
10. Absolute change in health status (quality of life), heart faulure symptoms and physical limitations from baseline to week 52, measured using change in KCCQ score.
11. All-cause mortality, cardiovascular mortality and hospitalization for heart failure will be recorded. Q12

Safety outcomes included treatment-emergent adverse events and changes in vital signs, physical examination, laboratory investigations and ECG measurements (Supplementary Table 2 provides further details).

Statistical analysis. We determined that 37 patients per group would provide the trial with 80% power to detect an absolute minimum difference, between pirfenidone and placebo groups, of 2% in terms of change in myocardial ECV from baseline following 52 weeks of treatment, at a 5% significance level (two-sided), assuming a standard deviation of the within-patient differences from baseline equal to 3% (ref. [36]). This effect size was based on an estimate of the magnitude of myocardial fibrosis regression that could be expected to translate into improved clinical outcomes based on the magnitude of fibrotic regression seen with renin–angiotensin inhibition in other conditions[25]. To allow for treatment discontinuation in up to 20% of patients prior to final follow-up[11,37], the number randomized to each group was adjusted to 47. An ECV threshold of 27% was chosen as the definition of myocardial fibrosis because it represents one standard deviation above that in healthy volunteers scanned using the same scanner and imaging sequence at the sponsor institution (Manchester University NHS Foundation Trust).

For the trial sub-study, we determined that 33 patients per group were required to detect an absolute minimum difference in PCr:ATP ratio of 0.37 between randomized (myocardial ECV ≥ 27%) and non-randomized (ECV < 27%) groups at baseline (80% power, 5% significance level, two-sided), assuming a standard deviation of the between-group differences of 0.52 (ref. [38]). This effect size was based on that seen in previous studies[38,39]. Additionally, 26 patients per group were required to detect an absolute minimum difference, between pirfenidone and placebo groups, of 0.4 in terms of absolute change in PCr:ATP ratio from baseline following 52 weeks of treatment (80% power, 5% significance level, two-sided), assuming a standard deviation of the within-patient differences from baseline equal to 0.5 (ref. [40]). This effect size was also based on that seen in other studies[39–42]. To allow for treatment discontinuation in up to 20% of patients prior to final follow-up, the number required in each group was inflated to 33.

PCr:ATP ratio and myocardial mechanical parameters at baseline were compared between patients that were due to be randomized (ECV ≥ 27%) and patients without myocardial fibrosis (ECV < 27%) but who were otherwise eligible using an independent *t*-test, with transformation as necessary. Correlation analysis was used to assess the relationships between PCr:ATP ratio, myocardial mechanical parameters and ECV at baseline. Similarly, correlation analysis was used to assess the relationships between change from baseline in each of these parameters with change in ECV from baseline. The PCr:ATP ratio was compared between treatment groups using analysis of covariance (ANCOVA), adjusting for baseline PCr:ATP ratio, stratification factor (sex) and treatment group.

The trial was analyzed and reported according to the 'Consolidated Standard of Reporting Trials' (CONSORT) and International Conference on Harmonisation E9 guidelines. All primary analyses were on an intention-to-treat basis, including all randomized patients retained in their randomized treatment groups. The primary and secondary outcomes were compared between treatment groups using ANCOVA, adjusting for baseline values of the outcome variable, stratification factor (sex) and treatment group. Repeated measures ANCOVA were used for NT-proBNP and high-sensitivity troponin T. The hypothesis testing on secondary outcomes was considered exploratory. Imputation methods were utilized in a sensitivity analysis to assess the robustness of the primary outcome results to missing data. The degree of missing data was assessed during the blind review phase, and imputation methods were only implemented if more than 5% of patients were missing primary outcome data. Multiple imputation, based on baseline NT-proBNP, smoking status, diabetes and hypertension, were used to adjust for missing primary outcome values in a sensitivity ANCOVA model, adjusting for the same baseline covariates and factors as for the primary analysis. A secondary causal analysis, according to dose and duration of pirfenidone, was undertaken using instrumental variable regression to assess the causal impact of pirfenidone received on the primary outcome. Adverse events were coded according to preferred terms in the Medical Dictionary for Regulatory Activities (version 19). The number and percentage of participants experiencing each safety outcomes were reported, and treatment-emergent changes in safety outcomes were described using summary

statistics. The conventional 5% significance level was used. All analyses were performed using SAS (Version 9.4, SAS Institute).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

De-identified participant data will be made available on reasonable request one year after the date of publication, with no end date to availability, and may be used for any purpose. Requests should be directed to the corresponding author. Requestors will be required to sign a data access agreement. The study protocol is provided with the manuscript.

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Author contributions

G.A.L. and C.A.M. had full access to all the data and take responsibility for the integrity of the data and accuracy of data analysis. Study concept and design was provided by G.A.L., S.D., E.B., E.B.S., J.H.N., B.D.J., S.G.W., C.C., F.Z.A., A.C., R.V., S.R., T.M., P.R.W. and C.A.M. Acquisition, analysis or interpretation of data was carried out by all authors. Drafting of the manuscript was performed by G.A.L. and C.A.M. All authors critically revised the manuscript for important intellectual content. Statistical analysis was carried out by S.D. and D.C. Funding was obtained by C.A.M. Administrative, technical or material support was provided by H.E., J.H.N. and B.D.J. The study was supervised by E.B., P.R.W. and C.A.M.

Competing interests

C.A.M. has previously received research support for other research from Guerbet, has served on an advisory board for Novartis and serves has an advisor for HAYA Therapeutics. None of these relationships are relevant to the content of this paper. The investigational medicinal product was gifted by Roche Products Limited. Immunoassay testing equipment and materials were gifted by Roche Diagnostics International Limited. Roche Products Limited and Roche Diagnostics International Limited had no role in the design and conduct of the study, the collection, management, analysis and interpretation of the data, the preparation or approval of the manuscript, and the decision to submit the manuscript for publication. Roche Products Limited and Roche Diagnostics International Limited conducted a factual accuracy check of this manuscript, but any decisions to incorporate comments were made solely at the discretion of the authors.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01452-0.

Correspondence and requests for materials should be addressed to C.A.M.

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Policy information about availability of computer code

Data collection	Software used for data collection were: CVI42 (Version 5.6, Circle Cardiovascular Imaging Inc, Canada) Argus tools (Version Syngo MR A25, Siemens AG, Erlangen, Germany) Java magnetic resonance user interface (Version 5.2, jMRUI, Katholieke Universiteit Leuven, Leuven, Belgium) Echopacs (Version 113, GE Vingmed Ultrasound, Norway) Pirouette Randomisation System (Version 1.7)
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Life sciences study design

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Sample size	We determined that 37 patients per group would provide the trial with 80% power to detect an absolute minimum difference, between pirfenidone and placebo groups, of 2% in terms of change in myocardial ECV from baseline following 52 weeks of treatment, at a 5% significance level (2-sided), assuming a standard deviation of the within-patient differences from baseline equal to 3%. (1) This effect size was based on an estimate of the magnitude of myocardial fibrosis regression that could be expected to translate into improved clinical outcomes based on the magnitude of fibrotic regression seen with renin–angiotensin inhibition in other conditions. (2) To allow for treatment discontinuation in up to 20% of patients prior to final follow-up, (3,4) the number randomised to each group was adjusted to 47. An ECV threshold of 27% was chosen as the definition of myocardial fibrosis because it represents one standard deviation above that in healthy volunteers scanned using the same scanner and imaging sequence at the sponsor institution (Manchester University NHS Foundation Trust). For the trial sub-study, we determined that 33 patients per group were required to detect an absolute minimum difference in Phosphocreatine (PCr) to adenosine triphosphate (ATP) ratio of 0.37 between randomised (myocardial extracellular volume (ECV) ≥27%) and non-randomised (ECV <27%) groups at baseline (80% power, 5% significance level, 2-sided), assuming a standard deviation of the between group differences of 0.52. (5) This effect size was based on that seen in previous studies.(5,6) Additionally, 26 patients per group were required to detect an absolute minimum difference, between pirfenidone and placebo groups, of 0.4 in terms of absolute change in PCr:ATP ratio from baseline following 52 weeks of treatment (80% power, 5% significance level, 2-sided), assuming a standard deviation of the within-patient differences from baseline equal to 0.5.(7) This effect size was also based on that seen in other studies.(6-9) To allow for treatment discontinuation in up to 20% of patients prior to final follow-up, the number required in each group was inflated to 33. (1) Garg, R. et al. Mineralocorticoid receptor blockade improves coronary microvascular function in individuals with type 2 diabetes. Diabetes 64, 236-242, doi:10.2337/db14-0670 (2015). (2) Diez, J. et al. Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chamber stiffness in hypertensive patients. Circulation 105, 2512-2517 (2002). (3) King, T. E., Jr. et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med 370, 2083-2092, doi:10.1056/NEJMoa1402582 (2014). (4) Noble, P. W. et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet 377, 1760-1769, doi:10.1016/S0140-6736(11)60405-4 (2011). (5) Phan, T. T. et al. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. Journal of the American College of Cardiology 54, 402-409, doi:10.1016/j.jacc.2009.05.012 (2009). (6) Hudsmith, L. E. & Neubauer, S. Magnetic resonance spectroscopy in myocardial disease. JACC Cardiovasc Imaging 2, 87-96, doi:10.1016/j.jcmg.2008.08.005 (2009). (7) Beadle, R. M. et al. Improvement in cardiac energetics by perhexiline in heart failure due to dilated cardiomyopathy. JACC Heart Fail 3, 202-211, doi:10.1016/j.jchf.2014.09.009 (2015). (8) Abozguia, K. et al. Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy. Circulation 122, 1562-1569, doi:10.1161/CIRCULATIONAHA.109.934059 (2010). (9) Beadle, R. M. et al. Metabolic manipulation in chronic heart failure: study protocol for a randomised controlled trial. Trials 12, 140, doi:10.1186/1745-6215-12-140 (2011).
Data exclusions	At the end of the trial, 12 patients had withdrawn from the study and 2 had died. Therefore, a total of 80 patients were included in the final efficacy analysis. Otherwise, no patient data were excluded from the analyses.
Replication	No experimental replication was attempted. This is essentially not applicable in a phase II randomised controlled clinical trial.
Randomization	Participants were randomised in a 1:1 ratio to treatment with either pirfenidone or matching placebo for 52 weeks using block randomisation, stratified by sex, with computer generated randomisation allocations and randomly varying block sizes. Randomisation was done using web randomisation software accessed using a secure website provided by the clinical trials unit.
Blinding	All trial team investigators were blinded to treatment allocation throughout the study and during data analysis. Participants remained blinded to treatment allocation throughout the duration of the study.

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☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	All patients were aged >40 years. All patients were required to have symptoms and signs of heart failure, a left ventricular ejection fraction of 45% or higher, and elevated natriuretic peptides at baseline (with different thresholds depending on the presence of atrial fibrillation of the occurrence of recent hospitalisation for heart failure). In order to be randomised, patients were required to have evidence of myocardial fibrosis, defined as myocardial ECV greater or equal to 27%; those without myocardial fibrosis were entered into a registry and did not undergo randomisation. The mean age of patients was 78 years, and 46% were female. Nearly all patients had New York Heart Association functional class II or III symptoms (95%), mean left ventricular ejection fraction was 64% and median NT-proBNP was 1104 pg/ml. Mean myocardial ECV was 30.1%.
Recruitment	Patients were identified at six NHS hospitals in the United Kingdom. Patients were identified from outpatient clinics and inpatient wards. All potentially eligible patients were invited to take part, thereby minimising any potential self-selection bias.
Ethics oversight	The study was approved by a regional ethics committee - NHS Health Research Authority, North West - Liverpool Central Research Ethics Committee (16/NW/0717). Trial conduct was overseen by a trial steering committee and an independent data and safety monitoring committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02932566
Study protocol	Study protocol has been submitted along with the manuscript.
Data collection	Patients were recruited between March 7th, 2017, to December 19th, 2018 at 6 NHS hospitals in the United Kingdom (1. Macclesfield District General Hospital, East Cheshire NHS Foundation Trust; 2. Manchester Royal Infirmary, Manchester University NHS Foundation Trust; 3. North Manchester General Hospital, Pennine Acute Hospitals NHS Trust; 4. Salford Royal Hospital, Salford Royal NHS Foundation Trust; 5. Stepping Hill Hospital, Stockport NHS Foundation Trust; 6. Wythenshawe Hospital, Manchester University NHS Foundation Trust). All study visits took place at Wythenshawe Hospital. The final follow-up visit was completed on November 29th, 2019.
Outcomes	All primary and secondary outcome measures were pre-specified in a statistical analysis plan (SAP). All statistical analyses performed on all outcome measures where thus predefined. The SAP has been submitted along with the study protocol and is freely available for review with this manuscript.