

# An exploratory randomized control study of combination cytokine and adult autologous bone marrow progenitor cell administration in patients with ischaemic cardiomyopathy: the REGENERATE-IHD clinical trial

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## Aims

The effect of combined cytokine and cell therapy in ischaemic cardiomyopathy is unknown. Meta-analyses suggest improved cardiac function with cell therapy. The optimal cell delivery route remains unclear. We investigated whether granulocyte colony-stimulating factor (G-CSF) alone or in combination with intracoronary (i.c.) or intramyocardial (i.m.) injection of autologous bone marrow-derived cells (BMCs) improves cardiac function.

## Methods and results

Ninety patients with symptomatic ischaemic cardiomyopathy and no further treatment options were enrolled in the randomized, placebo-controlled, single-centre REGENERATE-IHD study. Randomization was to one of three arms: peripheral, i.c., or i.m. In each arm, patients were randomized to active treatment or placebo. All patients, apart from the peripheral placebo group (saline only) received G-CSF for 5 days. The i.c. and i.m. arms received either BMCs or serum (placebo). The primary endpoint was change in LVEF at 1 year assessed by cardiac magnetic resonance imaging/computed tomography. The i.m. BMC group showed a significant improvement in LVEF of 4.99% (95% confidence interval 0.33–9.6%;  $P = 0.038$ ) at 1 year. This group also showed a reduction in NYHA class at 1 year and NT-proBNP at 6 months. No other group showed a significant change in LVEF. This finding is supported by post-hoc between-group comparisons.

## Conclusion

We have shown that G-CSF combined with autologous i.m. BMCs has a beneficial effect on cardiac function and symptoms. However, this result should be considered preliminary in support of a clinical benefit of i.m. stem cell infusion in 'no option' patients and needs further exploration in a larger study.

## Keywords

Bone marrow-derived cells • Ischaemic cardiomyopathy • Granulocyte colony-stimulating factor

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## Introduction

One of the principal causes of heart failure in developed countries is ischaemic heart disease.<sup>1</sup> Despite advances in treatment, the prognosis for patients who are admitted to hospital with heart failure remains poor, with a 5-year survival of ~50% and a 10-year survival of ~10%.<sup>2,3</sup> The increasing prevalence of heart failure poses a significant burden to patients, practitioners, and healthcare systems, and hence defines a need for new or improved treatments in patients with no further treatment options.

A number of small trials have evaluated the role of the cytokine granulocyte colony-stimulating factor (G-CSF) alone in improvement of cardiac function in ischaemic heart failure; however, the overall results have been mixed.<sup>4–8</sup> Following early trials showing beneficial effects of cell therapy in acute myocardial infarction (MI),<sup>9,10</sup> Phase I/II trials using infusion of autologous progenitor cells in ischaemic cardiomyopathy have shown promising results.<sup>11–13</sup> However, these studies have often lacked an appropriate comparison group for the interventions used. Meta-analysis confirms an improvement in cardiac function of patients with autologous cells and also that the intramyocardial route of delivery is most effective.<sup>14–17</sup> Only a small number of trials have assessed the use of cytokine therapy as an adjunct to cell therapy, of which none is fully controlled.<sup>15,17</sup>

In the exploratory randomized, placebo-controlled REGENERATE-IHD trial, we sought to address for the first time whether G-CSF alone or combined with cell therapy has a beneficial effect on cardiac function in patients with ischaemic cardiomyopathy who have no further treatment options but remain symptomatic. The trial design was modelled on the recently published REGENERATE-DCM trial,<sup>18</sup> a study assessing the combination of cytokine and cell therapy in patients with non-ischaemic cardiomyopathy.

## Methods

### Study design and patient selection

The REGENERATE-IHD trial is an investigator-initiated, single-centre, randomized, placebo-controlled trial. The trial assessed whether G-CSF administration alone or in combination with either the intracoronary (i.c.) or targeted intramyocardial (i.m.) injection of autologous bone marrow-derived cells (BMCs) leads to an additional benefit on cardiac function compared with placebo controls. Following a pilot study to address safety and feasibility,<sup>19</sup> the trial was approved by an independent ethics committee (REC no. 04/Q0603/13), the Medicines and Healthcare Products Regulatory Agency (MHRA), registered at approved registries (ClinicalTrials.gov: NCT00747708; EudraCT: 2005-002706-27), and was performed in accordance with the Declaration of Helsinki (1993) and the principles of the International Conference of Harmonization–Good Clinical Practice (ICH-GCP) guidelines. Details of the trial protocol have been published previously.<sup>20</sup>

Patients were referred to the trial with a confirmed diagnosis of heart failure from local heart failure clinics. Inclusion criteria were a confirmed diagnosis of ischaemic heart failure (including diffuse coronary stenoses and extensive infarcted myocardium), on optimal medical treatment (for at least 6 months), documented impaired LVEF, NYHA class II–IV, with no further treatment/revascularization

options (for a complete list, see the Supplementary material online, *Methods*).

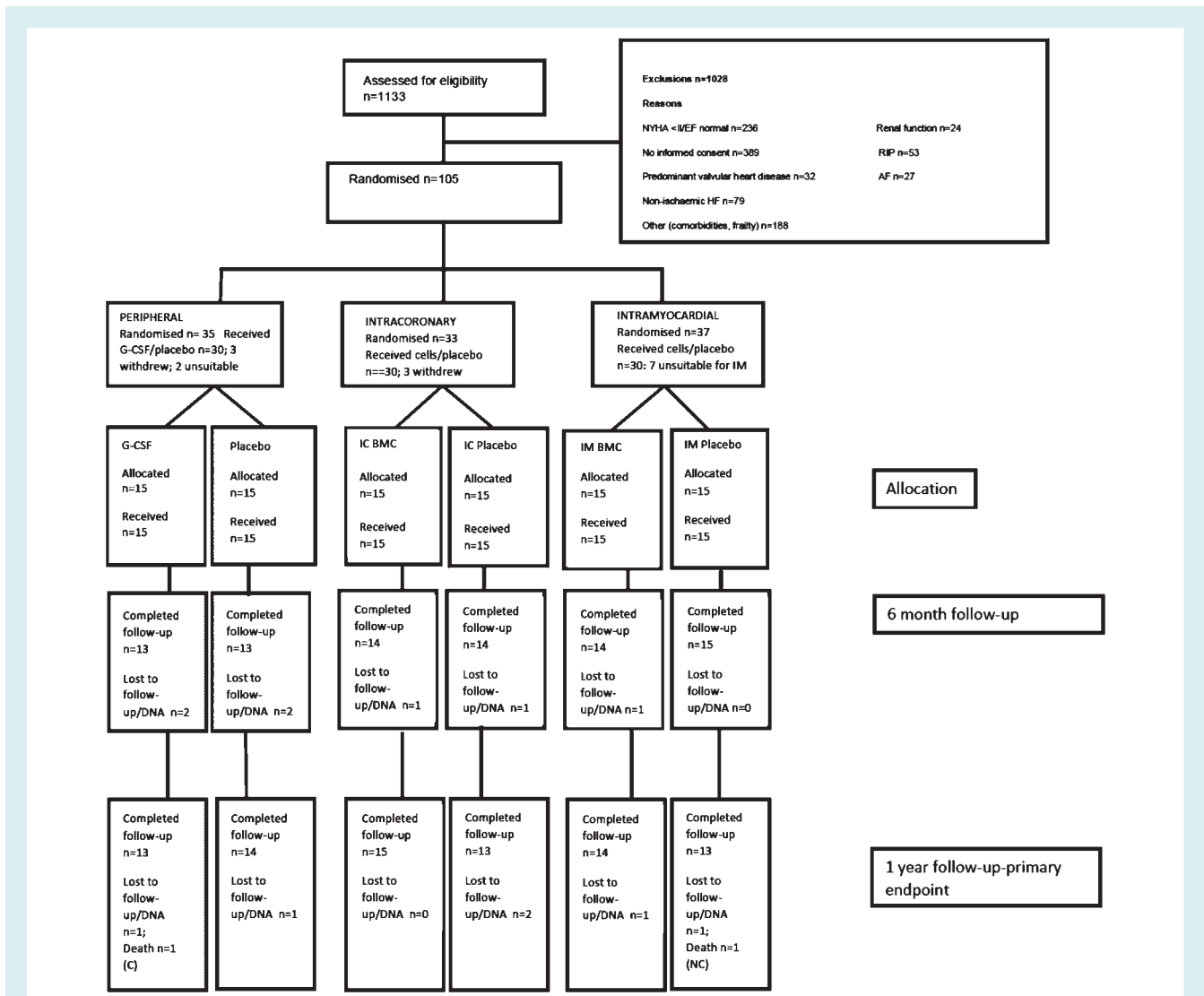
### Randomization, masking, and treatment

After consenting for the trial, patients underwent a two-stage randomization process using a dedicated trial software system (IHD, Bishops Stortford, Herts, UK). First, they were randomized to one of three treatment arms: the peripheral arm, the i.c. arm, or the i.m. arm independent of coronary anatomy. Once allocated to a treatment arm, patients were further randomized (1:1) to either the active treatment or the placebo group. If after first randomization, patients were found to be unsuitable for their assigned treatment arm on the basis of further trial investigations, they would be withdrawn at this stage. Accordingly, patients randomized to the peripheral arm were further randomized to receive 5 days of either subcutaneous injected saline or subcutaneous G-CSF (Granocyte™, Chugai Pharmaceutical Ltd, Mulliner House, London, UK) (10 µg/kg/day). Patients who were randomized to either the i.c. or i.m. treatment arms all received 5 days of subcutaneous G-CSF followed by bone marrow harvest within 24 h of completing treatment. A 50 mL aliquot of bone marrow was aspirated from the iliac crest under local anaesthetic, and an additional 30 mL of blood was taken to provide serum for the placebo group. The aspirate and blood were sent to the designated stem cell laboratory where patients were further randomized to receive either autologous BMCs or serum (1:1 within each arm). Bone marrow mononuclear cells were isolated by density gradient centrifugation, washed, and resuspended in autologous serum at a final volume of 10 mL (i.c.) or 2 mL (i.m.). The cell product/serum was injected via either i.c. or i.m. routes within 24 h of bone marrow harvest. The i.c. delivery was in equal amounts into three patent coronary vessels during stop flow conditions.<sup>9</sup> The i.m. cell product/serum was injected via the MyoStar™ catheter (Biologics Delivery Systems Group, Cordis Corporation, Diamond Bar, CA, USA) into border zones around scar tissue, guided by electromechanical mapping using the NOGA® XP Cardiac Navigation System. The total 2 mL volume was delivered equally to 10 target areas at ~1 cm intervals. (For further information see *Figure 1* and the Supplementary material online, *Methods*).

Patients within each arm remained blinded to the therapy they received. Both the i.c. and i.m. arms were double blinded, with patients, investigators, and treating clinicians blinded to group assignment (cells/serum). Due to daily full blood count monitoring during G-CSF treatment, the peripheral arm remained single blinded only. Data analysts were entirely masked to group assignment in all trial arms.

### Endpoints and definitions

The primary endpoint for all patients was the change in LVEF at 12 months compared with baseline (prior to commencing G-CSF, or placebo in the peripheral placebo group) as measured using cardiac magnetic resonance imaging (CMR) or computed tomography (CT; for those unable to undergo CMR). Secondary endpoints included change in NYHA class, quality of life (QoL) (EQ5D®, SF-36®, and MacNew® questionnaires), and LVEF by contrast transthoracic echocardiography at 6 months and 1 year compared with baseline, as well as NT-proBNP and, in the i.c. and i.m. arms only, quantitative left ventriculography at 6 months compared with baseline. Safety endpoints included major adverse cardiac events (MACE: cardiac death, MI, PCI, or coronary artery bypass grafting) or any significant arrhythmias (symptomatic ventricular tachycardia or survived cardiac death) at 6 months and 1 year.



**Figure 1** CONSORT diagram. Flow chart of the study summarizing flow of patients through the trial. BMC, bone marrow-derived cells; C, cardiac; G-CSF, granulocyte colony-stimulating factor; HF, heart failure; IC, intracoronary; IM, intramyocardial; NC, non-cardiac.

## Advanced cardiac imaging

Cardiac magnetic resonance imaging, or cardiac CT for those unable to undergo CMR (e.g. cardiac devices, claustrophobia, etc.) were performed at baseline and 12 months. Multiphase cardiac data sets with full LV coverage were acquired using standard protocols<sup>21,22</sup> (see the Supplementary material online, *Methods*). Images were analysed in blinded fashion by two experienced operators. The standard error of measurement of CMR and CT was 1.93% and 2.3%, respectively.

## Statistical design and analyses

The study was powered to detect a 3.5% within-group improvement in the primary endpoint, i.e. change in LVEF at 12 months based on changes seen in a contemporary trial of cell therapy.<sup>23</sup> Based on a power of 90%, a significance level of 5% and an estimated variance of 4% (assuming pre- and post-treatment means are uncorrelated), the calculated required number of patients in each group was 11. It was

estimated that an additional four per group would be needed in order to ensure that 11 patients reached the primary endpoint at 1 year, resulting in a size of 15 patients per treatment group.

A paired *t*-test was used to detect any statistical significance of within-group changes in LVEF. For additional analyses using continuous variables, appropriate parametric (paired *t*-test for paired, and independent samples *t*-test for unpaired data) and non-parametric (Wilcoxon sign-rank test for paired, Mann-Whitney for unpaired data, and Kruskal-Wallis test for more than two groups) tests were used.  $\chi^2$  or Fisher's exact tests were used for categorical variables. For post-hoc between-group comparisons, one-way analysis of variance (ANOVA) was used with Dunnett's adjustment for multiple comparisons with a significance level of 0.2 where all treatment groups were compared with the peripheral placebo group as a 'control'. Where post-hoc analysis was performed without a 'control' group comparison, a one-way ANOVA was used with Holm-Sidak adjustment for multiple comparisons with a significance level of 0.2. Pearson's linear regression was used for comparison between LVEF

**Table 1** Baseline characteristics

	Saline (n = 15)	G-CSF (n = 15)	i.c. serum (n = 15)	i.c. BMC (n = 15)	i.m. serum (n = 15)	i.m. BMC (n = 15)	P-value
Age, years (mean ± SD)	63.3 ± 9.3	63.1 ± 8.2	62.8 ± 10.7	62.1 ± 9.7	60.4 ± 11.2	65.3 ± 9.4	0.841
Sex M/F, n	14/1	13/2	14/1	14/1	15/0	15/0	0.896
BMI (kg/m <sup>2</sup> ) (mean ± SD)	29.5 ± 4.3	31.4 ± 6.0	31.7 ± 6.5	29.7 ± 4.8	29.6 ± 3.7	30.8 ± 4.0	0.739
Medical history, n (%)							
Hypertension	3 (20.0)	1 (6.7)	2 (13.3)	3 (20.0)	5 (33.3)	5 (33.3)	0.414
Diabetes	4 (26.7)	5 (33.3)	2 (13.3)	2 (13.3)	4 (26.7)	4 (26.7)	0.748
CABG	5 (33.3)	4 (26.7)	3 (20.0)	7 (46.7)	6 (40.0)	4 (26.7)	0.653
MI	13 (86.7)	12 (80.0)	14 (93.3)	13 (86.7)	13 (86.7)	13 (86.7)	0.949
Hypercholesterolaemia	4 (26.7)	6 (40.0)	5 (33.3)	5 (33.3)	8 (53.3)	4 (33.3)	0.715
Smoker/ex-smoker	12 (80.0)	8 (53.3)	13 (86.7)	11 (73.3)	14 (93.3)	11 (73.3)	0.150
Time from last MI, days median (IQR)	1307 (1064–5443)	2527 (966–4928)	2856 (1278–6041)	1805 (896–3855)	2406 (706–5402)	2684 (706–5402)	0.964
LVEF (%) (mean ± SD)	34.7 ± 10.1	27.9 ± 12.4	31.6 ± 7.4	31.7 ± 8.8	29.0 ± 9.2	28.6 ± 10.2	0.385
Number of devices, n (%)							
CRT-D	4 (26.7)	5 (33.3)	4 (26.7)	4 (26.7)	3 (20.0)	7 (46.7)	0.781
CRT-P	1 (6.7)	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0.999
ICD only	7 (46.7)	2 (13.3)	4 (26.7)	5 (33.3)	5 (33.3)	6 (40.0)	0.485
Medication history, n (%)							
Statin	12 (80.0)	13 (86.7)	13 (86.7)	13 (86.7)	14 (93.3)	13 (86.7)	0.995
ACE inhibitor/ARB	14 (93.3)	14 (93.3)	13 (86.7)	14 (93.3)	15 (100)	15 (100)	0.896
Beta-blocker	15 (100.0)	14 (93.3)	12 (80.0)	15 (100.0)	11 (73.3)	14 (93.3)	0.079
Aldosterone antagonist	9 (60.0)	13 (86.7)	9 (60.0)	12 (80.0)	9 (60.0)	12 (80.0)	0.351
Diuretics	10 (66.7)	11 (73.3)	13 (86.7)	12 (80.0)	8 (53.3)	12 (80.0)	0.363
NYHA at baseline, n (%)							0.393
II	10 (66.7)	9 (60.0)	5 (33.3)	8 (53.3)	11 (73.3)	8 (53.3)	
III	4 (26.7)	6 (40.0)	10 (66.7)	7 (46.7)	4 (26.7)	6 (40.0)	
IV	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.7)	
NT-proBNP (pg/mL) median (IQR)	600 (245–1691)	930 (457–1782)	812 (364–1632)	423 (241–577)	567 (237–1015)	634 (313–2038)	0.199

BMI, body mass index; CABG, coronary artery bypass graft; F, female; G-CSF, granulocyte colony-stimulating factor; i.c., intracoronary; ICD, implantable cardioverter defibrillator; i.m., intramyocardial; IQR, interquartile range; M, male; MI, myocardial infarction.

and cell function variables. Values are quoted as mean ± SD unless otherwise stated. All *P*-values are two sided, and *P* < 0.05 is considered to indicate statistical significance except for post-hoc between-group analysis. Statistical analyses were performed using SPSS<sup>®</sup> version 21 (IBM Corp., Armonk, NY, USA) and graphs were produced using Graphpad Prism<sup>®</sup> version 5.0 (GraphPad Software, San Diego, CA, USA).

## Results

### Patient population

A total of 1133 patients were referred from heart failure clinics throughout the UK. Of these, 1028 were ineligible: non-ischaeamic aetiology (*n* = 79), NYHA class < II/normal LVEF (*n* = 236), AF (*n* = 27), refusal to participate in the trial (*n* = 389), death before formal consent (*n* = 53), and other co-morbidities (*n* = 244). Of the 105 patients who were randomized, 15 patients were withdrawn because of either unsuitability for the intervention or patient withdrawal. Ninety received the allocated trial intervention (Figure 1).

The mean age of the patient population was 62.8 ± 9.6 years, while the majority of patients were male (94.4%). The baseline characteristics were similar across the groups, with a mean LVEF of 30.6% [95% confidence interval (CI) 28.53–32.74], a mean NT-proBNP of 1187.0 ± 179.9 pg/mL, and 97.8% of patients in NYHA class II/III. Baseline patient demographics are shown in Table 1. The majority of patients were on optimal medical therapy (as per ESC guidelines),<sup>24</sup> and there were no significant differences

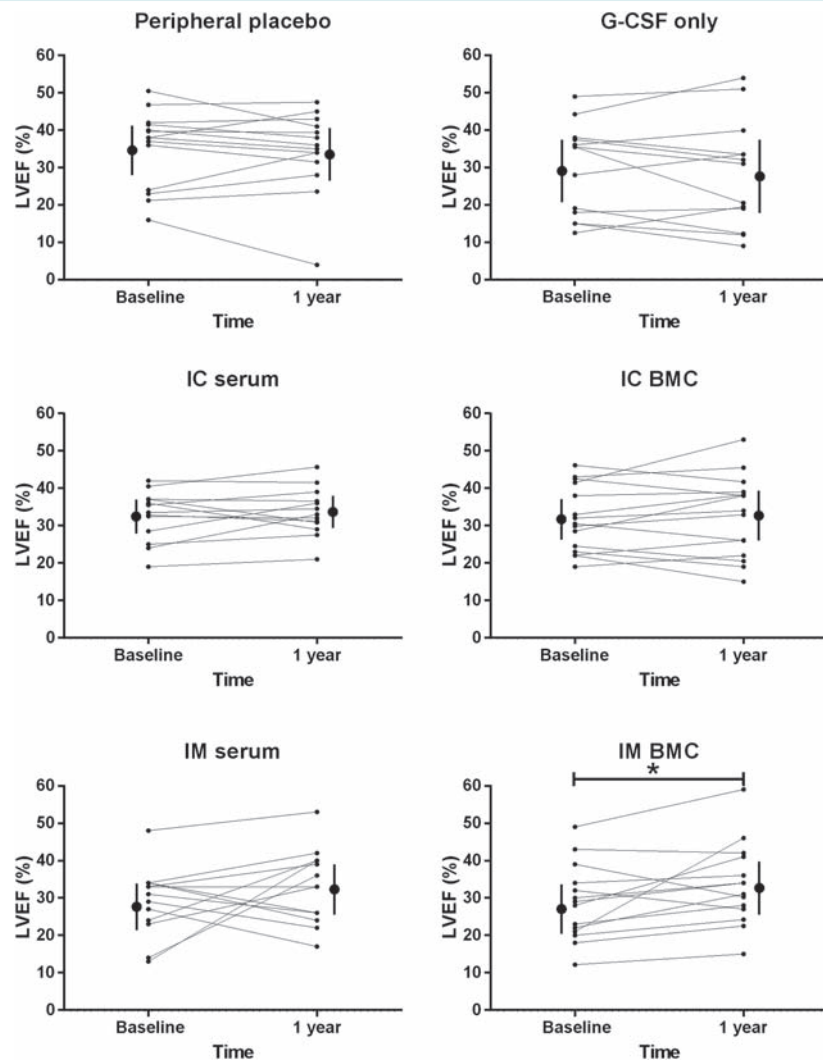
across groups in prescribed medication, implanted device therapy, or rates of LV assessment by CMR or cardiac CT. At 1 year, a total of 82 patients were assessed for the primary endpoint (Figure 1).

### Left ventricular ejection fraction

Twenty-six patients underwent CMR assessment of cardiac function and the remainder CT assessment. Only patients treated in the i.m. BMC group met the primary endpoint of change in LVEF (increase of 4.99%; 95% CI 0.33–9.6%; *P* = 0.038, Figure 2; Supplementary material online, Table S1). A trend to improvement in LVEF was seen in the i.m. serum group (4.15%; 95% CI –3.3–11.6%; *P* = 0.246). Importantly no change was seen in the i.c. BMC-treated group (0.89%; 95% CI –2.2 to –3.9%; *P* = 0.541) or the peripheral groups: G-CSF (–1.25; 95% CI –5.4 to –2.9%; *P* = 0.520) and placebo (–0.98%; 95% CI –4.4 to –2.5%; *P* = 0.551).

There were no significant changes in LV end-diastolic or end-systolic volumes (LVEDV and LVESV) over time in any treatment group (Supplementary material online, Table S1). The improvements in cardiac function in the i.m. BMC group were also seen using echocardiography, with no improvement seen in any other treatment group (Supplementary material online, Figure S1).

In post-hoc between-group analysis, there was an absolute improvement in LVEF of 5.97% in the i.m. BMC group compared with the peripheral placebo group (*P* = 0.1867), not seen in the other groups (Table 2). In post-hoc analysis comparing patients treated with cells (i.c./i.m.), G-CSF (alone or in combination with



**Figure 2** Primary endpoint analysis of LVEF. LVEF at baseline and 1 year as measured using cardiac magnetic resonance imaging/computed tomography in each of the treatment groups. BMC, bone marrow-derived cells; G-CSF, granulocyte colony-stimulating factor; IC, intracoronary; IM, intramyocardial. \* $P < 0.05$ . Large filled circles represent the means at baseline and 1 year for each group, respectively, error bars represent 95% confidence intervals.

i.c./i.m. serum), and peripheral placebo, there were no differences in improvement in LVEF. However, post-hoc comparisons between the three trial arms (peripheral, i.c., and i.m.) revealed that patients treated with combination G-CSF and i.m. injection (cells/serum) showed significant improvement in LVEF compared with the peripheral trial arm (absolute difference: 5.77%,  $P = 0.0165$ ) and the i.c. trial arm (absolute difference: 3.60%,  $P = 0.1477$ ) with an overall significant ANOVA of  $P = 0.0193$  (Supplementary material online, Table S1).

### Plasma N-terminal pro brain natriuretic peptide concentration

Statistical analysis was performed after logarithmic transformation of NT-proBNP values due to a non-normal distribution

(untransformed baseline values in Table 1). At 6 months, only patients in the i.m. BMC group showed a significant decrease in NT-proBNP ( $977.5 \pm 866.8$  to  $768.4 \pm 754.4$ ;  $P = 0.018$ ). The remaining groups showed no change (Figure 3). There were no between-group differences seen in post-hoc analysis (Supplementary material online, Table S5).

### New York Heart Association and Canadian Cardiovascular Society class

In the i.m. BMC group, there was a significant improvement in NYHA class at 6 months ( $-0.57$ ;  $P = 0.025$ ), maintained at 1 year ( $-0.50$ ;  $P = 0.048$ ). A significantly better improvement in NYHA class was also seen in the i.m. BMC group compared with peripheral

**Table 2** Post-hoc analysis of the between-group changes in left ventricular ejection fraction

Treatment groups compared with peripheral placebo	Within-group change	Absolute change	P-value
Peripheral placebo	-0.98	-	-
Peripheral G-CSF	-1.25	-0.43	0.9998
i.c. serum	1.10	+2.08	0.9272
i.c. BMC	0.89	+1.88	0.9439
i.m. serum	4.15	+5.14	0.2791
i.m. BMC	4.99	+5.97	0.1500*
Overall ANOVA (15 comparisons)			0.1645

Significance assessed by one-way analysis of variance (ANOVA) and Dunnett's adjustment for multiple comparisons with a significance level of <0.2.

G-CSF, granulocyte colony-stimulating factor; i.c., intracoronary; i.m., intramyocardial.

\*represents significance  $p < 0.2$  (Dunnett's adjustment for multiple comparisons).

placebo in post-hoc analysis ( $P = 1284$ ; Supplementary material online, Table). In the i.m. serum group, a significant reduction in NYHA class was seen at 6 months ( $-0.47$ ;  $P = 0.016$ ) but this was not sustained at 1 year ( $-0.15$ ;  $P = 0.451$ ). In all other groups, no change in NYHA class was seen (Figure 4; Supplementary material online, Table S2). There was no change in Canadian Cardiovascular Society (CCS) class at 6 months or 1 year in any group (Supplementary material online, Figure S2; Table S5).

## Quality of life questionnaires

The i.m. BMC group showed an improvement in QoL as assessed by the physical well-being score in the SF-36<sup>®</sup> questionnaire at 1 year. At 1 year, the i.c. BMC group showed a significant improvement in the MacNew<sup>®</sup> and the i.c. placebo group showed an improvement in the SF-36<sup>®</sup> physical well-being score. Neither the i.m. placebo group nor the peripheral groups showed improvement in QoL at 1 year (Supplementary material online, Table S3). In post-hoc analysis, the only significant between-group difference in QoL when compared with peripheral placebo was seen as an improvement at 6 months in the i.m. BMC group using the SF-36<sup>®</sup> physical well-being questionnaire ( $P = 0.0421$ ; Supplementary material online, Table S5). No other treatment groups showed a better response than peripheral placebo.

## Cell data

There was a trend towards a higher peripheral mononuclear cell count after 5 days of G-CSF in the i.m. peripheral group ( $P = 0.049$ ). No other differences were observed in G-CSF-treated patients. A breakdown of flow cytometric cellular analysis is given in the Supplementary material online, Table S6. The mean total number of cells injected in the two stem cell groups was  $115.1 \times 10^6$  mononuclear cells. The mean viability of processed cells was 98.2%. There were no significant differences in cell numbers injected

in the i.c. or i.m. group. No correlation was found between the cell type injected and change in LVEF in any of the groups. A significant correlation was observed between the number of colony-forming unit granulocyte-monocyte (CFU GM) colonies and the improvement in LVEF in the i.m. BMC group (Pearson  $r = 0.79$ ;  $P = 0.02$ ) (Figure 5). No correlation was observed between bone marrow CFU GM and change in LVEF in the i.c. BMC group.

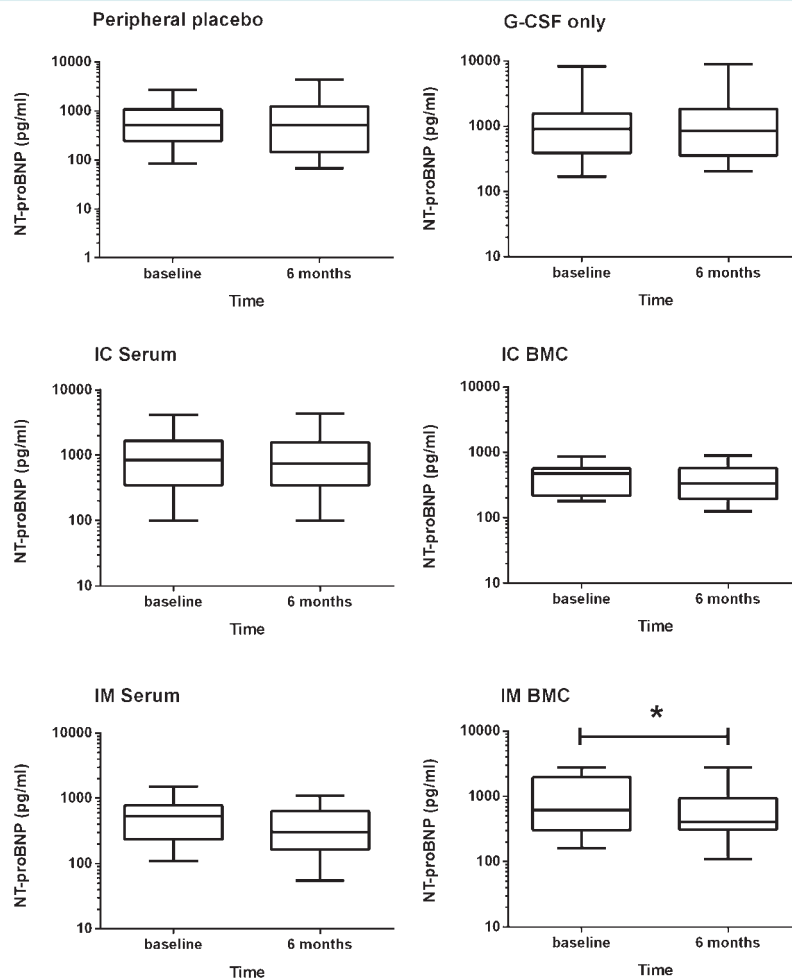
## Safety

A small increase in post-procedural troponin was detected in the i.m. arms ( $0.02 \pm 0.04$ ;  $P = 0.007$ ). However, there was no significant difference in troponin increase between the two groups and it was not associated with a rise in creatine kinase (from 85.1 to 129.5;  $P = 0.102$ ). No clinical events occurred during the procedures. No increases in cardiac enzymes were seen in any other treatment arm. No significant differences in procedural complication rates ( $P = 0.413$ ), significant arrhythmias ( $P = 0.896$ ), or rates of MACE ( $P = 0.539$ ) were noted between the groups. MACE rates were low for all groups (one cardiovascular death in the G-CSF group and one MI in the i.c. placebo group).

## Discussion

The REGENERATE-IHD randomized trial is an early stage exploratory placebo-controlled trial assessing the beneficial effects of a combination of cytokine and cell therapy directly comparing two different delivery routes in patients with advanced heart failure, a group with no further treatment options. Due to the sample size, the results should be seen as exploratory findings that will need confirmation in larger focused trials. Nevertheless, patients treated with G-CSF therapy and i.m. cell delivery for 5 days demonstrated a 4.99% within-group increase in LVEF at 1 year. This improvement in LV function was accompanied by a sustained reduction in NYHA class at 1 year, associated with significant improvement in QoL scoring and the biochemical marker NT-proBNP, making a clinical benefit in this treatment group more likely. Recognizing the limitations of within-group analyses with small sample size,<sup>25</sup> exploratory post-hoc between-group analyses were also performed. In support of the primary outcomes, post-hoc analysis demonstrated a 5.97% absolute improvement in LVEF in the i.m. BMC group compared with the peripheral placebo group, a group which could be considered the true control. No other treatment group showed evidence of either within-group improvement in LVEF at 1 year or between-group improvement in LVEF when compared with the peripheral placebo group. Importantly, although the change in LVEF would appear to be relatively low, the magnitude of effect is similar to that seen in other trials of heart failure therapies (e.g. ACE inhibitors), which are now part of standard practice.<sup>26</sup>

Together with inducing stem cell mobilization, G-CSF has also been postulated as an independent therapeutic agent in ischaemic cardiomyopathy, with previous studies suggesting an associated improvement in LVEF.<sup>7</sup> These studies have either lacked appropriate controls or have not been randomized.<sup>4-8,27</sup> In the exploratory

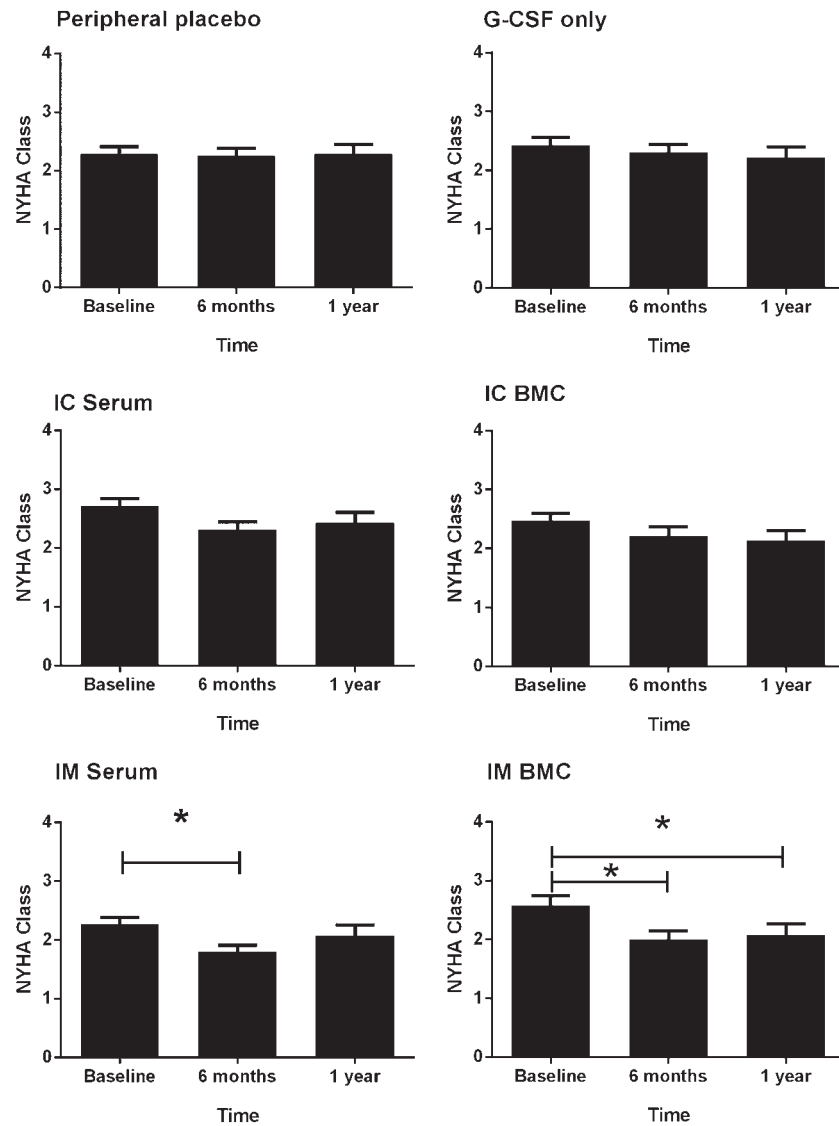


**Figure 3** NT-proBNP. Box and whisker plots showing NT-proBNP (pg/mL) at baseline and 6 months (median and range on a logarithmic scale). BMC, bone marrow-derived cells; G-CSF, granulocyte colony-stimulating factor; IC, intracoronary; IM, intramyocardial. \* $P < 0.05$ .

REGENERATE-IHD trial findings reported here, G-CSF treatment has no beneficial effect on functional, clinical and biochemical markers of heart failure in this patient population either within group or compared with peripheral placebo. However, it could be suggested that G-CSF has an incremental effect due to the additional benefit of a several fold increase in circulating mononuclear cell and CD34<sup>+</sup> cell counts or the increase in cell counts including CD34<sup>+</sup> cell counts in the final injected cell product as a result of G-CSF mobilization.<sup>28</sup> This is supported by the improvement seen in LVEF at 1 year in the i.m. placebo group of 4.1% which although non-significant may be attributed to higher G-CSF-induced peripheral mononuclear cell counts achieved in this treatment group compared with all other groups (0.049).

The findings of REGENERATE-IHD are in keeping with recent meta-analysis data in ischaemic heart failure, showing a 5.30% improvement in LVEF associated with i.m. cell injection compared with 3.19% improvement using the i.c. route.<sup>14–17</sup> Intramyocardial injection has been shown to be associated with higher cell retention rates within the myocardium than other delivery routes (11%

compared with 3% with i.c. delivery).<sup>29</sup> In chronic ischaemic cardiomyopathy, i.m. injection may be more effective by providing a targeted approach, thereby overcoming the lack of chemoattractive homing signals (that are more pronounced in acute ischaemia) as well as bypassing the requirement to adhere to and penetrate the endothelium. Furthermore, in this setting, the target myocardium may not be subtended by a patent coronary or collateral vessel, precluding the utility of i.c. delivery. It is interesting that the i.m. serum group shows a 4.15% improvement in LVEF at 1 year and an absolute change in LVEF of 5.14% over peripheral placebo, neither of which reach significance. However, when grouped by trial treatment arm (i.m. serum/cells) compared with the i.c. arm and peripheral arm, patients who received i.m. injection of either cells or serum demonstrate significantly improved LVEF. While this post-hoc analysis is exploratory and underpowered, it does support the superiority of the i.m. approach over i.c. delivery and seems to suggest a benefit of i.m. injection that is cell independent. It could therefore be hypothesized that the targeted i.m. injection of autologous serum after 5 days of treatment with



**Figure 4** NYHA class. Mean NYHA class at baseline, 6 months, and 1 year shown for each of the treatment groups. BMC, bone marrow-derived cells; G-CSF, granulocyte colony-stimulating factor; IC, intracoronary; IM, intramyocardial. \* $P < 0.05$ .

G-CSF may contain reparative cytokines and growth factors that play a role in recovery of cardiac function. However, the correlation between cell function (CFU assay) and change in LVEF in the i.m. BMC group also provides evidence for cellular involvement in sustained improvement in cardiac function. The observed improvement in LVEF would suggest favourable reverse LV remodelling, although the magnitude of the improvement and the small study size may explain the fact that changes in LVESV and LVEDV were not detected.

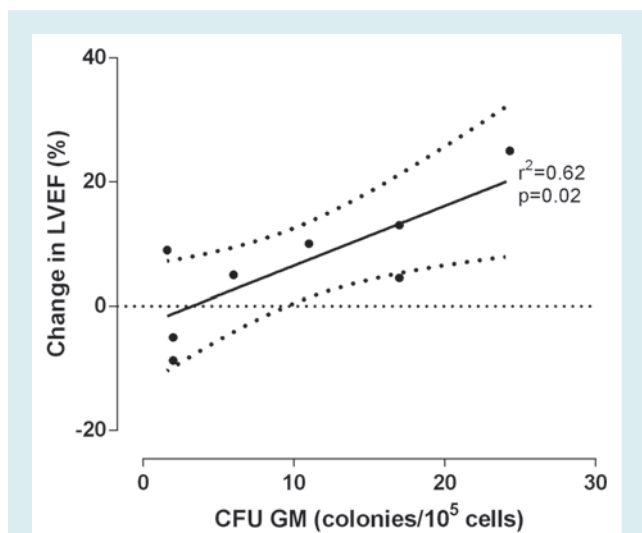
The baseline characteristics and disease parameters including LVEF (<30%) were consistent with an advanced ischaemic cardiomyopathy patient population and similar across the groups. Importantly, this trial provides evidence for the safety and feasibility of the use of autologous cells in patients with advanced heart failure with low peri-procedural complication rates and MACE

rates consistent with previous similar trials. Furthermore, the rise in troponin in the i.m. group is in keeping with previously published literature.<sup>30</sup>

### Study limitations

The REGENERATE-IHD trial was designed in the same way as the recently published REGENERATE-DCM trial.<sup>18</sup> In order to investigate three different modes of cell therapy with the appropriate control groups, the study population was divided into six individual groups of 15 patients each. Small sample size as well as a large exclusion rate among screened patients have the potential to create a clinical selection bias despite the fact that there are no significant differences in baseline disease markers between groups. Despite the small sample size, the trial met its statistical primary





**Figure 5** Colony-forming unit granulocyte–monocyte (CFU GM) count and LVEF. Significance is shown for correlations  $\pm$  95% confidence intervals.

endpoint of within-group improvement in LVEF only observed in the i.m. BMC group. It is reasonable to suggest that this evidence alone may not be enough to indicate benefit as within-group comparisons have often been demonstrated to be unreliable and even misleading, while between-group or two-sample methods are favoured.<sup>25</sup> However, clinical benefit in the i.m. BMC group is supported by the changes observed across independent surrogate endpoints: NT-proBNP, NYHA class, as well as exploratory albeit underpowered post-hoc between-group analysis showing benefit of i.m. BMCs over the true control group (peripheral placebo). This was a small Phase II trial powered around intermediate efficacy measures and was not powered to detect changes in MACE and mortality rates, as has been the case for similar trials before it. While the trial could not be fully blinded, data analysers were blinded to all study groups, and investigators and patients were blinded within each arm. For pragmatic and ethical reasons, the amount of bone marrow harvested was standardized at 50 mL rather than the dose of cells received by the patient.

## Conclusion

This is an early stage Phase II exploratory randomized controlled trial to assess whether G-CSF therapy alone or in combination with either i.m. or i.c. delivery of BMCs improves cardiac function in patients with advanced ischaemic heart failure with no other treatment options. We show that the combination of G-CSF and i.m. progenitor cell injection is safe, confers an improvement in LVEF at 1 year of 4.99%, and is accompanied by improvement in a panel of biochemical and symptom-related outcomes, while other treatment groups show no significant change. The results of REGENERATE-IHD should be considered preliminary in support of a clinical benefit of i.m. stem cell infusion as an adjunctive therapy in this ‘no option’ patient group. This finding should be explored in a larger study.

## Supplementary Information

Additional Supporting Information may be found in the online version of this article:

**Methods.** Supplemental methods.

**Figure S1.** LVEF (using transthoracic echocardiography).

**Figure S2.** Canadian Cardiovascular Society (CCS) angina score.

**Table S1.** Left ventricular volumes and ejection fraction.

**Table S2.** Between group changes in left ventricular ejection fraction: modified groups.

**Table S3.** NYHA class.

**Table S4.** Quality of life questionnaire scores.

**Table S5.** Between-group changes in secondary outcomes: NT-proBNP, NYHA class, CCS class and quality of life outcomes.

**Table S6.** Cell characterization in the study arms of REGENERATE-IHD.

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**Authors' contributions:** Anthony Mathur had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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