Granulocyte-Colony Stimulating Factor for Mobilizing Bone Marrow Stem Cells in Subacute Stroke

The Stem Cell Trial of Recovery Enhancement After Stroke 2 Randomized Controlled Trial

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Background and Purpose—Granulocyte-colony stimulating factor (G-CSF) is neuroprotective in experimental stroke and mobilizes $CD34^+$ peripheral blood stem cells into the circulation. We assessed the safety of G-CSF in recent stroke in a phase IIb single-center randomized, controlled trial.

Methods—G-CSF (10 μg/kg) or placebo (ratio 2:1) was given SC for 5 days to 60 patients 3 to 30 days after ischemic or hemorrhagic stroke. The primary outcome was the frequency of serious adverse events. Peripheral blood counts, $CD34^+$ count, and functional outcome were measured. MRI assessed lesion volume, atrophy, and the presence of iron-labeled $CD34^+$ cells reinjected on day 6.

Results—Sixty patients were recruited at mean of 8 days (SD $\pm$5) post ictus, with mean age 71 years ($\pm$12 years) and 53% men. The groups were well matched for baseline minimization/prognostic factors. There were no significant differences between groups in the number of participants with serious adverse events: G-CSF 15 (37.5%) of 40 versus placebo 7 (35%) of 20, death or dependency (modified Rankin Score: G-CSF 3.3$\pm$1.3, placebo 3.0$\pm$1.3) at 90 days, or the number of injections received. G-CSF increased $CD34^+$ and total white cell counts of 9.5- and 4.2-fold, respectively. There was a trend toward reduction in MRI ischemic lesion volume with respect to change from baseline in G-CSF–treated patients (P=0.06). In 1 participant, there was suggestion that labeled $CD34^+$ cells had migrated to the ischemic lesion.

Conclusions—This randomized, double-blind, placebo-controlled trial suggests that G-CSF is safe when administered subacutely. It is feasible to label and readminister iron-labeled $CD34^+$ cells in patients with ischemic stroke.

Clinical Trial Registration—URL: www.controlled-trials.com. Unique identifier: ISRCTN63336619.

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Key Words: granulocyte colony-stimulating factor ■ stroke recovery ■ CD34 ■ hematopoietic stem cell

Few interventions aid recovery for patients with acute stroke, and although earlier trials of putative neuroprotectants failed (eg, NXY-059[1]), new studies are ongoing, including focusing on colony-stimulating factors, such as granulocyte-colony stimulating factor (G-CSF) and erythropoietin, where promising data exist for preclinical experimental models of stroke.[2] G-CSF is a glycoprotein hormone encoded by a single gene located on chromosome 17 q11-22[3]; it plays a key role in the regulation of granulopoiesis and is responsible for the terminal maturation of neutrophils. Recombinant G-CSF is used for treatment of neutropenia and for production of $CD34^+$ hematopoietic stem cells (HSCs) for bone marrow transplantation. When considering G-CSF as a potential treatment for stroke, the mechanisms of action appear to be multimodal. Experimentally, neuroprotection occurs by reducing apoptosis in the ischemic penumbra and attenuating the inflammatory cascade.[4] It also appears to be neuroreparative through potentiating angiogenesis[5] and neurogenesis,[4] in part by activating brain endothelial cells[6] and mobilizing HSCs to migrate to the ischemic lesion.[7] More contentiously, bone marrow–derived stem cells have been shown to differentiate into neurons and glia,[8] and experimental transplants of HSCs have been observed to improve outcome post stroke.[9] The purpose of the present trial, Stem

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Cell Trial of Recovery Enhancement After Stroke 2, was to further evaluate the safety of G-CSF in stroke with emphasis on its effects on bone marrow–derived HSCs and their fate in the brain.

Methods

Design

We performed a prospective, single-center, double-blind, randomized, placebo-controlled, phase IIb trial of G-CSF in patients with subacute stroke. The trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonisation of Good Clinical Practice; received authorization from the Medicines and Healthcare Products Regulatory Agency (EudraCT 2006-005345-11) and the local research ethics committee; and was a registered clinical trial (ISRCTN 63336619).

Patients

Patients 3 to 30 days after ischemic and hemorrhagic stroke were recruited from the stroke service at Nottingham Universities Hospital National Health Service Trust from July 2007 to January 2010 (for inclusion and exclusion criteria, online-only Supplemental Table 1; http://stroke.ahajournals.org). Written informed consent was obtained from each patient. If the patient was unable to consent (eg, because of confusion or dysphasia), proxy consent was obtained from a relative or carer.

Intervention

Participants were randomly assigned 2:1 to receive subcutaneous human recombinant G-CSF (1×10^6 units/kg, equivalent to 10 μg/kg, Neupogen, Amgen, the maximum dose in Stem Cell Trial of Recovery Enhancement After Stroke 1[10]) or matching subcutaneous saline once per day for 5 days. Treatment was prepared centrally and both the participants and those administering the injections were blinded to treatment and its assignment. Randomization involved computerized minimization (online-only Supplemental Table 1), and randomized treatment was administered in addition to best medical care.

Clinical Outcomes

The primary outcome measure was safety assessed as the number of participants having a serious adverse event (SAE) by day 90. Clinical secondary outcomes included tolerability, feasibility, impairment (National Institutes of Health Stroke Scale), grip strength, dependency (modified Rankin Score), disability (Nottingham Extended Activities of Daily Living and Barthel Index), cognition (Mini-Mental State Examination), and mood (Zung depression score); measurements were made at days 10 and 90. All of the assessments were blinded to treatment assignment. The Data Monitoring Committee assessed unblinded safety data after recruiting 20 and 40 patients into the trial.

Laboratory Measures

Peripheral blood $CD34^+$ cell count was measured at day 5 using flow cytometry (FACScalibur, Becton Dickinson, Oxford, UK) and complied with International Society for Hematotherapy and Graft Engineering guidelines.[11] Full blood counts and putative markers of neuroprotection efficacy (D-dimer, B-natriuretic peptide, matrix metalloproteinase 9, and protein S-100β; Triage Stroke Panel, Biosite Inc, San Diego, CA) were assessed on days 0, 5, and 10.

Volumetric Analysis

MRI of the brain was performed at baseline and day 90 ($\pm$7) in patients with ischemic stroke, except in those who were intolerant, had contraindications to MRI, or refused imaging. The volume in acutely cerebral infarcted tissue was calculated at baseline based on diffusion-weighted imaging hyperintense lesion outlines at day 0 and at day 90, at which time point intermediately T2-weighted scans were used (online-only, Supplemental Table 2). Contralateral ventricular volume on diffusion-weighted images (including the lateral ventricles to the level of the third ventricle) was also assessed as an indirect measure of cerebral atrophy. Two assessors blinded to treatment allocation (T.J.E., M.A.) were trained by an experienced neuroradiologist (D.P.A.) and measured volumes independently.

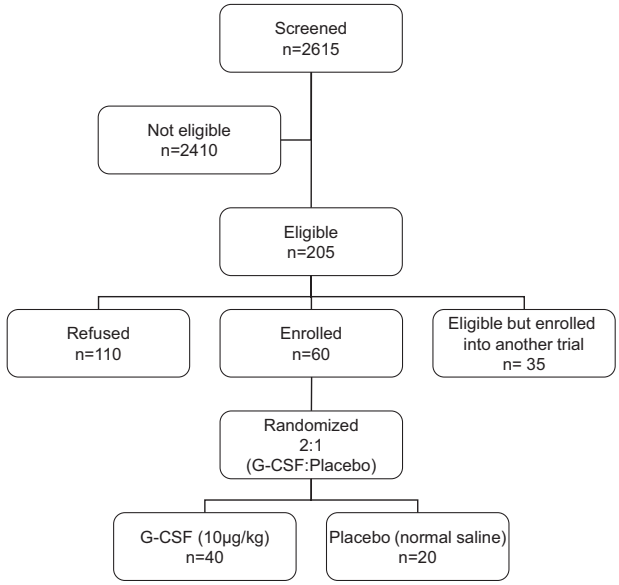

Figure 1. Flow of participants into the trial.

$CD34^+$ Cell Labeling

All of the patients were offered the opportunity to take part in the HSC labeling substudy. For this procedure, 150 mL of blood was collected on day 6 and processed in an HTA licensed clean room. $CD34^+$ cells were immunomagnetically separated from the blood using a CliniMACS Separator (Miltenyi Biotec, Bergisch Gladbach, Germany). The $CD34^+$ antibody used for immunoseparation contained a dextran-coated iron-oxide nanobead,[12] effectively labeling the cells and allowing visualization with gradient echo (T2*-weighted) imaging.[7] After labeling, the $CD34^+$ cells were reinjected intravenously back into the donor patient on the same day and tracked with MR T2* imaging on day 10 (echo time: 16.11 ms; repetition time: shortest; slice thickness: 3 mm). These images are highly sensitive to iron and, therefore, to iron-labeled cells. A neuroradiologist blinded to treatment allocation and cell counts interpreted prelabeling and postlabeling scans.

Statistical Analysis

The primary outcome was the number of participants with $\geq$1 SAE; this was chosen because G-CSF was associated with a nonsignificant increase in SAEs in our earlier phase IIa trial.[10] The sample size was calculated as 60 assuming α5%, power 80%, SAE rate G-CSF 50% versus placebo 20%, ratio G-CSF:placebo 2:1, and losses 5%. Data were analyzed using Fisher exact test, Mann-Whitney *U* test, repeated-measures ANOVA, or ANCOVA as appropriate. Data are presented as number (percentage), mean ($\pm$SD), or median (interquartile range). Stroke lesion volume data were positively skewed; it was, therefore, transformed using log(10) to normalize the distribution and antilogged to present the geometric mean. All of the analyses were performed using PASW statistics data editor (version 18.0) and MediStat; analysis was by intention to treat (according to randomization), and statistical significance was taken at $P<0.05$.

Results

Subjects

Of 205 screened participants who were eligible, 60 were included, and the groups (40 G-CSF and 20 placebo) were well matched at baseline (Figure 1 and Table 1). The first

Table 1. Baseline Patient Characteristics by Treatment Group

Characteristic	All	G-CSF	Placebo
No.	60	40	20
Age, y*	71.5 (11.8)	71.1 (12.9)	72.3 (9.6)
Male, %*	32 (53.3)	22 (55.0)	10 (50.0)
NIHSS	10.4 (6.1)	10.6 (6.5)	9.9 (5.4)
Time to randomization, d*	8.1 (4.8)	8.3 (5.0)	7.8 (4.3)
Stroke type, %*			
Ischemic	51 (85)	34 (85)	17 (85)
Hemorrhagic	9 (15)	6 (15)	3 (15)
Clinical syndrome, %*			
Lacunar	13 (21.7)	8 (20.0)	5 (25.0)
Partial anterior circulation	19 (31.7)	12 (30.0)	7 (35.0)
Total anterior circulation	27 (45.0)	19 (47.5)	8 (40.0)
Posterior circulation	1 (1.7)	1 (2.5)	0 (0.0)
TOAST subgroup, %†			
Small vessel	8 (15.7)	7 (20.6)	1 (5.9)
Large vessel	15 (29.4)	8 (23.5)	7 (41.2)
Cardioembolic	19 (37.3)	14 (41.2)	5 (29.4)
Unknown	8 (15.7)	5 (14.7)	3 (17.6)
Past medical history, %			
Hypertension	31 (51.7)	18 (45.0)	13 (65.0)
Hyperlipidemia	27 (45.0)	17 (42.5)	10 (50.0)
Diabetes mellitus	6 (10.0)	3 (7.5)	3 (15.0)
Atrial fibrillation	9 (15.0)	5 (12.5)	4 (20.0)
Stroke	9 (15.0)	5 (12.5)	4 (20.0)
TIA	6 (10)	4 (10)	2 (10)
Ischemic heart disease	17 (28.3)	10 (25.0)	7 (35.0)
Peripheral vascular disease	0	0	0
Thrombolysis at admission, %	4 (6.7)	3 (7.5)	1 (5.0)
Antiplatelet treatment, %			
Aspirin	49 (81.7)	34 (850)	15 (75.0)
Dipyridamole	40 (66.7)	28 (70.0)	12 (60.0)
Clopidogrel	1 (1.7)	0 (0.0)	1 (5.0)
Statin treatment, %	48 (80)	32 (80)	16 (80)
Infection present, %*	14 (23.3)	10 (25.0)	4 (20.0)
Time stroke to first dose, d	9.7 (4.9)	9.7 (5.1)	9.8 (4.5)

Data are no. (%) or mean (SD).

NIHSS indicates National Institutes of Health Stroke Scale; TIA indicates transient ischemic attack; G-CSF, granulocyte-colony stimulating factor; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

*Data show minimization variables.

†Hemorrhagic stroke was excluded.

dose of trial drug was received, on average, 10 days after stroke onset. The mean number of treatment doses received was 4.5 (90.5% in total) in participants randomized to G-CSF and 5.0 (100%) for placebo; 2 participants randomized to G-CSF refused any treatment (but agreed to continued follow-up) before they received their first dose. Other participants stopped treatment after receiving G-CSF because of lower back pain (n=1, 2 of 5 doses received), acute illness not related to the trial drug (n=1), and administration error (n=2, missed doses). One patient withdrew from the trial; although he or she refused follow-up, he or she allowed information on vital status and modified Rankin Score to be obtained from the general practitioner.

Clinical Outcomes

No significant difference in the number of patients with SAE (the primary outcome) or in the mean number of SAEs and adverse events per patient was observed between treatment groups (Table 2 and online-only Supplemental Table 3). There were 3 deaths in the G-CSF group (pulmonary embolus, ischemic bowel, and community pneumonia), but all occurred after the end of treatment phase; no deaths occurred in the placebo group. There were no differences in vascular events (nonfatal stroke, nonfatal myocardial infarction, pulmonary embolus, and deep vein thrombosis) or infection rates by day 90 (Table 2). Fifteen percent (6 G-CSF and 3 placebo) of the participants were included with a primary intracerebral hemorrhage, and no safety concerns were present in this subgroup (online-only Supplemental Table 3).

No significant difference emerged between treatment and placebo groups with respect to measurements of dependency (modified Rankin Score), disability (Barthel Index and Nottingham Extended Activities of Daily Living), impairment (National Institutes of Health Stroke Scale, Motoricity Index, and grip strength), cognition (Mini-Mental State Examination), or mood (Zung depression score; Table 2). When modified Rankin Score is dichotomized (poor outcome >2), no difference is observed between groups (G-CSF 28 of 40 versus placebo 14 of 20; $P=1.0$).

Volumetric Analysis

Stroke lesion volumes at baseline and day 90 were analyzed in 20 participants (14 G-CSF and 6 placebo; Table 2). Scans were not indicated in 9 recruits (primary hemorrhage) and not performed because of patient refusal (n=17), contraindication (n=4), scan intolerance (n=1), and severe illness or death (n=5). Four scans were not analyzable (eg, because of poor quality or the acute lesion becoming embedded in chronic ischemic change). Stroke lesion territory and size varied considerably, but there was no significant difference in baseline diffusion-weighted imaging lesion volume (Table 2). When adjusted for baseline diffusion-weighted imaging lesion volume, a trend toward reduced lesion volume at day 90 was present in G-CSF–treated patients (ANCOVA, $P=0.06$). With respect to change in ventricular volume over 90 days, no significant difference was seen between groups (Table 2).

Laboratory Measures

In comparison with placebo, G-CSF significantly elevated $CD34^+$ cell count (peak: 31.4 versus 3.3 cells per microliter), white cell count (peak: 40.2×10^9 versus 9.5×10^9 cells per liter), and white cell components, including neutrophils (peak: 34.1×10^9 versus 6.9×10^9 cells per liter), at day 5 (online-only Supplemental Table 4 and Figure 2). By day 10, the counts were returning to normal. There were apparent absolute differences between groups in baseline values in B-natriuretic peptide and D-dimer, but they did not reach statistical significance ($P=0.08$ and 0.07, respectively). Change in hemoglobin, platelet count, red cell count, hemat-

Table 2. Outcome Encompassing Serious Adverse Events (Primary Outcome), Functional Measures, and Volumetric Analysis by Treatment Group

Event	G-CSF (N=40)	Placebo (N=20)	*P*
Mean n of SAEs	0.6 (0.9)	0.7 (1.2)	0.96
Mean n of adverse events	1.2 (1.2)	1.0 (1.2)	0.59
No. of patients with SAE	15 (37.5)	7 (35)	0.54
Nonfatal	12 (30)	7 (35)	0.77
Death	3 (7.5)	0 (0)	0.54
Vascular event	6 (15)	3 (15)	1.0
Fatal	2 (5)	0 (0)	0.55
Nonfatal stroke	1 (2.5)	0 (0)	1.0
Nonfatal MI	1 (2.5)	1 (5)	1.0
Venous thromboembolism†	3 (7.5)	2 (10)	1.0
All infections	7 (17.5)‡	5 (25)	0.51
Lower respiratory tract	6 (15)	2 (10)	0.71
Urinary tract	2 (5)	2 (10)	0.60
Other	0 (0)	1 (0)§	0.33
Timing			
During treatment/washout	5 (12.5)	5 (25)	0.28
During follow-up	10 (25)	2 (10)	0.30
Functional measures	N=40	N=20	
Modified Rankin scale, /6	3.3 (1.3)	3.0 (1.3)	0.45
Barthel index	60.1 (37.1)	66.8 (30.2)	0.49
NIHSS	5.9 (6.0)	6.8 (5.7)	0.60
ΔNIHSS (days 0–90)	4.7 (4.2)	3.3 (2.3)	0.16
Motoricity Index, /100	59.9 (38.9)	55.8 (37.2)	0.70
Grip strength, kg	5.5 (7.5)	4.1 (4.3)	0.45
NEADL, /66	24.8 (19.8)	20.7 (17.4)	0.44
Zung depression score, /100¶	48.3 (21.5)	51.3 (14.1)	0.60
MMSE, /30¶	23.0 (8.7)	23.6 (6.0)	0.80
Stroke lesion volume, cm^3‖	N=14	N=6	
Day 0, DWI	9.79 (9.0)	26.9 (4.2)	0.32
Day 90 (T2-weighted)	5.22 (10.2)	27.6 (4.0)	0.51
Difference (day 90-d 0)*	−1.88 (2.03)	1.03 (1.2)	0.06
Contralateral ventricular vol, cm^3			
Day 0 (DWI)	23.1 (12.6)	22.7 (8.2)	0.96
Day 90 (DWI)	24.3 (13.5)	23.2 (8.9)	0.87
Difference (day 90-0)*	1.21 (1.8)	0.46 (2.0)	0.47

Data are no. (%), mean (SD); comparison by Fisher exact test, Mann-Whitney *U* test, *t* test, or ANCOVA.

NEADL indicates Nottingham Extended Activities of Daily Living; MMSE, Mini-Mental State Examination; DWI, diffusion weighted imaging; NIHSS, National Institutes of Health Stroke Scale; MI, myocardial infarction; SAE, serious adverse event.

*Data were adjusted for baseline volume (ANCOVA).

†Data include deep vein thrombosis and pulmonary embolism.

‡One participant had both urinary tract and lower respiratory tract infections.

§Patient had clostridium difficile diarrhea.

¶Dysphasic participants who were unable to answer questions were excluded.

‖Data show geometric means and SDs.

ocrit, D-dimer, B-natriuretic peptide, matrix metalloproteinase 9, and protein S100-β did not differ significantly between the treatment groups.

$CD34^+$ Cell Labeling

A phantom model was used to demonstrate that iron-oxide microbead-labeled $CD34^+$ cells (harvested from a volunteer) dispersed in 0.5% agar (4×10^3 cells per mL) were visible on 3T T2* magnetic resonance scanning. Agar alone and unlabeled CD34 cells were not detectable (Figure 3).

Eight participants (G-CSF 6 and placebo 2) with an ischemic event underwent $CD34^+$ cell labeling. There was marked variation in each participant's response to G-CSF; after 5 days of treatment, between 50 and 430×10^4 cells were harvested from 150 mL of whole blood. Participants receiving placebo yielded between 2 and 7×10^4 $CD34^+$ cells. Labeled cells were then introduced intravenously on day 6. One G-CSF-treated participant developed a hypodensity compatible with iron deposition within his or her left gangliocapsular infarct that was evident on both day 10 and 90 T2* scans (Figure 3). The hypodensity was not present at baseline, and this participant received the highest number (430×10^4) of labeled $CD34^+$ cells with administration 14 days after stroke onset. Three participants (G-CSF 2 and placebo 1) had evidence of hypodensities compatible with hemorrhagic transformation at days 0, 10, and 90 (T2*-weighted images). The remaining 4 participants receiving labeled cells had no hypodensities in their infarct zones at any time point.

Discussion

The Stem Cell Trial of Recovery Enhancement After Stroke 2 was designed to test the safety of G-CSF and explore mechanisms by which it might work in participants with subacute stroke. There were no significant differences in SAEs (primary outcome) between 2 well-matched groups, supporting that treatment with G-CSF is safe. The inclusion of 9 hemorrhagic strokes also provides initial safety data, although further phase II trials designed specifically in this subgroup are warranted. The majority of subcutaneous injections were received suggesting that the treatment is also feasible to administer, at least when given subacutely. Adverse events experienced that were probably or definitely related to the study drug were typical expected adverse effects of G-CSF (musculoskeletal pain and dizziness, see online-only Supplemental Table 3). Despite significant increases in white cell count, no difference in the frequency of vascular events (arterial and venous) or infection was seen. Two of 3 deaths in the G-CSF group were vascular in nature and did not appear to be secondary to active treatment; the overall vascular event rates were identical in each group (15%).

A secondary aim of the trial was designed to establish whether G-CSF mobilized iron-labeled $CD34^+$ cells migrate to the site of the stroke lesion and, therefore, contribute to neurorepair, as seen experimentally.[13] The complexity of recovering, labeling, and readministering $CD34^+$ cells meant that we only studied 8 participants. Of these, T2* MRI suggested the presence of migrated-labeled cells in 1 participant, as demonstrated by new signal loss within the infarct on days 10 (4 days after reinjection)

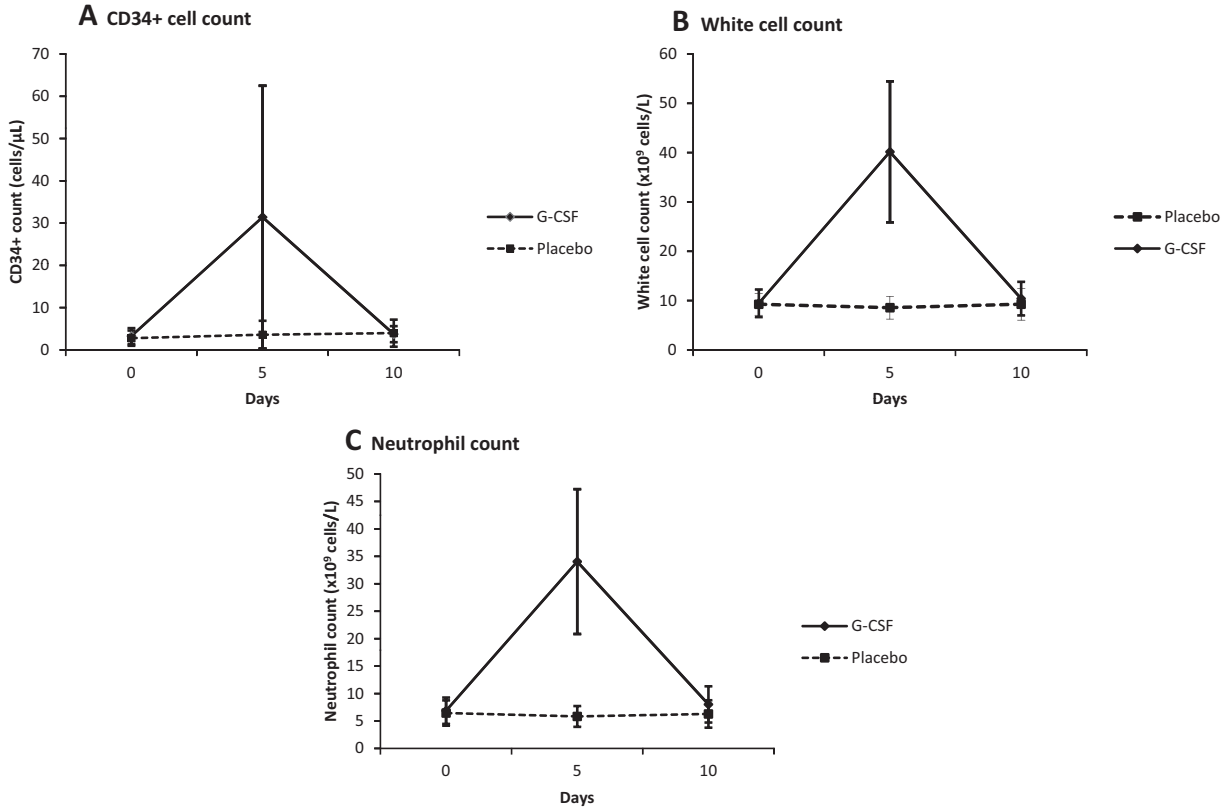

Figure 2. CD34$^+$ cell, white cell, and neutrophil count at days 0, 5, and 10 by treatment: granulocyte-colony stimulating factor vs placebo. Mean (SD). Analysis by repeated-measures ANOVA, $P<0.001$ for **A**, **B**, and **C**.

and 90 and without evidence of baseline hemorrhagic transformation (at day 14 after stroke). This followed intravenous injection of 430×10^4-labeled CD34$^+$ cells (the highest number recovered from any participant). There was no evidence of T2* signal loss in 4 participants, and baseline hemorrhagic transformation occurred in 3. Bone marrow–derived stem cells have been tracked successfully in animal models of stroke using extracellular and intracellular iron labels,[7,14] a method that

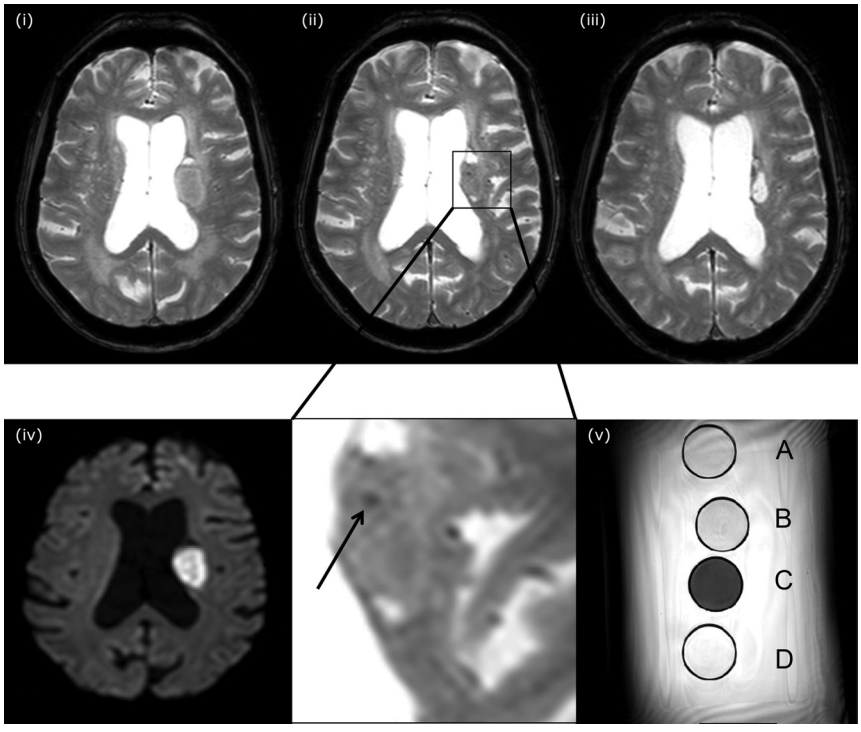

Figure 3. Gradient echo imaging at days (**i**) 0, (**ii**) 10, and (**iii**) 90 in a participant with left gangliocapsular infarction. **iv**, Corresponding diffusion-weighted image at day 0. The participant received 4.3×10^6 intravenous immunomagnetically labelled CD34$^+$ cells on day 6. Arrow indicates new area of negative enhancement. **v**, Phantom model comparing (**A**) labelled CD34$^+$ cells dispersed in 0.5% agar (4×10^3 cells per milliliter), (**B**) agar alone, (**C**) iron-oxide microbeads alone, and (**D**) unlabelled CD34$^-$ cells.

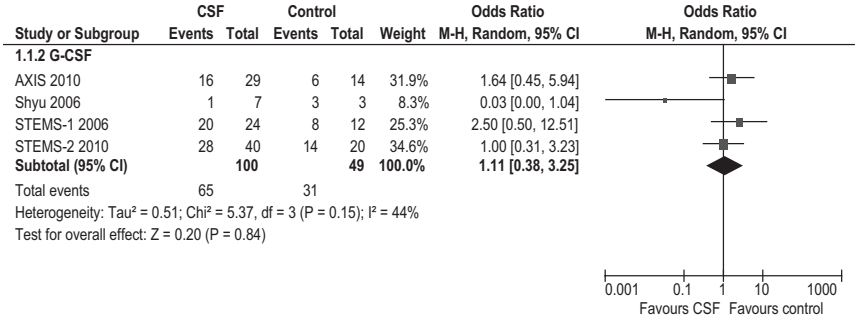

Figure 4. Meta-analysis of the effect of granulocyte-colony stimulating factor on functional outcome events assessed as dependency (modified Rankin score >2 or Barthel index <60).

confers visibility on gradient echo MRI. Nevertheless, qualitative interpretation of T2* images was limited by scan quality and preexisting signal loss (eg, because of hemosiderin deposition from previous bleeds). New petechial or parenchymal bleeding from hemorrhagic transformation further limited specificity of interpretation of new signal loss. Future mechanistic cell-labeling trials should exclude patients with any degree of baseline hemorrhage and consider further the dose of cells administered an alternative route (eg, intra-arterial) and using an intracellular cell label.[15]

Although no significant differences between groups were seen with respect to functional outcome (and the trial was not powered to detect this), a trend toward improvement was observed in change in the National Institutes of Health Stroke Scale over 90 days in G-CSF–treated subjects. This is consistent with other measured clinical parameters (Motoricity Index, grip strength, and Nottingham Extended Activities of Daily Living), whereby the mean value in the G-CSF group was nonsignificantly better when compared with placebo. Further phase III trials are, therefore, required to test efficacy. Similarly, a trend to reduced stroke lesion volume and growth in the G-CSF group was observed. This is unexpected considering that G-CSF administration in the subacute phase is aimed to improve outcome via promotion of neurogenesis[4] and angiogenesis[5] and not through neuroprotection. This should be interpreted with caution because of the small sample size and heterogeneity of case mix, although this was accounted for, in part, by adjusting for baseline stroke volume. In addition, preclinical concern on the effects of G-CSF on cerebral atrophy[16] was not demonstrated here; subgroup analysis revealed no significant difference between groups in change in contralateral ventricular volume, although the numbers are small and 90 days may be an insufficient follow-up period.

Altogether, 4 trials have evaluated G-CSF in ischemic stroke, this and the first Stem Cell Trial of Recovery Enhancement After Stroke, as well as 2 others.[17,18] Of these, the first assessed 10 patients (7 in the G-CSF arm dosed at 15 μg/kg OD for 5 days, 3 in the control arm) randomized within 7 days of ictus and no safety concerns were raised.[17] In the Treatment with AX200 for Acute Ischemic Stroke Trial (n=44), where treatment was instituted with 12 hours of stroke onset at a cumulative dose range of 30 to 180 μg/kg,[18] there was no significant difference between treatment groups with respect to SAEs and thromboembolic complications. Although G-CSF transiently increases total white cell count, therefore potentially leading to vascular complications, there is currently no evidence to suggest that G-CSF causes thromboembolic events or aggravates stroke symptoms.[19] All 4 of the trials were too small individually to assess the effect of G-CSF on functional outcome; when data from the 4 trials are aggregated, no safety concern is obvious with G-CSF (odds ratio: 1.11 [95% CI: 0.38–3.25]; Figure 4). In light of the above evidence and a recent negative trial assessing administration of erythropoietin (another colony-stimulating factor[20]) within 6 hours of ischemic stroke,[21] further phase II/III trials are required evaluating the safety and efficacy of G-CSF.

In summary, this randomized, double-blind, placebo-controlled trial suggests that G-CSF is safe when administered subacutely. It is feasible to label and readminister iron-labeled CD34$^+$ cells in patients with ischemic stroke.

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Disclosures

None.

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Granulocyte-Colony Stimulating Factor for Mobilizing Bone Marrow Stem Cells in Subacute Stroke: The Stem Cell Trial of Recovery Enhancement After Stroke 2 Randomized Controlled Trial

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SUPPLEMENTAL MATERIAL

Supplementary Table 1. Inclusion and exclusion criteria into STEMS-2 and computerised minimisation factors used for randomisation.

Inclusion criteria
Age > 18
Clinical diagnosis of stroke
Limb weakness present at randomisation (SNSS score in arm and/or leg <4)
Brain scan compatible with ischaemic or haemorrhagic stroke
Time from ictus 3-30 days
Exclusion criteria
Pre-morbid dependency (modified Rankin scale, mRS >3),
Coma (SNSS consciousness <4)
Dementia
Malignancy
Sickle cell disease
Pregnancy
Neutropenia
Computerised minimisation factors
Age (<70, ≥70 years)
Sex
Time since stroke (<8, ≥8 days)
Stroke type (ischaemic, haemorrhage)
Stroke severity (SNSS <30, ≥30),
Cortical signs (cortical, sub-cortical)
Presence of infection
Intention to perform MRI

SNSS, Scandinavian Neurological Stroke Score

Supplementary Table 2. MRI sequence parameters

Scans were performed on a 3T Achieva (Philips, Netherlands) using a 8-channel phased array coil or on a 1.5T Signa Excite (GE Medical Systems, US) for baseline scans when participant could not be transferred for 3T imaging. Lesion volume was measured using semi-automated software developed locally (NeuRoi, Dr C Tench, Department of Clinical Neurology, Nottingham, UK [1]).

MRI Scan Sequence	Parameter	3T Achieva	1.5T Signa Excite
Diffusion weighted (baseline, day 0)	Echo Time (ms)	53	92
	Repetition time (ms)	6605	8000
	Slice thickness (mm)	3	3
	Field of View (mm)	240×240	256x256
	Scan Matrix	96×96	128x128
	Voxel Size (mm)	2.5x2.5x2.5	2x2x3
	Diffusion weighting (s/mm^2)	1000	1000
T2 weighted	Echo Time (ms)	32	74
	Repetition time (ms)	2000	4120
	Slice thickness (mm)	3	3
	Pixel size (mm)	0.38x0.38	0.47x0.47

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Supplementary table 3. (i) Serious Adverse Events (SAE) and (ii) Adverse Events (AE) by treatment group and their relationship to study drug (blinded adjudication).

(i)

Group	Subject N^o	Serious Adverse Event	Days post Rx	Relationship to drug
Placebo	9	UTI	15	Unlikely
	10	Aspiration pneumonia	2	Possible
	17	Functional weakness	8	Possible
		Clostridium Difficile diarrhoea	16	Not related
	30	UTI	0	Not related
		Urinary retention	4	Unlikely
		Lower GI Bleed	17	Unlikely
		DVT	70	Unlikely
	31	Aspiration Pneumonia	8	Possible
	36	Neurological deterioration	6	Possible
		PE	9	Possible
		DVT	20	Possible
	37 †	STEMI	15	Unrelated
		Seizure	66	Possible
G-CSF	5	Aspiration pneumonia	26	Not related
	6	ARF 2^o to outflow obstruction	15	Possible
		UTI	16	Possible
		Post stroke psychosis	7	Possible
		NSTEMI	11	Possible
	7	Muscular Chest pain	58	Not related
		Ischaemic stroke	62	Not related
	8	Aspiration pneumonia	0	Not related
		DVT	17	Unlikely
	13	Cardiac arrest 2^0 to massive PE	15	Unlikely
	14	Admission for Inguinal Hernia	11	Not related
	15 †	Aspiration pneumonia	2	Unlikely
	20	Aspiration pneumonia	1	Possible
		UTI	44	Not related
	23	Anaemia under investigation	13	Possible
	25 †	DVT	22	Not related
		PE	26	Not related
	26	Community pneumonia causing death	86	Not related
	35	Ischaemic Bowel causing death	24	Unlikely
	41	UTI	31	Unlikely
		Aspiration Pneumonia	49	Unlikely
	45	Admitted for investigation of PE	26	Not related
	46	Hyperaesthesia	6	Unlikely

† haemorrhagic stroke; UTI, urinary tract infection; DVT, deep vein thrombosis; PE pulmonary embolus; STEMI, ST elevation myocardia infarction; ARF, acute renal failure; NSTEMI, non-ST elevation myocardia infarction.

(ii)

Group	Subject N^o	Adverse Event (not serious)	Days post Rx	Relationship to drug
Placebo	4	Depression	18	Unlikley
	10	Neuropathic pain	7	Unlikely
		Depression	20	Unlikely
	11	Exacerbation COPD	34	Not related
		Oral candidiasis	45	Not related
	17	Angina	4	Possible
		Functional weakness	75	Not related
		Functional weakness	80	Not related
	19	UTI	17	Unlikely
	22	Abdominal bruising	0	Definite
	24	Hypokalaemia 2^0 to thiazide	5	Unrelated
		UTI	7	Possible
		Neuropathic pain	22	Not related
		Spasticity	56	Not related
	30	LRTI	10	Possible
		LRTI	67	Not related
	31	Hypothyroidism	5	Not related
	40 †	Intertrigo	5	Possible
		Back pain	67	Not related
	52	Bad dreams	4	Unlikely
G-CSF	2	Minor epistaxis 2^0 anticoagulation	8	Unlikely
		UTI	18	Unlikely
		Hypokalaemia 2^0 to thiazide	24	Not related
		Statin induced rise in liver enzymes	25	Not related
	3	Back pain post trial drug injections	0	Probably
	8	Dizziness post injection	0	Probable
		Oral candidiasis	5	Possible
		Abdo pain 2^0 constipation	16	Unlikely
	12	UTI	53	Not related
		Vasovagal Syncope	41	Not related
	13	Abdominal bruising	0	Definite
	16	Headache after 3rd dose only	2	Possible
	18 †	Viral diarrhoea	21	Not related
	20	UTI	52	Not related
	21 †	UTI	7	Possible
		UTI	46	Not related
		UTI and haematuria	67	Not related
	21	Deranged LFTs	67	Not related
	25 †	Deranged LFTs	9	Possible
		Oral Candidiasis	15	Possible
	29 †	Muscular shoulder pain	55	Not related
	32	UTI	3	Possible
	38	UTI	6	Possible
		Abdominal bruising	1	Definite
	39	Transient hypotension	5	Possible

41	UTI	89	Not related
43	Back pain	0	Probable
	Frozen shoulder	66	Not related
	Swollen hand 2^0 to osteoarthritis	40	Not related
45	Thrombocytopenia	7	Probable
	Leucopenia	18	Possible
46	UTI	6	Possible
	UTI	20	Unlikely
49	UTI	10	Possible
	Transient left arm pain	4	Possible
50	Epistaxis	8	Possible
	UTI with renal impairment	12	Possible
	UTI	33	Not related
51	Simple fall	9	Unlikely
54	Photopsia	4	Possible
	Muscular chest pain	6	Unlikely
	Allergic rash to morphine	7	Unrelated
55	Dizziness	3	Possible
57	Neuropathic pain	19	Unlikely
	UTI	26	Unlikely
	Deranged LFTs	33	Unlikley
58	Gout	90	Not related

† haemorrhagic stroke; UTI, urinary tract infection; COPD, chronic obstructive pulmonary disease; LFT, liver function tests.

Supplementary Table 4. Laboratory measures by treatment group - granulocyte-colony stimulating factor versus placebo. Mean (standard deviation); comparison by repeated measures analysis of variance

	G-CSF			Placebo			ANOVA
Day of treatment	**0**	**5**	**10**	**0**	**5**	**10**	**p**
Haemoglobin (g/dL)	13.6 (1.9)	13.4 (1.8)	13.4 (1.9)	14.1 (1.2)	13.8 (1.4)	13.6 (1.3)	0.49
White cells ($x10^9$/L)	9.45 (2.8)	40.2 (14.3)	10.41 (3.4)	9.26 (2.2)	8.56 (2.3)	9.26 (3.2)	<0.001
Neutrophils ($x10^9$/L)	6.88 (2.4)	34.1 (13.2)	8.03 (3.3)	6.46 (2.3)	5.84 (1.9)	6.29 (2.5)	<0.001
Lymphoctyes ($x10^9$/L)	1.56 (0.5)	2.74 (0.9)	1.58 (0.6)	1.81 (0.5)	1.80 (0.4)	1.85 (0.5)	0.22
Monocytes ($x10^9$/L)	0.79 (0.3)	2.39 (1.1)	0.78 (0.9)	0.77 (0.2)	0.71 (0.3)	0.76 (0.3)	<0.001
Eosinophils ($x10^9$/L)	0.15 (0.2)	0.36 (0.4)	0.15 (0.1)	0.23 (0.2)	0.22 (0.2)	0.20 (0.1)	0.99
Basophils ($x10^9$/L)	0.02 (0.04)	0.07 (0.16)	0.01 (0.03)	0.02 (0.04)	0.03 (0.05)	0.03 (0.05)	0.61
CD34+ (/µL)	3.27 (1.9)	31.40 (31.1)	3.74 (1.9)	2.78 (1.8)	3.59 (3.3)	3.97 (3.2)	<0.001
Platelets ($x10^9$/L)	240 (93)	279 (89)	238 (93)	260 (63)	272 (42)	302 (61)	0.17
Red cell count ($x10^{12}$/L)	4.53 (0.6)	4.43 (0.6)	4.51 (0.6)	4.55 (0.5)	4.46 (0.5)	4.22 (1.0)	0.63
Haematocrit (L/L)	0.41 (0.05)	0.41 (0.05)	0.40 (0.05)	0.42 (0.04)	0.41 (0.04)	0.41 (0.04)	0.51
D-dimer (ng/ml) †	2952 (2009)	2560 (1819)	2440 (1811)	1844 (1682)	1635 (1768)	1586 (1789)	0.10
BNP (pg/ml) †	281.9 (501.6)	219.9 (315.9)	206.8 (303.4)	89.9 (101.7)	97.1 (102.5)	118.1 (134.6)	0.15
MMP-9 (ng/ml) †	284.2 (325.3)	259.1 (265.3)	231.6 (174.4)	374.8 (407.4)	202.5 (204.1)	163.9 (102.8)	0.87
S100-β (pg/ml) †	107.4 (30.7)	105.5 (17.0)	113.0 (34.0)	103.1 (11.1)	109.1 (31.0)	104.9 (18.8)	0.60

BNP: ß-natriuretic peptide; MMP-9: matrix metalloproteinase-9. †, n=41 (G-CSF 26, placebo 15)