Stroke
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Stroke Association
A Division of American Heart Association

Granulocyte-Colony-Stimulating Factor Mobilizes Bone Marrow Stem Cells in Patients With Subacute Ischemic Stroke: The Stem Cell Trial of Recovery EnhanceMent After Stroke (STEMS) Pilot Randomized, Controlled Trial (ISRCTN 16784092)

Nikola Sprigg, Philip M. Bath, Lian Zhao, Mark R. Willmot, Laura J. Gray, Marion F. Walker, Martin S. Dennis and Nigel Russell

Stroke 2006;37;2979-2983; originally published online Nov 2, 2006;
DOI: 10.1161/01.STR.0000248763.49831.c3
Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0039-2499. Online ISSN: 1524-4628

Granulocyte-Colony–Stimulating Factor Mobilizes Bone Marrow Stem Cells in Patients With Subacute Ischemic Stroke

The *S*tem Cell *T*rial of Recovery *E*nhance*M*ent After *S*troke (STEMS) Pilot Randomized, Controlled Trial (ISRCTN 16784092)

Nikola Sprigg, MRCP; Philip M. Bath, MD; Lian Zhao, PhD; Mark R. Willmot, MRCP; Laura J. Gray, MSc; Marion F. Walker, PhD; Martin S. Dennis, MD; Nigel Russell, MD

Background and Purpose—Loss of motor function is common after stroke and leads to significant chronic disability. Stem cells are capable of self-renewal and of differentiating into multiple cell types, including neurones, glia, and vascular cells. We assessed the safety of granulocyte-colony–stimulating factor (G-CSF) after stroke and its effect on circulating CD34+ stem cells.

Methods—We performed a 2-center, dose-escalation, double-blind, randomized, placebo-controlled pilot trial (ISRCTN 16784092) of G-CSF (6 blocks of 1 to 10 μg/kg SC, 1 or 5 daily doses) in 36 patients with recent ischemic stroke. Circulating CD34+ stem cells were measured by flow cytometry; blood counts and measures of safety and functional outcome were also monitored. All measures were made blinded to treatment.

Results—Thirty-six patients, whose mean±SD age was 76±8 years and of whom 50% were male, were recruited. G-CSF (5 days of 10 μg/kg) increased CD34+ count in a dose-dependent manner, from 2.5 to 37.7 at day 5 (area under curve, $P=0.005$). A dose-dependent rise in white cell count ($P<0.001$) was also seen. There was no difference between treatment groups in the number of patients with serious adverse events: G-CSF, 7/24 (29%) versus placebo 3/12 (25%), or in their dependence (modified Rankin Scale, median 4, interquartile range, 3 to 5) at 90 days.

Conclusions—G-CSF is effective at mobilizing bone marrow CD34+ stem cells in patients with recent ischemic stroke. Administration is feasible and appears to be safe and well tolerated. The fate of mobilized cells and their effect on functional outcome remain to be determined. (***Stroke*. 2006;37:2979-2983.)**

Key Words: ischemic stroke ■ stem cells ■ colony-stimulating factors ■ stroke recovery

Recovery after stroke is improved with thrombolysis or aspirin use and management in a stroke unit. The brain's capacity to undergo dynamic and plastic change means that it may be possible to enhance recovery by pharmacological means (pharmacological rehabilitation, eg, with amphetamine) or the use of stem cells (neuroreparative therapy).[1]

Stem cells have the capacity to self-renew and differentiate into different cell types, including neurons, astrocytes, and endothelial cells. Stem and progenitor cells are present in fetal cells, immortalized cell lines, umbilical cord blood, bone marrow, and specific organs, including the brain. Animal studies suggest that stem cells (including those from bone marrow) can survive, integrate, and function as neurons in experimental models of stroke.[2–4] Nevertheless, between- and within-species transplantation of cells is fraught with problems (including infection, rejection, risk of malignancy, and ethical considerations),[5] and stimulation of endogenous stem cell pools might be preferable.[6]

Granulocyte-colony–stimulating factor (G-CSF) is a growth factor that acts on hematopoietic stem (CD34+) cells to regulate neutrophil progenitor proliferation and differentiation. G-CSF is routinely used to mobilize stem cells for transplantation in patients with hematological malignancy. Data support its use in healthy donors and older people with hematological malignancy, whereas experimentally, G-CSF has been assessed in patients with multiple sclerosis.[7–9] G-CSF does not appear to induce platelet aggregation or microembolism.[10,11] In experimental models of stroke (in mice and rats), G-CSF exhibited neuroprotective and regenerative activity, including recruiting neural progenitor cells, reducing cerebral edema, improving survival, and enhancing sensorimotor and functional recovery.[12–18] This multimodal

Received April 6, 2006; final revision received June 9, 2006; accepted July 5, 2006.

From the Institute of Neuroscience (N.S., P.M.B., L.Z., M.R.W., L.J.G., M.F.W.) and Department of Haematology (N.R.), University of Nottingham, Nottingham, England, and the Department of Clinical Neuroscience (M.S.D.) University of Edinburgh, Edinburgh, Scotland.

Correspondence to Prof Philip Bath, Stroke Trials Unit, University of Nottingham, D Floor, South Block, Queen's Medical Centre, Nottingham NG7 2UH UK. E-mail philip.bath@nottingham.ac.uk

***Stroke* is available at http://www.strokeaha.org** **DOI: 10.1161/01.STR.0000248763.49831.c3**

behavior of G-CSF means that it is a candidate treatment for enhancing recovery after stroke, although no clinical studies addressing this treatment paradigm have been reported to date.

Subjects and Methods

Design

We performed a prospective, 2-center, double-blind, dose-escalation, randomized, placebo-controlled, Phase IIa trial of G-CSF in patients with subacute ischemic stroke. A dose-escalation design was used, because the effects of G-CSF on stem cell mobilization are poorly documented in elderly patients with significant comorbid disease, and it was conceivable that the marrow would be either under- or supersensitive to therapy. Such designs are frequent in stroke.[19]

The study was approved by the Nottingham Local Research Committee (June 5, 2003), had a Medicines and Healthcare Products Regulatory Agency Clinical Trial Authorization (March 10, 2003), was registered for a trial number (ISRCTN 16784092), and was performed according to the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice.

Subjects

Adult patients with recent (7 to 30 days postictus) ischemic stroke and motor weakness (arm and/or leg, MRC grade <5/5) were identified and enrolled from Nottingham City Hospital (NCH) and Queen's Medical Centre (QMC) by M.R.W. and N.S. Treatment was not given during the first 7 days after ictus because we did not wish to exacerbate the normal leukocytosis seen during acute stroke with G-CSF, which also increases leukocyte count. The principal exclusion criteria included premorbid dependency (modified Rankin scale [mRS] >3), primary intracerebral hemorrhage, dementia, coma, malignancy, sickle cell disease, and pregnancy. Full written, informed consent was obtained from patients before randomization, or assent was received from a relative/caregiver if the patient was incompetent owing to being obtunded, confused, or dysphasic.

Intervention

Patients were randomized to receive either subcutaneous human recombinant G-CSF (filgastrim, Amgen; purchased from the hospitals' pharmacies) or placebo (saline) in a dose-escalation design. Dose blocks comprised 6 patients (4 active and 2 placebo in random order) and ranged from 1 dose of 1 μg/kg (10^5 U/kg) to 5 daily doses of 10 μg/kg (10^6 U/kg; Figure 1); the latter dose is standard after bone marrow transplantation. When designing the protocol, we had allowed higher doses (30 μg/kg given either once or daily for 5 days) to be given, depending on the achieved CD34+ count; review by the Data Monitoring Committee advised that testing of these doses would be unnecessary. Computerized randomization was performed with minimization on age, sex, baseline severity (Scandinavian Neurological Stroke Scale [SNSS]), and baseline CD34+ and leukocyte counts.

Outcomes

The primary outcome was peak circulating blood CD34+ count, with the aim of achieving a CD34+ count of >10 cells/μL; measurements were made on days 1, 3, 5, 7, and 10. Full blood counts and assessment of tolerability were performed in parallel with CD34+ counts. Safety was assessed as mortality, impairment (SNSS), disability (Barthel Index [BI]), dependence (mRS), and serious adverse events at days 10 and 90. Specific clinical information on musculoskeletal pain, splenomegaly, thrombocytopenia, proteinuria, infection, and venous thromboembolism was also recorded. Laboratory and clinical measurements were performed blinded to each other and to treatment assignment.

Laboratory Measures

CD34+ count was performed by flow cytometry (FACScalibur, Becton Dickinson, Oxford, UK). Blood cells were labeled with fluorescein isothiocyanate- and phycoerythrin-tagged antibodies against CD34+ and added to tubes containing latex beads (TruCount, Becton Dickinson).[20] Full blood counts were analyzed by the NCH and QMC Hematology Department staff using standard hematology analysers. C-reactive protein (CRP) was measured with a commercial high-sensitivity, wide-range ELISA kit (Kalon Biological Ltd, Aldershot, UK).

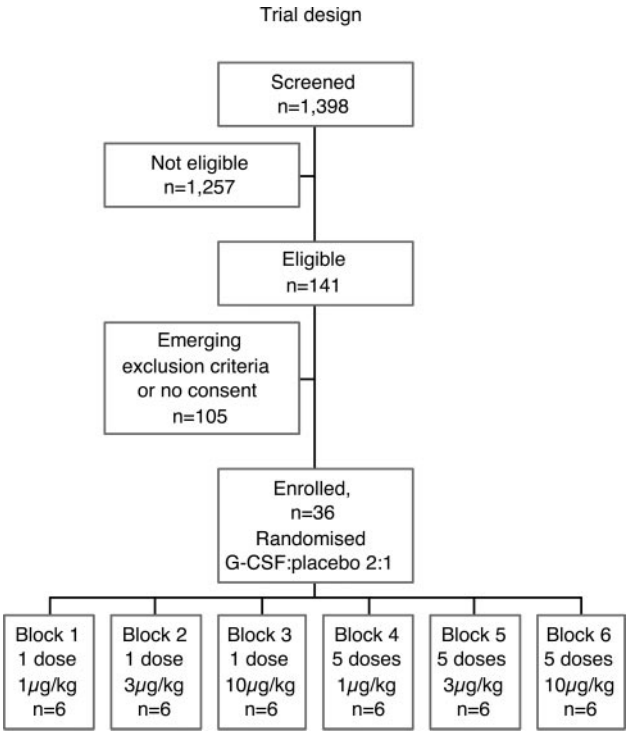

Figure 1. Trial design.

Statistical Methods

Data on CD34+, full blood count, and safety (serious adverse events, death, impairment) were assessed after each dosing block of 6 patients by the Data Monitoring Committee (comprising M.S.D., N.R., and P.M.B.). A decision was then made on whether to proceed to the next dosing block. Data on other measures were pooled by treatment group. The primary analysis was peak CD34+; peak blood count parameters and comparison of areas under the curve (AUCs) across 10 days were also performed for CD34+, blood counts, and temperature. Data are presented as mean (with SD), median (with interquartile range [IQR]), or number (and percentage) and were analyzed with Fisher's exact test, Mann-Whitney *U* test, ANCOVA, Kruskal-Wallis test, or ordinal logistic regression, as appropriate. All analyses were performed with SPSS (Apple Mac, version 11; SPSS Inc). Analysis was by intention to treat, and significance was taken at $P<0.05$.

Results

Subjects

Thirty-six patients were enrolled between August 2003 and November 2005 (Figure 1). The baseline characteristics were matched for age, sex (Table 1), and baseline CD34+ (Table 2). Patients randomized to G-CSF had a trend to milder stroke (SNSS) and were more likely to have a history of diabetes ($P=0.07$). Patients were enrolled between 7 and 28 days after stroke, G-CSF median of 14 days (IQR, 10–18), and control median of 12 days (IQR, 10 to 17). No patients were lost to

TABLE 1. Baseline Characteristics of Patients.

Characteristic	Placebo (n=12)	G-CSF (n=24)
Age (years)†*	74 (8)	76 (9)
Male*	6 (50)	12 (50)
SNSS*	21 [14–33]	26 [21–41]
Time to treatment (days)†	13 (4)	14 (5)
Clinical stroke syndrome[29]		
Lacunar	4 (33)	5 (21)
Partial anterior circulation	3 (25)	11 (46)
Total anterior circulation	5 (42)	8 (33)
TOAST aetiological group[30]		
Small vessel disease	5 (42)	7 (29)
Large artery disease	1 (8)	2 (8)
Cardio-embolic stroke	2 (17)	11 (46)
Previous hypertension	6 (50)	18 (75)
Hyperlipidaemia	2 (17)	8 (33)
History of diabetes	0 (0)	7 (29)
Atrial fibrillation	2 (17)	10 (42)
Previous stroke	3 (25)	6 (25)
Previous ischaemic heart disease	2 (17)	8 (33)
Peripheral arterial disease	1 (8)	1 (4)
Anti-platelet treatment‡	9 (75)	18 (75)
Statin treatment‡	11 (92)	15 (63)

No. (%), mean† (SD) or median [IQR].

SNSS (low numbers signify severe stroke); *Minimization variables; ‡concurrent treatment at time of randomization.

follow-up, and all patients received all prescribed G-CSF or placebo injections.

CD34+, Blood Counts, and CRP

G-CSF increased CD34+ in a dose-dependent manner, with the peak level occurring at day 5; the highest dose of G-CSF (5 days of 10 μg/kg) achieved a 15-fold increase in CD34+ compared with placebo (AUC, $P=0.005$; Table 2 and Figure 2). The total leukocyte count also increased in a dose-dependent manner ($P<0.001$); most of this response was driven by increases in neutrophil count (data not shown). There was no significant relation with platelet count, although laboratory thrombocytopenia (platelet count <150 without clinical features) was noted in 2 (8%) G-CSF patients versus 0 in the control group; no cases of clinical thrombocytopenia or hemorrhage were observed. Erythrocyte counts did not change with G-CSF. Additionally, G-CSF did not alter CRP levels at 5 days (with adjustment for levels at baseline): G-CSF mean 42.9 μg/L (SD, 25.1) versus placebo 41.9 μg/L (SD, 30.1) (ANCOVA, $P=0.747$).

Safety

Five patients (3 G-CSF and 2 placebo) died during the course of the study (Table 3). One patient died on day 10, and the remaining 4 died after completion of the treatment phase (Table 4). During the study, 1 recurrent stroke occurred (1 G-CSF 1) after completion of the treatment phase. Rates of infection and venous thromboembolism did not differ between patients randomized to G-CSF and placebo (Table 3). Adverse events were not related to dose (Table 3). Temperature did not differ between the treatment groups. Splenomegaly was not detected in any patient.

Impairment (SNSS), disability (BI), and dependence (mRS) were also assessed as measures of safety and did not differ between the treatment groups at either day 10 or day 90 (Table 4). There was no difference in functional outcome with different doses of G-CSF (Kruskal-Wallis $P=0.837$; data not shown). The apparent difference in median BI score at day 90 (G-CSF 43 versus control 63) was nonsignificant in both univariate (Table 4) and baseline covariate-adjusted (age and SNSS; ordinal logistic regression, $P=0.18$; data not shown) analyses.

Discussion

This is the first Phase IIa dose-escalation clinical trial to assess G-CSF in patients with stroke. We found that G-CSF was effective in mobilizing CD34+ stem cells into the peripheral bloodstream in subacute ischemic stroke. In particular, 5 daily doses of 10 μg/kg G-CSF, as is used in hematological malignancies, increased CD34+ counts by 15-fold and achieved a mean peak of 37.7 cells/μL. Preclinical data suggest that G-CSF has both neuroprotective and neuroreparative properties.[12–18,21] Mobilized bone marrow stem cells could home into the damaged brain after stroke and promote cytogenesis, either by direct clonal expansion and transdifferentiation into neurones, glia, and vascular cells or through stimulation of local brain progenitor cells and enrichment of the local milieu.[17] Our experimental protocol was solely based around a neuroreparative paradigm, and we did

TABLE 2. Peripheral Blood CD34+ Count (cells/μl) by Treatment Dose and No. of Treatments

Dose	Doses	Day 0*	Day 1	Day 3	Day 5	Day 7	Day 10
0	1 or 5	3.0 (1.2)	3.9 (1.2)	3.6 (1.1)	3.3 (1.0)	3.8 (2.3)	3.5 (1.2)
1 μg/kg	1	3.4 (2.4)	6.1 (8.4)	5.5 (4.1)	3.6 (1.3)	1.8 (0.8)	2.2 (1.1)
3 μg/kg	1	3.1 (0.8)	4.0 (0.8)	6.3 (2.5)	6.1 (2.5)	4.3 (1.8)	3.0 (1.7)
10 μg/kg	1	2.8 (1.7)	3.0 (1.5)	5.0 (2.5)	3.7 (2.1)	3.0 (1.7)	4.3 (2.7)
1 μg/kg	5	3.9 (1.1)	3.4 (0.7)	4.1 (1.9)	6.3 (0.5)	8.2 (2.1)	4.8 (1.4)
3 μg/kg	5	2.2 (1.1)	3.1 (1.1)	11.2 (1.5)	19.1 (7.9)	20.0 (6.0)	8.7 (9.3)
10 μg/kg	5	2.5 (1.3)	10.1 (7.3)	22.4 (17.2)	37.7 (36.7)	19.8 (11.1)	6.5 (1.4)
All	–	3.0 (1.4)	4.6 (4.0)	7.0 (8.1)	9.8 (15.9)	7.4 (7.8)	4.3 (3.2)

The dose and No. of treatments reflect the order used in the trial. Mean (SD).

Day 0 is baseline. *Minimization variable.

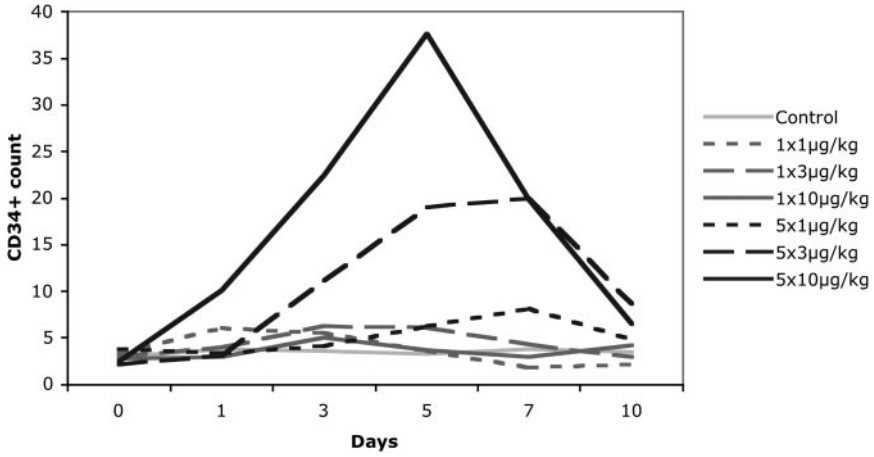

Figure 2. CD34+ count (cells/μL) per treatment block.

not test the neuroprotective hypothesis, because we deliberately started treatment 7 or more days after ictus to avoid exacerbating the normal leukocytosis seen after ischemic stroke.

Treatment with G-CSF was associated with a dose-dependent increase in the mean leukocyte count from 8.5 to 36.4 at the highest dose ($P<0.001$). Moderate laboratory thrombocytopenia, another recognized effect of G-CSF, occurred in 2 patients but did not lead to any clinical sequale. No difference in serious adverse events was seen between the treatment groups, and G-CSF treatment was not associated with infection or thromboembolic events. Other recognized adverse effects of G-CSF, such as musculoskeletal pain and splenomegaly, were not seen. Overall, the drug was well tolerated. All doses of treatment were administered, supporting the tolerability and feasibility of the treatment. No differences in functional outcome were noted between treatment groups or G-CSF dose, although the trial had minimal power to detect these.

A very small, nonplacebo-controlled, randomized trial involving just 10 patients (7 G-CSF and 3 control) with acute stroke has been published recently.[22] The investigators found that G-CSF treatment was well tolerated, was not associated with serious adverse events, and might improve neurological function. G-CSF has also been administered to patients with other vascular disease. The results of the 4 published randomized trials in patients with acute myocardial infarction vary, in part because of their small size and different dosing regimes.[23–26] However, G-CSF treatment has been associated with potential benefits (apparent infarct healing[24]) and hazard (increased restenosis[23]), although the results are inconsistent across studies, and further larger trials are needed.[27] Nevertheless, all of the studies to date, including published unblinded data from another ongoing trial,[28] found that G-CSF was well tolerated and increased CD34+ and leukocyte counts.

In summary, the results of this study suggest that G-CSF, at standard hematology doses, mobilizes peripheral blood stem cells in older patients with acute ischemic stroke. Treatment appears to be well tolerated and safe. Further evaluations are now required to assess whether mobilized stem cells migrate into the brain and whether treatment improves functional outcome.

TABLE 3. No. of Patients Experiencing SAEs or Selected Adverse Events by Treatment Group

Event	Placebo (n=12)	G-CSF (n=24)	2p	G-CSF events by dose regimen, Block (No. of patients with adverse event)
Death	2 (17)	3 (13)	1.0	Block 1(1), Block 3 (1), Block 4 (1)
Complication of incident stroke	2 (17)	0		
Recurrent stroke	0	1 (4)		
Pneumonia	0	2 (8)		
Infection	3 (25)	7 (29)	1.0	Block 1 (3), Block 2 (1), Block 3 (2), Block 5 (1)
Urinary tract infection	2 (17)	5 (21)		
Pneumonia	2 (17)	3 (13)		
Other	0	1 (4)		
Venous thromboembolism	1 (8)	2 (8)	1.0	Block 5 (1), Block 6 (1)
Hospital re-admission	0	1(4)	1.0	Block 2 (1)
Total patients experiencing a SAE	3 (25)	7(29)	1.0	

Note: Some patients had more than one event.
Frequency (%); comparison by Fisher Exact test.
SAE indicates serious adverse events.
Block 1: 1 dose 1 μg/kg, Block 2: 1 dose 3 μg/kg, Block 3: 1 dose 10 μg/kg,
Block 4: 5 doses 1 μg/kg, Block 5: 5 doses 3 μg/kg, Block 6: 5 doses 10 μg/kg.

TABLE 4. Death, Impairment (SNSS), Disability (BI) and Dependency (mRS) by Treatment Group

	Placebo (n=12)	G-CSF (n=24)	2P
Day 10			
Death (%)	0 (0)	1 (4)	1.0
SNSS	29 (26–38)	34 (25–47)	0.43
BI	35 (1–54)	35 (10–59)	0.66
mRS	4 (3–5)	4 (3–5)	0.97
Day 90			
Death (%)	2 (17)	3 (13)	1.0
SNSS	32 (25–44)	32 (19–50)	0.75
BI	63 (0–85)	43 (6–84)	0.89
mRS	4 (2–5)	4 (3–5)	0.95

Frequency (%) or median (IQR); analysis by Fisher exact test or Mann-Whitney *U* test.

BI (low numbers signify disability); mRS (high numbers signify dependency); SNSS (low numbers signify severe impairment).

Acknowledgments

We thank the Hematology Laboratory (NCH) and Dr Sue Fox (QMC) for help with CD34+ measurements and Dr Ulvi Bayraktutan for CRP assays

Sources of Funding

The trial was supported by the Stroke Association (01/03). Support was also received from the BUPA Foundation (N.S., L.J.G.) and the Hypertension Trust (M.R.W.). P.M.B. is a Stroke Association Professor of Stroke Medicine.

Disclosures

None.

References

1. Sprigg N, Bath PMW. Pharmacological enhancement of recovery from stroke. *Curr Med Lit: Stroke Rev*. 2005;8:33–39.
2. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science*. 2000;290:1779–1781.
3. Brazelton TR, Rossi FMV, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*. 2000;290: 1775–1779.
4. Chen J, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001;32:1005–1011.
5. Weissman IL. Stem cells—scientific, medical and political issues. *N Engl J Med*. 2002;346:1576–1579.
6. Haas S, Weidner N, Winkler J. Adult stem cell therapy in stroke. *Curr Opin Neurol*. 2005;18:59–64.
7. Morris CL, Siegel E, Barlogie B, Cottler-Fox M, Lin P, Fassas A, Zangari M, Anaissie E, Tricot G. Mobilization of CD34+ cells in elderly patients (>70years) with multiple myeloma: influence of age, prior therapy, platelet count and mobilization regime. *Br J Haematol*. 2003;120:413–423.
8. Cavallaro AM, Lilleby K, Majolino I, Storb R, Appelbaum FR, Rowley SD, Bensinger WI. Three to six year follow-up of normal donors who received recombinant human granulocyte colony-stimulating factor. *Bone Marrow Transplant*. 2000;25:85–89.
9. Fassas A, Passweg JR, Anagnostopoulos A, Kazis A, Kozak T, Havrdova E, for the Autoimmune Disease Working Party of the EBMT (European Group for Blood and Marrow Transplantation). Hematopoietic stem cell transplantation for multiple sclerosis: a retrospective multicenter study. *J Neurol*. 2002;249:1088–1097.
10. Schattner M, Pozner RG, Gorostizaga AB, Lazzari MA. Effect of thrombopoietin and granulocyte colony-stimulating factor on platelets and polymorphonuclear leukocytes. *Thromb Res*. 2000;99:147–154.
11. Sohngen D, Wienen S, Siebler M, Boogen C, Scheid C, Schulz A, Kobbe G, Diehl V, Heyll A. Analysis of rhG-CSF-effects on platelets by in vitro bleeding test and transcranial Doppler ultrasound examination. *Bone Marrow Transplant*. 1998;22:1087–1090.
12. Corti S, Locatelli F, Strazzer S, Salani S, Del Bo R, Soligo D, Bossolasco P, Bresolin N, Scarlato G, Comi GP. Modulated generation of neuronal cells from bone marrow by expansion and mobilization of circulating stem cells with in vivo cytokine treatment. *Exp Neurol*. 2002;177:443–452.
13. Six I, Gasan G, Mura E, Bordet R. Beneficial effect of pharmacological mobilization of bone marrow in experimental cerebral ischemia. *Eur J Pharmacol*. 2003;458:327–328.
14. Schabitz WR, Kollmar R, Schwaninger M, Juettler E, Bardutzky J, Scholzke MN, Sommer C, Schwab S. Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. *Stroke*. 2003;34:745.
15. Shyu WC, Lin SZ, Yang HI, Tzeng YS, Pang CY, Yen PS, Li H. Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated cells. *Circulation*. 2004;110:1847–1854.
16. Park HK, Kon Chu K, Lee ST, Jung KH, Kim EH, Lee KB, Song YM, Jeong SW, Kim M, Roh JK. Granulocyte colony-stimulating factor induces sensorimotor recovery in intracerebral hemorrhage. *Brain Res*. 2005;1041: 125–131.
17. Schneider A, Kruger IC, Steigleder IT, Weber D, Pitzer C, Laage R, Aronowski IJ, Maurer MH, Gassler N, Mier W, Hasselblatt M, Kollmar R, Schwab S, Sommer C, Bach A, Kuhn HG, Schabitz WR. The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J Clin Invest*. 2005;115:2083–2098.
18. Gibson CL, Jones NC, Prior MJW, Bath PMW, Murphy S. G-CSF suppresses edema formation and reduces interleukin-1β expression after cerebral ischemia in mice. *J Neuropathol Exp Neurol*. 2005;64:1–7.
19. The Abciximab in Acute Ischemic Stroke Investigators. A randomized, double-blind, placebo-controlled, dose-escalation study. *Stroke*. 2000;31: 601–609.
20. Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT. Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. *Clin Lab Haematol*. 1999;21:301–308.
21. Gibson CL, Bath PMW, Murphy SP. G-CSF reduces infarct volume and improves functional outcome after transient focal cerebral ischemia in mice. *J Cereb Blood Flow Metab*. 2005;25:431–439.
22. Shyu WC, Lin SZ, Lee CC, Liu DD, Li H. Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. *Can Med Assoc J*. 2006;174:927–933.
23. Kang HJ, Kim HS, Zhang SY. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet*. 2004; 363:751–756.
24. Ince H, Petzsch M, Kleine HD, Schmidt H, Rehders T, Korber T, Schymichen C, Freund M, Nienaber CA. Preservation From Left Ventricular Remodeling by Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Use of Granulocyte-Colony Stimulating Factor (FIRSTLINE-AMI). *Circulation*. 2005;112:3097–3106.
25. Valgimigli M, Rigolin GM, Cittanti C, Malagutti1 P, Curello S, Percoco G, Bugli AM, Della Porta M, Bragotti LZ, Ansani1 L, Mauro E, Lanfranchi A, Giganti M, Feggi L, Castoldi G, Ferrari R. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. *Eur Heart J*. 2005;26:1838–1845.
26. Zohlnhöfer D, Ott I, Mehilli J, K S, Michalk F, Ibrahim T, Meisetschläger G, von Wedel J, Bollwein H, Seyfarth M, Dirschinger J, Schmitt C, Schwaiger M, Kastrati A, Schömig A, for the REVIVAL-2 Investigators. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction. *JAMA*. 2006;295:1003–1010.
27. Kloner RA. Attempts to recruit stem cells for repair of acute myocardial infarction: a dose of reality. *JAMA*. 2006;295:1058.
28. Ripa RS, Wang Y, Jorgensen E, Johnsen HE, Grande P, Kastrup J. Safety of bone marrow stem cell mobilization induced by granulocyte-colony stimulating factor: 30 days blinded clinical results from the Stem Cells in Myocardial Infarction (STEMMI) Trial. *Heart Drug*. 2005;5:177–182.
29. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. 1991;337:1521–1526.
30. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, Investigators TT. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial. *Stroke*. 1993;24: 35–41.