

Joint UKBTS / NIBSC Professional Advisory Committee (1) Summary Sheet

1. Paper for the JPAC meeting on:	21 st June 2007
2. Date submitted:	25 th May 2007
3. Title (including version no.):	The Use of G-CSF in Volunteer Stem Cell Donors
4. Author(s):	Derwood Pamphilon on behalf of the SAC on Stem Cells
5. Brief summary:	G-CSF is given to unrelated volunteers registered on the UK Stem Cell Donor Registries who are selected to donate peripheral blood stem cells. Concerns have been raised that its use might cause or precipitate myelodysplasia or acute myeloid leukaemia in susceptible individuals. Although there is currently no data to support this, it is recommended that all stem cell donors are followed up for a minimum of 10 years. The results of more detailed UK and US studies on genomic instability associated with G-CSF are awaited.
6. Action required by the Joint Professional Advisory Committee EWG: (What do you want JPAC to do in response to this paper?) e.g. <ul style="list-style-type: none"> • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within the work plan • provide a steer on policy 	For information.
7. Any other relevant information:	

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at: Transfusion, Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

(1) Joint United Kingdom Blood Transfusion Services and National Institute for Biological Standards and Control Professional Advisory Committee

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

Prepared by: Dr Derwood Pamphilon on behalf of the Standing Advisory Committee on Stem Cells

Summary

Granulocyte colony stimulating Factor (G-CSF) is licensed for the mobilisation of haemopoietic stem cells (HSC) in healthy donors. It has a number of common side effects such as bone pain, which resolve rapidly after administration is discontinued. Recent publications have raised concern that it may act as a trigger for the development of haematological malignancy in susceptible donors possibly by causing genomic instability but conclusive evidence is lacking. Ongoing studies aim to confirm whether or not G-CSF causes chromosomal abnormalities in healthy donors. Blood services give G-CSF to donors enrolled on the British Bone Marrow Registry (BBMR) and Welsh Bone Marrow Donor Registry (WBMDR) who are selected to donate peripheral blood stem cells (PBSC).

It is recommended that all stem cell donors are given updated information explaining the current uncertainties with regard to the use of G-CSF before they give informed consent to its administration. The World Marrow Donor Association (WMDA) has agreed a statement that may be used by individual donor registries. Further, all PBSC donors who receive G-CSF as well as bone marrow donors who do not should be under regular review for at least 10 years to allow the occurrence of long-term problems such as malignancy to be documented. The total dose per course and number of courses of G-CSF should be limited although at the present time it is unclear what the maximum exposure should be.

Background

G-CSF is a glycoprotein which stimulates the development of primitive stem cells, their release from the marrow into the circulation and an increase in circulating granulocytes, monocytes and lymphocytes.¹ It also has a number of other effects which include

- Increased mononuclear cell (MNC) procoagulant activity and thrombin generation²
- Modulation of effector molecules on monocytes and monocyte activation³
- Cytokine responses: increased interleukin (IL)-1ra and soluble TNF receptors,^{4,5} increased IL-6, IL-8 and IL-10; decreased TNF, GM-CSF and interferon (IFN) γ release
- Skewed helper cell (Th1:Th2) ratio⁶
- Proliferation of endothelial cells⁷ and mobilisation of endothelial progenitor cells⁸

G-CSF is usually given at a dose of 5-10 μ g/kg/d for 4-6 days for stem cell mobilisation. If

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

further donations of stem cells are required then further courses may be given, usually after a minimum interval of 1 month; most further donations of PBSC are given in the first 12 months after the primary donation. Moreover donors may remain on the registry and donate for a second patient if they are willing.

A number of side effects are commonly associated with the use of G-CSF, these include bone pain, headache and myalgia¹ (see Table 1). Rarer effects include spontaneous rupture of the spleen and acceleration of autoimmune disease. It has been suggested that G-CSF administration might precipitate myeloid malignancy in susceptible individuals, possibly by causing genomic instability.⁹ At the present time there is a relative lack of long term follow up data on donors who have received G-CSF (see Table 2). In addition, cases of leukaemia and other cancers occur at a well defined rate in previously healthy individuals irrespective of the use of G-CSF and it would at this stage be wrong to assume a cause and effect relationship.

Table 1

Acute side-effects associated with G-CSF administration

Common	Bone pain Headache Myalgia
Less common	Nausea Vomiting Diarrhoea Fatigue Redness at injection site Insomnia
Rare	Splenic rupture Acceleration of autoimmune disease
Hypothetical	MDS / AML

Data Relating to G-CSF and Myeloid Malignancy

a) Studies in Normal Donors

In 2004 Nagler et al reported that G-CSF caused loss of synchrony in allelic replication and aneuploidy in lymphocytes collected from a group of 18 healthy allogeneic stem cell donors who had received G-CSF.⁹ During 9 months of follow up, the occurrence of allele specific replication was a transient phenomenon but aneuploid cell clones remained in

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

some donors. The authors used interphase Fluorescence In Situ Hybridisation (iFISH) analysis of chromosome (Chr)17. The changes were detected in stimulated lymphocytes and were of the type previously documented in patients with haematological malignancies and pre-malignant disorders. Nagler suggested that by causing ASR and therefore monoallelic gene expression G-CSF might either unmask mutated recessive genes or leave cells vulnerable to a second 'hit' as described in Knudson's two-hit cancer-initiation hypothesis.⁹ This group subsequently reported transient changes in gene expression (53 genes increased and 69 genes decreased using Affymetrix Gene Chip array) in donors who received G-CSF. Hernandez et al investigated the gene expression profiles in MNC from 9 stem cell donors who received G-CSF and found that the expression of 374 genes was increased whilst expression of 387 genes was decreased, the latter including CD11b, ICAM-2 and immune response genes, consistent with its known biological effects.¹⁰ The authors stressed the need for rigorous follow-up of PBSC donors. In December 2006 Bennett and colleagues reported 5 cases of haematological malignancy occurring in haemopoietic growth factor recipients. In 3 cases lymphoproliferative disease followed the administration of recombinant human megakaryocyte growth and development factor (rhuMGDF) but there were also 2 cases of acute myeloid leukaemia (AML) that occurred in donors who received G-CSF when donating PBSC for siblings with AML. These cases occurred 4 and 5 years after receipt of G-CSF and in one case the donor's mother had also been diagnosed with secondary AML.¹¹ One further case of AML in a PBSC donor who received G-CSF was reported in 2004. In this case leukaemia occurred 14 months after donation for a sibling with multiple myeloma.¹²

b) In Vitro Studies of G-CSF

It has been reported that in vitro G-CSF treatment induces expansion of monosomy 7 clones in the bone marrow of patients with pre-existing monosomy 7 cells. Bone marrow samples of 28 patients with myelodysplasia and pre-existing monosomy 7 were cultured in vitro for 7 days with G-CSF and evaluated by FISH. At higher doses (400ng/ml) there was a steady increase in monosomy 7 cells over a 15 day period. However, abnormal cell clones, e.g. monosomy 7, could not be induced by G-CSF.¹³ Kaplinsky et al using a multiparametric cell scanning system assessed the effect of G-CSF administration on the morphology and genotype of leucocytes from normal donors. They showed that 0.6% of myeloid cells, but not purified CD34+ progenitor cells became tetraploid. This effect was transient (<30d.) and suggests that the G-CSF could induce alterations of chromosomal numbers in a small subset of mature myeloid cells.¹⁴

c) Mutations in the G-CSF receptor in patients with neutropenia

The G-CSF receptor is present on pluripotent and committed myeloid progenitors, neutrophils, monocytes, endothelial cells and possibly some lymphocyte subsets.¹ It is known that patients with severe congenital neutropenia (SCN Kostmann's Syndrome)

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

may develop structural abnormalities of the G-CSF receptor, mainly due to point mutations. These result in a truncated receptor and are associated with a high risk of subsequent development of MDS or AML.¹⁵ The cumulative incidence is 36% after 12 years of treatment. Follow up of these patients is undertaken by the Chronic Neutropenia International Registry. Other patient groups reported to the registry e.g. those with cyclical neutropenia, do not have an increase in MDS/AML after treatment with G-CSF, although 2 cases of AML occurred in patients with Schwachman-Diamond Syndrome 1 month and 2.2 years after initiation of G-CSF therapy.¹⁶

d) Use of G-CSF in patients with breast cancer

Two reviews have discussed the occurrence of MDS/AML in breast cancer patients who received G-CSF to minimise neutropenia after adjuvant chemotherapy. The National Surgical Adjuvant Breast and Bowel Cancer Program (NSABP) reported an increased risk of MDS/AML (1.2% v 0.3%) in 1694 breast cancer patients who received G-CSF support compared to breast cancer patients who did not. In a second study of 5510 women reported in the Medicare Surveillance epidemiology and End Results (SEER) database, there was a twofold increase in MDS/AML where G-CSF support had been given.¹⁷

c) Studies in Stem Cell donors

These are shown in Table 2. They do not show any increase in MDS/AML in PBSC donors who receive G-CSF and in some cases, e.g. the US National Marrow Donor Program (NMDP) no cases of haematological malignancy at all have been recorded. One criticism of the data is that much of it is retrospective in nature e.g. the EBMT survey of Halter et al, and this may lead to under-reporting. In addition the period of follow up is relatively short. Nonetheless it is reassuring that the incidence of haematological malignancy in general and MDS/AML in particular is low. In fact there are only 3 reported cases of AML following the donation of G-CSF mobilised PBSC and all occurred in siblings who might anyway have a shared genetic susceptibility.^{11,12} Recent EBMT data includes 3 individuals developing AML after donation (2 BM; 1 PB) but further information on these cases is not available at present.²⁰

Table 2

Report	Ref.	Registry/ institution	BM donors			PB donors		
			No.	Follow-up	HM*	No.	Follow-up	HM*
Anderlini P et al	22	MD Anderson	-	-	-	281	3.3 years (median)	None
Confer DC	18	NMDP ⁽²⁾ Registry	1160	3 years ⁽¹⁾	None	4005	Up to 8 years ⁽¹⁾	None
Schmidt A	19	DKMS ⁽²⁾ (German)	3713	5 years max 18,000	1 CLL	7236	5 years max 18,000	1 HD

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

		Registry		observation years (BM + PB)			observation years (BM + PB)	
Halter J ⁽⁴⁾	20	EBMT ⁽³⁾ Survey	23,254	Data collected 1993-2005	8 incl 2 AML	27,770	Data collected 1993-2005	11 incl 1 AML
Kodera A	21	JMDP (Japanese) Registry	-	-	-	3264	-	1 AML in a sibling ⁽¹²⁾

* Haematological malignancy

- (1) ≥ 1 year minimum
- (2) Unrelated donors
- (3) Related donors > 90%
- (4) Incidence of AML occurrence not significant for BM v PB

Proposed Studies

The BBMR and Anthony Nolan Trust (ANT) donor registries in the UK and the National Marrow Donor Program (NMDP) with the University of Minnesota in the USA are now about to start detailed chromosomal studies in selected donor populations.

- (i) NMDP-University of Minnesota study. This aims to replicate the study of Nagler et al and evaluate chromosomal end points that have specifically been associated with higher dose or longer term G-CSF therapy in patients with aplastic anaemia and breast cancer as well as patients with constitutional chromosome instability syndromes. Both unstimulated and PHA stimulated peripheral blood mononuclear cell cultures will be studied for abnormalities of chromosomes 7 and 17. Twenty healthy non-donors and 20 healthy G-CSF stimulated sibling donors will be studied and followed for up to 24 months.²³
- (ii) BBMR-ANT study. This study aims to assess if G-CSF is associated with long-term damage to the lymphocytes of healthy donors. It will also employ more sensitive methods to assess genetic damage, i.e. the presence of allele specific replication and aneuploid cell clones. There will be both retrospective and prospective arms each including 100 donors. Bone marrow donors will be included in this study to answer the question – is rapid displacement of stem cells itself associated with chromosomal damage? Donors will be aged 18-60 years and will have no family history of haematological malignancy. Fifty PBSC and 50 bone marrow donors will be studied retrospectively at 3-5 years post-donation. In the prospective arm 50 BM and 50 PBSC donors will be sampled pre-donation, 3 and 12 months post-donation and subsequently at 2 and 3 years if aneuploidy is detected. The methods employed will be FISH analysis of chromosome 7, 17 and 8, the latter representing the most frequently gained autosome in haematological malignancies. For samples that tested positive for aneuploidy, high resolution array comparative genomic hybridisation (CGH) will be undertaken. The array CGH uses

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

oligonucleotide probes and can detect any imbalances with a resolution of about 80 bp but only if the abnormal cell population is represented at 35% level. Two control groups are proposed:

- Samples taken from the donors prior to donation (+/- G-CSF) will act as negative controls
- There will be one positive control group of 25 patients with newly diagnosed haematological malignancies (patients with AML, ALL, CLL, CML) asking the question – do their lymphocytes show the same changes as G-CSF treated donors, i.e. genomic instability?

Recommendations

1. The continued use of G-CSF is acceptable in unrelated PBSC donors. At the present time there is no evidence to show that they are at an increased risk of developing MDS/AML compared to donors who do not receive G-CSF. They should receive appropriate counselling including explanation of the statement issued by the WMDA which states that, "Normal individuals are at risk for developing cancer, including leukemia, lymphoma or other blood diseases throughout their life time. G-CSF stimulates normal blood cell growth. In some patients with cancer or abnormal blood cells, it has been shown to stimulate leukemic blood cells. It is unknown whether G-CSF increases or decreases an individual's risk of developing cancer. Based on available data from healthy people who have received G-CSF, no long-term risks have been found so far. The data being collected during follow-up will help establish if there are any positive or negative long-term effects from receiving G-CSF."
2. All such donors and, in addition, bone marrow donors should be subject to a minimum follow-up period of 10 years to assess the incidence of leukaemia and other malignancies that occur in them. This will provide valuable data. It is important to include bone marrow donors who have not received G-CSF as a critical control group.
3. Detailed chromosome studies should be performed on selected donors to determine whether G-CSF causes chromosomal instability and increases the susceptibility to subsequent development of haematological malignancy.

Dr. Derwood H. Pamphilon
14th May 2007

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS**The Use of G-CSF in Unrelated Volunteer Stem Cell Donors****21st June 2007**

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at *Transfusion* Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

References

1. Demetri GD and Griffin JD. Granulocyte Colony-Stimulating Factor and its receptor. *Blood*, 1991 Dec, 78, 11, 2791-2808
2. Falanga A, Marchetti M, Evangelista V et al. Neutrophil activation and hemostatic changes in healthy donors receiving granulocyte colony-stimulating factor. *Blood*, 1999 April 15, 93(8), 2506-2514
3. Ohsaka A, Saionji K, Kuwaki T et al. Granulocyte colony-stimulating factor administration modulates the surface expression of effector cell molecules on human monocytes. *British Journal of Haematology*, 1995 Mar, 89(3), 465-72
4. Hartung T, Docke WD, Gantner F et al. Effect of granulocyte colony-stimulating factor treatment on ex-vivo blood cytokine response in human volunteers. *Blood*, 1995, 85, 9, 2482-2489
5. Pollmacher T, Korth C, Mullington J et al. Effects of granulocyte colony-stimulating factor on plasma cytokine and cytokine receptor levels and on the in vivo host response to endotoxin in healthy men. *Blood*, 1996, 87, 3, 900-905
6. Klansinsirkul P, Russell NH. Peripheral blood stem cell harvests from G-CSF-stimulated donors contain a skewed Th2 CD4 phenotype and a predominance of type 2 dendritic cells. *Experimental Haematology*, 2002 May, 30(5), 495-501
7. Bocchietto E, Guglielmetti A, Silvagno F et al. Proliferative and migratory responses of murine microvascular endothelial cells to granulocyte-colony-stimulating factor. *Journal of Cellular Physiology*, 1993 Apr, 155(1), 89-95
8. Korbling M, Reuben JM, Gao H et al. Recombinant human granulocyte-colony-stimulating factor-mobilized and apheresis-collected endothelial progenitor cells: a novel blood cell component for therapeutic vasculogenesis. *Transfusion*, 2006 Oct; 46(10) : 1795-802
9. Nagler A, Korenstein-Ilan A, Amiel A, Avivi L. Granulocyte-colony stimulating factor generates epigenetic and genetic alterations in lymphocytes of normal volunteer donors of stem cells. *Exp Haematol*, 2004, 32, 1221-130
10. Hernandez JM, Castilla C, Gutierrez NC et al. Mobilisation with G-CSF in healthy donors promotes a high but temporal deregulation of genes. *Leukaemia*, 2005, 19,

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at *Transfusion* Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

1088-1091

11. Bennett CL, Evens AM, Andritsos LA et al. Haematological malignancies developing in previously healthy individuals who received haematopoietic growth factors: Report from the research on adverse drug effects and reports (RADAR) project. *Brit J Haem*, 2006 Dec, 135(5), 642-650
12. Makita K, Ohta K, Mugaita NA et al. Acute myelogenous leukaemia in a donor after granulocyte colony-stimulating factor-primed peripheral blood stem cell harvest. *Bone Marrow Transplantation*, 2004 Mar, 33(6), 661-665
13. Sloand EM, Yong AS, Ramkissoon S et al. Proceedings of National Academy of Science, 2006 Sep 26, 103(39), 14483-8
14. Kaplinsky C, Trakhtenbrot L, Hardan I et al. Tetraploid myeloid cells in donors of peripheral blood stem cells treated with rhG-CSF, *Bone Marrow Transplant*, 2003, 32, 31-34
15. Germeshausen M, Ballmaier M, Velt EK. Instance of CSF 3R mutations in severe congenital neutropenia and relevance for leukaemogenesis: Results of a long-term study. *Blood*, 2007, 107, 93-99
16. Rosenberg PS, Alter BP, Bolyard AA et al. The incidence of leukaemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. *Blood*, 2006 Jun 15(12), 107, 4628-4635
17. Tigue CC, Trifilio SM, Tallman MS, Goldman JM, Bennett CL. Long-term safety of Filgrastim (rhG-CSF) administration: Response to Confer and Miller and Bacher and Zander. *Brit J Haematol*, 2007, 137, 79-80
18. Confer DL, Miller JP. Long-term safety of Filgrastim (rhG-CSF) administration. *Brit J Haem*, 2007, 137, 77-78
19. Schmidt et al. Presented at 33rd meeting of EBMT Lyon, March 2007
20. Halter et al. Presented at 33rd meeting of EBMT Lyon, March 2007
21. Kodura et al. Presented at 33rd meeting of EBMT Lyon, March 2007
22. Anderlini P, Chan FA, Champlin R, Korbeling M, Strom S. Long-term follow-up of normal peripheral blood progenitor cell donors treated with Filgrastim: no evidence of increased risk of leukaemia development. *Bone Marrow Transplantation*, 2002, 30,

Joint UKBTS / NIBSC Professional Advisory Committee (1)

Discussion Document for the Joint Professional Advisory Group (JPAC) of the UKBTS

The Use of G-CSF in Unrelated Volunteer Stem Cell Donors

21st June 2007

This is the pre-peer-reviewed version of the following article: "The use of granulocyte-colony-stimulating factor in volunteer unrelated hemopoietic stem cell donors", which has been published in final form at Transfusion Volume 48, Issue 7, Date: July 2008, Pages 1495 - 1501).

661-663

23. Hirsch B. Personal communication, March 2007

(1) **Joint United Kingdom Blood Transfusion Services and National Institute for Biological Standards and Control Professional Advisory Committee**