

# Haematological malignancies developing in previously healthy individuals who received haematopoietic growth factors: report from the Research on Adverse Drug Events and Reports (RADAR) project

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Thrombopoietic growth factors have been administered to healthy individuals in an effort to improve platelet yields from platelet donors (Kuter *et al*, 2001). Similarly, granulocyte colony-stimulating factors (G-CSF) have been administered to more than 60 000 healthy donors who provide peripheral blood stem cells for persons undergoing haematopoietic stem cell transplantation (HSCT) (Horowitz & Confer, 2005; Pulsipher *et al*, 2006). The recently identified platelet cytokine, thrombopoietin, is the ligand for the c-Mpl receptor on platelets and megakaryocytes. Thrombopoietin promotes pro-

## Summary

Pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) and granulocyte colony-stimulating factor (G-CSF) promote haematopoietic progenitor cell maturation. We reviewed the findings for healthy volunteers/donors who developed haematological malignancies following PEG-rHuMGDF or G-CSF administration. Information was reviewed for three of 538 volunteers who received PEG-rHuMGDF in clinical trials and two of 200 donors who underwent G-CSF mobilised stem cell harvesting procedures for sibling stem cell transplants. Mantle cell, diffuse large B-cell lymphoma and chronic lymphocytic leukaemia were diagnosed 1–5 years after PEG-rHuMGDF exposure among three volunteers. For one patient, thrombocytopenia due to autoantibodies to PEG-rHuMGDF developed shortly after PEG-rHuMGDF administration and persisted until chemotherapy was administered. All three achieved complete remission, although one patient relapsed. Acute myeloid leukaemia was diagnosed 4 and 5 years after G-CSF mobilisation in two donors who underwent peripheral blood stem cell donation for sibling allogeneic haematopoietic stem cell transplantation. Following intensive chemotherapy, one died from acute leukaemia and the second is in complete remission. Controversy exists over the appropriateness of administering haematopoietic growth factors to healthy individuals. While a causal relationship with haematological malignancies cannot be demonstrated, long-term follow-up among healthy individuals who receive haematopoietic growth factors is needed.

**Keywords:** haematopoietic growth factors, megakaryocyte growth and development factor, granulocyte colony-stimulating factor, Research on Adverse Drug Events and Reports.

liferation of megakaryocyte progenitors and their maturation into platelet-producing megakaryocytes (Harker *et al*, 2000). Two recombinant thrombopoietin products, recombinant human thrombopoietin and pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) have been developed. One of them, PEG-rHuMGDF, was administered to 584 healthy subjects in two clinical trials (Harker *et al*, 2000; Kuter *et al*, 2001; Li *et al*, 2001; Nagler *et al*, 2004). Experience with recombinant G-CSF among healthy volunteers is far more extensive, as this cytokine has

been administered to several thousand healthy donors who undergo peripheral blood stem cell mobilisation procedures as part of unrelated and related allogeneic peripheral blood HSCT regimens.

Safety and ethical concerns have been raised regarding administration of haematopoietic colony-stimulating factors to healthy volunteers or healthy donors (Verdijk, 2002; Pulsipher *et al*, 2006). G-CSF is accepted for widespread use among healthy unrelated donors for allogeneic HSCT largely because of the absence of reports of G-CSF related complications among healthy donors who participate in allogeneic stem cell transplantation. When the phase I/II clinical trials with PEG-rHuMGDF were initiated, concern was raised over the possibility of developing neutralising antibodies as well as the ethics of administering a cytokine that did not have extensive safety assessments to healthy individuals who participated in early phase clinical trials (Verdijk, 2002). As part of a series of studies on serious adverse events, the Research on Adverse Drug Events and Reports (RADAR) group identified five previously healthy individuals who received PEG-rHuMGDF or G-CSF and subsequently developed haematological malignancies (Bennett *et al*, 2005). The clinical details of these cases are reported herein.

## Methods

The RADAR project is a National Cancer Institute-funded research programme that identifies and disseminates clinical information on adverse drug reactions, with a particular emphasis on drugs used in haematology and oncology (Bennett *et al*, 2005). RADAR investigators identified haematological malignancies developing in three of 584 individuals who participated in two Institutional Review Board-approved clinical trials with PEG-rHuMGDF and two of 200 individuals who were stem cell donors for siblings undergoing allogeneic peripheral blood stem cell transplantation for the treatment of acute myeloid leukemia (AML). The two PEG-rHuMGDF studies evaluated the safety and thrombopoietic effects of this investigational drug in healthy human subjects. In one study, a single dose of 1 µg/kg or 3 µg/kg of PEG-rHuMGDF was administered to 46 healthy platelet apheresis donors (Horowitz & Confer, 2005). In a second study, one to three doses of 3 µg/kg PEG-rHuMGDF were administered monthly to 538 healthy human volunteers (Kuter *et al*, 2001; Shapira *et al*, 2003; Nagler *et al*, 2004).

## Results

### Case #1

In 1998, a 41-year-old man with no prior toxin or chemical exposure participated as a volunteer in the multiple dose administration of PEG-rHuMGDF clinical trial. Screening history, including family history and the physical examination, was unremarkable. The complete blood count prior to

administration of PEG-rHuMGDF showed a white blood cell count of  $6.8 \times 10^9/l$  (normal differential), haemoglobin 16.1 g/dl, and platelet count of  $130 \times 10^9/l$ . Following three doses of PEG-rHuMGDF, the platelet count rose to levels between  $224 \times 10^9/l$  and  $388 \times 10^9/l$ , while the white blood cell count and haemoglobin were unchanged. However, over the subsequent 2 weeks, a steady drop in platelet counts reaching  $61 \times 10^9/l$  at 1-month follow-up and  $52 \times 10^9/l$  at 3 months were noted. Platelets remained in the  $50\text{--}70 \times 10^9/l$  range over the next 5 years. Laboratory studies identified antibodies to PEG-rHuMGDF that cross-reacted and neutralised endogenous thrombopoietin. In May 2003, the patient presented to his family physician with anaemia. The physical examination was notable for palpable inguinal lymphadenopathy. White blood cell count was  $4.8 \times 10^9/l$ , haemoglobin 10.8 g/dl and platelets  $79 \times 10^9/l$ . White blood cell differential included 37% neutrophils, 3% eosinophils, 57% lymphocytes and 2% monocytes. A computerised tomography (CT) scan revealed splenomegaly (20 cm) and diffusely enlarged mediastinal, hilar, abdominal and pelvic lymphadenopathy. A bone marrow biopsy revealed marked hypercellularity with normal megakaryocyte morphology, while flow cytometry demonstrated lymphocytes with a phenotype  $CD5^+$ ,  $CD23^-$  consistent with mantle cell lymphoma. Molecular studies confirmed the diagnosis with  $t(11;14)(q13;q32)$  in 38% of the interphase nuclei. In June 2003, the presence of massive splenomegaly led to a splenectomy. The patient received rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone (R-CHOP) for six cycles and achieved complete remission, including normalisation of platelet counts and no evidence of antibodies to PEG-rHuMGDF. However, the patient relapsed 5 months later and received salvage therapy with cisplatin and cytarabine, attaining complete remission. In June 2004, the patient underwent matched sibling allogeneic HSCT and achieved a complete remission with cyclophosphamide and total body irradiation conditioning. As of December 2005, 18 months post-transplant, the patient has maintained complete remission with mild chronic graft-versus-host disease.

### Case #2

In 1998, a 51-year-old female with no prior exposure to toxins or chemicals, participated as a volunteer in the multi-dose administration of PEG-rHuMGDF clinical trial. Screening history, family history, physical examination and baseline complete blood count were unremarkable. Following each of two PEG-rHuMGDF injections, the platelet count transiently rose above normal, while the white blood cell count and haemoglobin were unchanged. Upon completion of the study, the platelet count returned to normal. In September 2000, the patient reported abdominal pain and a several month history of a vascular-appearing skin rash on the buttocks and legs. The white blood cell count was  $5.0 \times 10^9/l$  with an absolute neutrophil count of  $4.6 \times 10^9/l$ , haemoglobin 12.5 g/dl, and platelet count of  $276 \times 10^9/l$ . CT scans revealed diffusely

enlarged lymphadenopathy and a spleen that measured 16 cm. Lymph node biopsy showed diagnosis of diffuse large B-cell lymphoma. The patient received R-CHOP for six cycles with complete remission, including resolution of the rash. The patient was without evidence of disease for 3.5 years, after which time she presented with isolated central nervous system relapse with a solitary temporal lobe brain mass positive for lymphoma without evidence of other disease on staging studies. The patient received high-dose methotrexate and high-dose cytarabine followed by high dose carmustine, etoposide, cytarabine and melphalan conditioning with autologous HSCT. She is now 1 year following transplantation without evidence of disease.

#### Case #3

In June 1997, a 50-year-old male with no prior exposure to toxins or chemicals participated as a volunteer in the single dose administration of PEG-rHuMGDF clinical trial. Screening history, including family history and physical examination, were unremarkable. The patient had in the past been a frequent platelet donor. At baseline, white blood cell count was  $5.2 \times 10^9/l$  (normal differential), haemoglobin 14.1 g/dl, and platelet count  $223 \times 10^9/l$ . Following a single injection of PEG-rHuMGDF, the platelet count increased to  $596 \times 10^9/l$ , while the white blood cell count and haemoglobin remained unchanged. The platelet count returned to  $229 \times 10^9/l$  3 weeks following treatment. Subsequently, the patient continued to donate platelets as a volunteer. A complete blood count in December 1997 (6 month after PEG-rHuMGDF administration) was notable for a white blood cell of  $6.3 \times 10^9/l$  with 49% lymphocytes and 40% neutrophils and normal haemoglobin concentration and platelet counts. In June 1998, a complete blood count revealed thrombocytopenia that spontaneously resolved. However in November 1998, lymphadenopathy was noted with a concurrent white blood cell count of  $22 \times 10^9/l$  (61% lymphocytes and 37% neutrophils), haemoglobin 14.2 g/dl, and platelet count of  $212 \times 10^9/l$ . Chronic lymphocytic leukaemia (CLL) was diagnosed by peripheral blood flow cytometry and bone marrow biopsy. Cytogenetics were not done at this time. From 1998 until 2001, the patient remained clinically well without therapy. In July 2000, the white blood cell count was  $60 \times 10^9/l$  and haemoglobin 11.6 g/dl. In mid-2001, anaemia was treated with packed red blood cell transfusions, which in July 2001 prompted two cycles of single agent fludarabine therapy ( $25 \text{ mg/m}^2$ ). After treatment, the white blood cell count decreased to  $19 \times 10^9/l$ , while the haemoglobin was 10.9 g/dl and platelet count  $113 \times 10^9/l$  with the clinical course complicated by a mild Coombs-positive autoimmune haemolytic anaemia. In November 2001, progression of CLL was noted with white blood cell count  $78 \times 10^9/l$ , haemoglobin 9.7 g/dl, and platelet count  $166 \times 10^9/l$ . The patient did not tolerate fludarabine re-treatment due to persistent fevers. The anaemia was treated with packed red blood cell transfusions and recombinant

erythropoietin and the patient received 4 months of intravenous cyclophosphamide and G-CSF. The patient remained asymptomatic for 2 years with mild anaemia that did not require therapy. In March 2004, a rapid rise in white blood cell count to  $274 \times 10^9/l$  was accompanied by a symptomatic increase in adenopathy and mild splenomegaly was observed. Further cytogenetic evaluation revealed a 17p deletion. The patient was treated with five cycles of cyclophosphamide and prednisone and achieved a partial remission.

#### Case #4

In 1996, a 55-year-old female with no prior exposure to chemicals, but a possible prior exposure to asbestos, donated peripheral blood stem cells for her human leucocyte antigen (HLA)-identical brother who was being treated for AML. Her medical history was only remarkable for hypertension and hyperlipidemia. Family history was notable for secondary AML in her mother and her brother with *de novo* AML. She received G-CSF  $10 \text{ }\mu\text{g/kg/d}$  for 5 d followed by apheresis, collecting  $2.22 \times 10^6 \text{ CD34}^+$  cells/kg. She was in good health until December of 2000, when she had a syncopal episode secondary to anemia. A bone marrow biopsy showed AML French-American-British (FAB) type M6 with a complex karyotype, including monosomy 7. She underwent standard induction chemotherapy with idarubicin and cytarabine followed by two cycles of high dose cytarabine-based consolidation. Because of her unfavourable cytogenetics, a matched unrelated allogeneic donor HSCT was performed in first complete remission in May of 2001. Her transplant course was uncomplicated. Her post-transplant course was complicated by the development of ciclosporin-associated central nervous system toxicity with reversible leucoencephalopathy. She experienced no acute or chronic graft-versus-host disease. In November 2001, the patient presented with focal neurologic symptoms and a lumbar puncture confirmed central nervous system recurrence of AML. She expired from progressive disease the same month.

#### Case #5

In 1998, a 40-year-old man with no prior exposure to toxins or chemicals donated peripheral blood stem cells for his HLA-identical brother who was being treated for *de novo* AML. His medical history was significant only for lumbar disc disease requiring surgery, and his family history was significant only for a brother with AML. He underwent peripheral stem cell mobilisation twice, 2 weeks apart, with G-CSF  $10 \text{ }\mu\text{g/kg/d}$  for 5 d followed by apheresis. He remained in his usual state of health until 5 years later, when he developed a rash and leucopenia, with a white blood cell count of  $1.8 \times 10^9/l$ . A bone marrow biopsy was performed and revealed the presence of myeloblasts (AML FAB M5a), and a skin biopsy was consistent with leukaemia cutis. Cytogenetics showed a normal male karyotype. He received induction chemotherapy on a clinical protocol including idarubicin, etoposide and cytarabine,

achieving a first complete remission. As part of postremission therapy he was randomised to undergo autologous HSCT as consolidation. He underwent chemomobilisation with cytarabine, etoposide, and G-CSF and in February 2004 received high dose chemotherapy with busulfan and etoposide followed by autologous HSCT. The patient remains in complete remission, with his most recent complete blood count showing a white blood cell count of  $5.8 \times 10^9/l$  (normal differential), haemoglobin of 14.9 g/dl and a platelet count of  $341 \times 10^9/l$ .

## Discussion

This report describes five previously healthy individuals who received PEG-rHuMGDF or G-CSF and developed haematological malignancy within 5 years of exposure. In interpreting our findings, several factors should be considered.

Short-term safety concerns regarding administration of PEG-rHuMGDF have been reported. Thrombocytopenia occurred in four of 665 cancer, stem cell transplantation, or leukaemia patients given multiple doses and in two (1.0%) of 204 healthy volunteers who received two doses and in 11 (8.9%) of 124 healthy volunteers given three doses of PEG-rHuMGDF (Yang *et al*, 1999; Li *et al*, 2001). No subject developed neutralising antibodies or thrombocytopenia after a single dose of PEG-rHuMGDF; this included all 46 apheresis subjects and 210 healthy volunteers who received only one dose. The thrombocytopenia resulted from the formation of an IgG antibody to PEG-rHuMGDF that cross-reacted with endogenous TPO and neutralised its biological activity. In two patients, thrombocytopenia was associated with anaemia and neutropenia, suggesting an effect on a stem cell population (Li *et al*, 2001; Basser *et al*, 2002). Because endogenous thrombopoietin is produced in a constitutive fashion by the liver, thrombocytopenia ensues. Although PEG-rHuMGDF was withdrawn from clinical trials in 1998 after cases of thrombocytopenia were identified, long-term safety assessments for the 538 healthy volunteers have yet to be reported. With respect to B-cell disorders, the association with PEG-rHuMGDF is far more tenuous, particularly as c-mpl is not at all, or at least only minimally, expressed on lymphoma cells (Graf *et al*, 1996). In some model systems, thrombopoietin may actually have a protective effect (Shibuya *et al*, 2004).

Several groups have evaluated laboratory models that looked at the effect of G-CSF on DNA function and stability. Because malignancies demonstrate changes in DNA stability, if long-term DNA changes occur in normal donors, concern would be raised about possible increased risk of cancer developing in these donors. Haematological malignancies exhibit disruption of the normal mode of tight synchrony during cell replication. Nagler *et al* (2004) measured allele specific replication and aneuploidy in 40 normal volunteer donors and 20 patients with haematological malignancies mobilised with G-CSF following G-CSF mobilisation. These studies demonstrated that lymphocytes of G-CSF mobilised donors displayed levels of allele-specific replication and aneuploidy similar to those

observed in lymphocytes of leukaemic patients. Although the loss of replication synchrony in the lymphocytes of G-CSF mobilised donors returned to baseline after a few months, aneuploidy was still seen in a small number of donors up to 9 months after G-CSF administration. The G-CSF effect was also observed after G-CSF incubation *in vitro*, which was inhibited by 5-azacytidine. Shapira *et al* (2003) showed similar findings using a double-stranded DNA relaxation technique. *De novo* synthesis of DNA in the white blood cell population of healthy donors increased with G-CSF administration, but returned to baseline levels 1–2 months after completion of G-CSF therapy. Using a combined multiparametric cell scanning system to assess the effect of G-CSF administration to normal donors on morphology and genotype of white blood cells, Kaplinsky *et al* (2003) demonstrated that up to 0.6% of myeloid cells, but not purified CD34<sup>+</sup> stem progenitor cells, became tetraploid, indicating that G-CSF may induce alterations of chromosomal numbers in a small subset of mature myeloid cells in G-CSF-mobilised normal donors. Severe functional alterations have also been demonstrated in circulating lymphocytes of normal peripheral blood stem cell donors treated with G-CSF. (Rutella *et al*, 2000) While these observations raise concern, they do not demonstrate a definite link between G-CSF and leucemogenesis. It should be noted that molecular studies have raised a cautionary note about possible interactions between acquired mutations in the G-CSF receptor, G-CSF therapy, and the risk of leukaemia (Dong *et al*, 1995). However, the prevalence and pathogenicity of these mutations in unselected patients is unclear (Ancliff *et al*, 2003).

Studies of G-CSF therapy administered to healthy peripheral blood stem cell donors have evaluated safety concerns. In a prospective series of 20 healthy donors for peripheral blood stem cell transplantation mobilisation procedures who received G-CSF, almost all experienced an increase in spleen size (Stroncek *et al*, 2003). Spleen length increased by 20% or more in 30% of individuals (Casadevall *et al*, 2004). In rare instances, splenic ruptures occurred among G-CSF-treated donors undergoing stem cell mobilisation (Balaguer *et al*, 2004; Dincer *et al*, 2004). A 2-year follow-up study of 24 neonates treated with G-CSF for sepsis found no cases of leukemia (Rosenthal *et al*, 1996). Single-site studies of adult peripheral blood stem cell donors have not shown late effects associated with short-term G-CSF therapy, with the longest follow-up now at 5 years (Sakamaki *et al*, 1995; Cavallaro *et al*, 2000; Anderlini *et al*, 2002; Makita *et al*, 2004; Tassi *et al*, 2005) (Table I). Another study of white cell subsets in nine donors, 1 year after donation, found no change in B, T and natural killer (NK) cells by flow cytometric analysis (Storek *et al*, 2000). A mild decrease in monocytes was noted compared with the donor's baseline labs and when compared with 103 healthy normal volunteers (Storek *et al*, 2000). Clinical trials of persons with myelodysplastic syndrome (MDS) or AML have not identified an association between cytokine use and leukemogenesis (Dombret *et al*, 1995; Rowe

Table 1. Long-term evaluation of leukaemia development among G-CSF treated blood stem cell donors and persons with chronic neutropenia, myelodysplasia or cancer.

| Study Type   | Population  | No. of patients                                 | Median duration of follow-up | Evidence related to leukaemogenesis  |
|--|---|---|------------------------------|--|
| <b>Healthy Donors</b>  |   |   |                              |  |
| Case report (This study)   | Leukaemia in healthy donors after PBSC collection   | 2   | 6-7 years                    | Case reports of leukaemia  |
| Case report (Makita <i>et al</i> , 2004)                         | Leukaemia in a healthy donor after PBSC collection  | 1   | 1-2 years                    | Case report of leukaemia   |
| Retrospective survey (Cavallaro <i>et al</i> , 2000)             | Healthy PBSC or Granulocyte donors  | 101   | 3-6 years                    | None   |
| Prospective survey (Tassi <i>et al</i> , 2005)                   | Healthy PBSC donors   | 90  | 2-8 years                    | None   |
| Retrospective survey (Anderlini <i>et al</i> , 2002)             | Healthy PBSC donors   | 281   | 3-3 years                    | None   |
| Retrospective examination (Sakamaki <i>et al</i> , 1995)         | Bone marrow of healthy PBSC donors  | 3   | 5 years                      | None   |
| Retrospective study (Horowitz & Confer, 2005)                    | Haematopoietic stem cell donors   | 28 134  | Not stated                   | None   |
| Retrospective study (Pulsipher <i>et al</i> , 2006)              | Normal paediatric haematopoietic cell donors  | 60 000 sibling donors,<br>3000 unrelated donors | Not stated                   | None   |
| <b>Breast cancer patients</b>                                    |   |   |                              |  |
| Review (Smith <i>et al</i> , 2003)                               | NSABP experience in breast cancer studies   | 8563  | 7-4 years                    | 5-0-fold increase in AML/MDS compared with patients who did not receive G-SCF (1.01% vs. 0.2%)   |
| Review (Hershman <i>et al</i> , 2006)                            | Surveillance Epidemiology End Results-Medicare experience with older women with breast cancer             | 5515  | 4 years                      | 1.8-fold increase in AML/MDS risk compared with patients who did not receive G-CSF (1.8 vs. 0.7%).   |
| <b>Severe chronic neutropenia or Schwachman Diamond syndrome</b> |   |   |                              |  |
| Prospective review (Donadieu <i>et al</i> , 2005)                | Patients with severe congenital neutropenia followed by the French Severe Chronic Neutropenia Study Group | 101   | > 10 years                   | 11% cumulative incidence of MDS/AML/ALL at 20 years.   |
| Prospective review (Donadieu <i>et al</i> , 2005)                | Patients with Schwachman Diamond syndrome followed by the French Severe Chronic Neutropenia Study Group   | 55  | > 10 years                   | 19% cumulative incidence of MDS/AML/ALL at 20 years.   |
| Prospective review (Rosenberg <i>et al</i> , 2006)               | Patients with severe congenital neutropenia followed by the Chronic Neutropenia International Registry    | 374   | > 10 years                   | 21% cumulative incidence for MDS/AML after 10 years of G-CSF therapy and 36% after 12 years. Higher rates among patients who were less responsive to G-CSF |
| Prospective review (Rosenberg <i>et al</i> , 2006)               | Patients with Schwachman Diamond syndrome followed by the Chronic Neutropenia International Registry      | 29  | > 10 year                    | Two cases (1 month and 2.2 years after initiation of G-CSF). Incidence estimate could not be derived because of small sample size.                         |

Table I. Continued

| Study Type   | Population                                    | No. of patients | Median duration of follow-up | Evidence related to leukaemogenesis |
|--|---|-----------------|------------------------------|-------------------------------------|
| Myelodysplastic syndrome patients<br>Database analysis (Jadersten <i>et al</i> , 2005) | Three prospective Nordic<br>MDS group studies | 129             | 3-8 years                    | None                                |
| Prospective randomised phase II study (Hellstrom-Lindberg <i>et al</i> , 1998)         | MDS   | 71              | 3-7 years                    | None                                |
| Prospective study (Negrin <i>et al</i> , 1996)   | MDS   | 55              | 3 years                      | None                                |
| Prospective study (Mantovani <i>et al</i> , 2000)                                      | MDS   | 33              | 0-67 year                    | None                                |
| Prospective randomised study (Casadevall <i>et al</i> , 2004)                          | MDS   | 30 in G-CSF arm | 1 year                       | None                                |

PBSC, peripheral blood stem cells; NSABP, National Surgical Adjuvant Breast and Bowel Project; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; G-CSF, granulocyte colony-stimulating factor.

*et al*, 1995; Stone *et al*, 1995; Negrin *et al*, 1996; Heil *et al*, 1997; Hellstrom-Lindberg *et al*, 1998; Mantovani *et al*, 2000; Casadevall *et al*, 2004; Donadieu *et al*, 2005; Jadersten *et al*, 2005).

Registries that contain information on large numbers of healthy donors who receive G-CSF prior to stem cell mobilisation procedures have not identified an association with subsequent development of AML or MDS. A retrospective survey of the European Blood and Bone Marrow Transplant Registry of 28 134 normal donors harvested between 1990 and 2003 found that nine individuals developed a haematological malignancy after donation for an overall reported rate of 0.03%. Those receiving G-CSF for collection of peripheral blood stem cells had a similar reported rate of haematological cancers. (Pulsipher *et al*, 2006) The Combined International Bone Marrow Transplant Registry contains clinical information on more than 60 000 sibling-donors and 3000 unrelated National Marrow Donor Program donors who have received G-CSF and to date, there is no *in vivo* evidence for an increased incidence of leukemia or other haematological malignancies in the donors. If the median follow-up on these cases is 3-4 years, then there are about 240 000 patient years of available observation data. If the incidence of AML in the general population is five cases per 100 000 patient-years, then 12 cases should have occurred (Hasenclever & Sextro, 1996). However, registry experiences, while large, have limitations. The European Blood and Marrow Transplant Registry review was conducted in a voluntary survey fashion and some events could have been missed. During the survey years, clinical practice shifted from bone marrow to peripheral blood stem cells as the primary stem cell source. Therefore, the median follow-up of the peripheral blood stem cell cohort is shorter and the incidence of cancer in the groups could be different. The Combined International Blood and Marrow Transplant Registry experience is more reliable, as reporting centres are required to submit follow up blood test results. Data are not obtained by a central monitoring group in direct contact with donors and some events may have not been reported.

In contrast, four recent database studies with fairly comprehensive follow-up information on large numbers of patients have reported increased risks of AML/MDS development with G-CSF therapy. These databases included women with breast cancer and persons with non-malignant conditions known to be associated with increased risk of leukaemia. (Table I) Patients with severe congenital neutropenia evaluated in the Severe Chronic Neutropenia Study Group had a risk of 2.9% per year after 6 years and 8.0% per year after 12 years for progression to MDS/AML with G-CSF therapy. Among a subgroup of severe congenital neutropenia patients that was less responsive to G-CSF, 40% developed MDS or AML after 10 years, in comparison to an 11% rate after 10 years among the subgroup of patients that was more responsive to G-CSF. (Rosenberg *et al*, 2006) A second study of severe chronic neutropenia patients identified an 11% rate of AML/MDS at 20-year among the subgroups of patients with severe congen-

ital neutropenia and 19% for persons with Schwachman Diamond syndrome. This compared to a 1% rate among persons with cyclic neutropenia or glycogen storage disease type 1b. Of note, owing to their particular susceptibility to infections, patients with severe congenital neutropenia had the highest exposure to G-CSF and the risk of acute leukaemia increased with the degree of G-CSF exposure in this subgroup (Donadieu *et al*, 2005). The authors of both studies suggested that, if leukaemogenic effects of high cumulative doses of G-CSF are confirmed, then consideration should be given to extending indications for stem cell transplantation (currently recommended for G-CSF failure and leukaemic transformation) to include severe congenital neutropenia patients requiring high doses of G-CSF. The National Surgical Adjuvant Breast and Bowel Project identified a fivefold increase in incidence of AML/MDS among breast cancer patients who received more intense chemotherapy regimens with G-CSF support (1.01% vs. 0.21%). The analyses suggested that G-CSF administration was independently correlated with increased AML risk independent of cumulative dose of chemotherapy (Smith *et al*, 2003). Similarly, analyses of women with breast cancer in the Surveillance Epidemiology and End Results – Medicare database identified a twofold increase in incidence of AML/MDS at 4 years among women who received chemotherapy with G-CSF support (1.8% vs. 0.7%,  $P < 0.05$ ) (Hershman *et al*, 2006).

Our study has limitations. First, no *in vitro* or *in vivo* data support a causal relationship between administration of PEG-rHuMGDF and subsequent development of lymphoproliferative disease and without comprehensive pharmacovigilance efforts, it is not possible to derive incidence estimates for rates of haematological malignancies developing among healthy volunteers who received PEG-rHuMGDF. However, even the occurrence of B-cell lymphoproliferative disorders in three of 538 healthy volunteers represents a high incidence. The histopathology and cytogenetic findings for the three cases differed. Also, the first patient had a mildly low platelet count prior to enrolling in the PEG-rHuMGDF study, suggesting a possible underlying haematological disorder. Of interest, antibodies to thrombopoietin and thrombocytopenia persisted until the patient received immunochemotherapy for his non-Hodgkin lymphoma. Second, shared environmental exposures, heritable conditions and genetic susceptibility can lead to the development of AML in stem cell donors for siblings with leukaemia. For example, HLA-identical siblings of patients with AML appear to have a higher incidence of AML than the general population. (Bortin *et al*, 1987) The observation that the fourth case had more than one immediate family member with AML and also had a complex leukaemic karyotype suggests a genetic predisposition. There is one previous report of a healthy peripheral blood stem cell donor who developed AML 14 months after stem cell mobilisation with G-CSF prior to performing allogeneic HSCT for a brother with multiple myeloma (Makita *et al*, 2004). Leukaemia cells from the donor were positive for G-

CSF receptors without karyotypic abnormalities and responded *in vitro* to G-CSF.

Formal comprehensive long-term safety studies are needed to evaluate healthy individuals who receive haematopoietic colony stimulating factors. While the National Marrow Donor Program prospective long-term registry is the best source of safety information for G-CSF mobilised peripheral blood stem cell donors, more than 2000 normal donors will have to be followed for at least 10 years in order to detect a 10-fold increase in leukemia risk following G-CSF administration and detection of a smaller risk would require an even greater sample size (Hasenclever & Sextro, 1996). Recently, Pulsipher *et al* (2006) called for similar prospective tracking of adverse events occurring in paediatric donors in all studies involving administration of G-CSF to normal donors, noting that one quarter of matched sibling transplants for paediatric patients utilise G-CSF mobilised peripheral blood stem cells as the stem cell source. These prospective studies should include prospective evaluation of any patients who develop haematological malignancies to assess the status of growth factor receptor expression in their malignant cells and any responses or adverse outcomes attributable to potential administration of G-CSF during the course of their treatments. Longer and more complete follow-up of sibling donors would identify opportunities for additional laboratory study, such as instances where another member of the family has or develops a haematological malignancy. Similarly, as new thrombopoietic cytokines are administered to volunteers in an effort to improve yield from healthy platelet donors, comprehensive short-term and long-term pharmacovigilance efforts are advised (Jenkins *et al*, 2005).

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