**Title: Incidence of Hematologic and Non-Hematologic Malignancies, Thrombotic Events, and Autoimmune Disorders in Unrelated Normal Donors Undergoing Bone Marrow Harvest Versus Peripheral Blood Stem Cell Mobilization with Recombinant Human Granulocyte Colony-Stimulating Factor**

***Neupogen***

Amgen Protocol Number *20130209*

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**Protocol Synopsis (Amgen)**

**Study Title:** Incidence of Haematologic and Non-Haematologic Malignancies, Thrombotic Events, and Autoimmune Disorders in Unrelated Normal Donors Undergoing Bone Marrow Harvest Versus Peripheral Blood Stem Cell Mobilization with Recombinant Human Granulocyte Colony-Stimulating Factor

**Indication:** Peripheral Blood Stem Cell Mobilization among unrelated donors

**Study Background and Rationale:** Allogeneic hematopoietic cell transplantation (alloHCT) is a life-saving procedure for the treatment of a variety of diseases. However, not all patients in need of alloHCT have a suitable related donor. Haematopoietic stem cells are typically collected from unrelated donors in two ways: via unstimulated bone marrow (BM) harvest or by filgrastim-mobilized peripheral blood stem cell (PBSC) collection. Filgrastim-mobilized PBSC collection involves administering daily subcutaneous injections of filgrastim to the donor for approximately 5 days, followed by harvesting of PBSCs. The latter involves the use of a cell separator that collects a cell fraction rich in hematopoietic stem cells and returns other blood components back to the donor circulation. This apheresis procedure generally requires administration of anticoagulants including citrate, and intravenous volume expansion with a duration of approximately 4-6 hours for a median of 1 day.

The National Marrow Donor Program (NMDP) in the USA, through its research program, the Center for International Blood and Marrow Transplant Research (CIBMTR), collects data on outcomes of alloHCT donors and recipients. Both unstimulated BM and filgrastim-mobilized PBSC donors are contacted by the donor center two days post-donation and then weekly until the donor states he or she has recovered. Since 2006, all donors are then contacted annually for as long as possible, unless the donor formally withdraws from long term follow-up.

To date, there is no convincing evidence that filgrastim administration results in long term sequelae in normal, healthy donors. However, there are theoretical concerns that filgrastim could be associated with malignant, thrombotic, or autoimmune disorders. There is little data regarding these concerns in the normal, healthy population.

**Primary Objective:** To describe the long-term incidence of malignant myeloid haematologic disorders in donors who received and in those who did not receive filgrastim.

**Secondary Objectives:**

1) To describe the long-term incidence of malignant haematologic disorders in donors who received and in those who did not receive filgrastim
2) To describe the long-term incidence of non-haematologic malignant disorders in donors who received and in those who did not receive filgrastim
3) To describe the long-term incidence of thrombotic events in donors who received and in those who did not receive filgrastim
4) To describe the long-term incidence of autoimmune diseases in donors who received and in those who did not receive filgrastim

**Hypothesis/Hypotheses:** Estimate the incidence rates of malignant myeloid and non-myeloid haematologic disorders, non-haematologic malignant disorders, thrombotic events, and autoimmune diseases in donors who received and in those who did not receive filgrastim.

**Primary Endpoint or Outcome:** Malignant myeloid haematologic disorders

**Secondary Endpoints or Outcomes:** Malignant haematologic disorders, non-haematologic malignant disorders, thrombotic events (venous and arterial), and autoimmune disorders (multiple sclerosis [MS], systemic lupus erythematosus [SLE], rheumatoid arthritis [RA], and others)

**Study Design/Type:** The study is an observational cohort study, including unrelated normal donors who donated haematopoietic cells between July 1999 and study activation in October 2010, as well as prospectively enrolled unrelated normal donors who donated haematopoietic cells between 2010 and 2015.

**Study Population or Data Resource:** Study subjects are unrelated donors from the U.S. whose unstimulated BM or filgrastim-mobilized PBSC donation was facilitated by the NMDP between July 1, 1999 and approximately five years post study activation in

2010. (This also includes those donors who receive at least one injection of filgrastim during this timeframe but do not actually proceed to collection.)

**Summary of Subject Eligibility Criteria:**

*Inclusion criteria*:

- Unrelated donor who donated either unstimulated BM or filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation
- Unrelated donor who received at least one injection of filgrastim or more, but did not donate filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation
- Donation was managed by a U.S. NMDP donor centre
- Signed informed consent from the donor for participation in this long-term donor follow-up study
- Concurrent enrolment on other studies is permitted

*Exclusion criteria*:

- Unrelated donor who donated filgrastim-mobilized bone marrow
- Donation was managed by a non-U.S. donor centre
- Donor is unable to verbally communicate in any of the following languages: English, Spanish, Mandarin Chinese, Cantonese Chinese, Vietnamese, Korean or Portuguese

**Follow-up:** The first follow-up assessment for donors who donated prior to this study being implemented will occur either in the first or second year of the study near the anniversary date of their donation and biennially thereafter until approximately 10 years post study activation. Those donors who donate after the study is implemented will undergo their first assessment near the first anniversary of their donation and biennially thereafter until approximately 10 years post study activation. All donor follow-up assessments will be administered using a standardized telephone interview script. In the

event a donor reports a targeted malignant, thrombotic or autoimmune disorder, a designated staff member will seek consent from the donor for release of the donor's pertinent medical records. The study's medical monitor will review the medical records to verify the diagnosis.

**Sample Size:** It is anticipated that approximately 90% of the eligible donors projected to donate during the study accrual period will enrol in the study for a total enrolment of approximately 10,956 unstimulated BM and 21,172 filgrastim-mobilized PBSC donors. Assuming that the incidence rate of haematological malignancies in the normal population is 1 case per thousand person-years (based on one study), then this study should have approximately 80% power to detect a rate ratio of 1.25 associated with filgrastim use.

**Statistical Considerations:** Incidence rates of outcome events will be estimated among donors of unstimulated BM and donors of filgrastim-mobilized PBSC. An attempt will be made to adjust for potential confounders.

CIBMTR® CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

# Incidence of Hematologic and Non-Hematologic Malignancies, Thrombotic Events, and Autoimmune Disorders in Unrelated Normal Donors Undergoing Bone Marrow Harvest Versus Peripheral Blood Stem Cell Mobilization with Recombinant Human Granulocyte Colony-Stimulating Factor (rHuG-CSF)

**NMDP IRB-2009-0241**
Version 4.1

**Sponsored by**
National Marrow Donor Program (NMDP)
and
Center for International Blood and Marrow Transplant Research (CIBMTR)

[1] National Marrow Donor Program (NMDP)
[2] Center for International Blood and Marrow Transplant Research (CIBMTR)
(An Affiliation of the National Marrow Donor Program® (NMDP) and
the Medical College of Wisconsin's International Bone Marrow Transplant Registry and
Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR)



## PROTOCOL SYNOPSIS – NMDP IRB-2009-0241

**Incidence of Hematologic and Non-Hematologic Malignancies, Thrombotic Events, and Autoimmune Disorders in Unrelated Normal Donors Undergoing Bone Marrow Harvest Versus Peripheral Blood Stem Cell Mobilization with Recombinant Human Granulocyte Colony-Stimulating Factor (rHuG-CSF)**



## STUDY DESIGN

This is an observational study of unstimulated bone marrow (BM) and filgrastim-mobilized peripheral blood stem cell (PBSC) donors. The primary goal is to evaluate the hypothesis that the incidence of targeted malignant, thrombotic and autoimmune disorders after unrelated hematopoietic stem cell donation are similar between unstimulated BM and filgrastim-mobilized PBSC donors. Donors will undergo biennial surveys until study completion. Cases of targeted disorders will be reviewed by the medical monitors to confirm the veracity of the report.

The study population will be comprised of unrelated donors from the U.S. whose unstimulated BM or filgrastim-mobilized PBSC donation was facilitated by the National Marrow Donor Program (NMDP) between July 1, 1999 and approximately five years post study activation. (This also includes those donors who receive at least one injection of filgrastim during this timeframe but do not actually proceed to collection). It is anticipated that the study will enroll approximately 10,956 unstimulated BM donors and 21,172 filgrastim-mobilized PBSC donors.

## STUDY OBJECTIVES

**Primary Objective:**

- To describe the long-term incidence of malignant myeloid hematologic disorders in donors who received and in those who did not receive filgrastim.

**Secondary Objectives:**

- To describe the long-term incidence of malignant hematologic disorders in donors who received and in those who did not receive filgrastim.
- To describe the long-term incidence of non-hematologic malignant disorders in donors who received and in those who did not receive filgrastim.
- To describe the long-term incidence of thrombotic events in donors who received and in those who did not receive filgrastim.
- To describe the long-term incidence of autoimmune diseases in donors who received and in those who did not receive filgrastim.
- To assess the drop-out rate (lost to follow-up, withdrawal of consent) of long-term donor follow-up over time.

**Inclusion Criteria:**

1) Unrelated donor who donated either unstimulated BM or filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation.
2) Unrelated donor who received at least one injection of filgrastim or more, but did not donate filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation.
3) Donation was managed by a U.S. NMDP donor center.
4) Signed informed consent for participation in this long-term donor follow-up study.

Concurrent enrollment on other studies is permitted

**Exclusion Criteria:**

1) Unrelated donor who donated filgrastim-mobilized bone marrow.
2) Donation was managed by a non-U.S. donor center.
3) Donor is unable to verbally communicate in any of the following languages: English, Spanish, Mandarin Chinese, Cantonese Chinese, Vietnamese, Korean, or Portuguese.

**Accrual Objective:**

To enroll all unstimulated BM and filgrastim-mobilized PBSC donors whose collections were facilitated by a U.S. NMDP donor center and occurred between July 1, 1999 and approximately five years post study activation. (This also includes those donors who receive at least one

injection of filgrastim during this timeframe but do not actually proceed to collection). It is anticipated that approximately 90% of the eligible donors projected to donate during the study accrual period will enroll on the study for a total enrollment of approximately 10,956 unstimulated BM and 21,172 filgrastim-mobilized PBSC donors.

**Accrual Period:** Accrual to the study will commence from date of study initiation. Accrual is anticipated to be completed approximately five years post study activation.

**Study Duration:** The study duration will be approximately 10 years.

**Study Schema:** See Appendix B.

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## 1.0 BACKGROUND AND RATIONALE

Allogeneic hematopoietic cell transplantation (alloHCT) is a life-saving procedure for the treatment of a variety of devastating diseases. However, not all patients in need of alloHCT have a suitable related donor. The National Marrow Donor Program (NMDP) was founded in 1987 to facilitate alloHCT from anonymous unrelated donors. Hematopoietic stem cells are typically collected from unrelated donors in two ways: via unstimulated BM harvest or by filgrastim-mobilized peripheral blood stem cell (PBSC) collection. Since the recognition that filgrastim (Neupogen®, Amgen, Thousand Oaks, CA) is a highly effective PBSC mobilizing agent, the proportion of filgrastim-mobilized PBSC collections in normal donors has continually increased (Lipton 2003).

Filgrastim-mobilized PBSC collection involves administering subcutaneous injections of filgrastim to the donor for approximately 5 days. These injections are generally administered once daily and subsequently lead to mobilization of hematopoietic stem cells from marrow to the peripheral blood stream. The donor is then connected to a cell separator that collects a cell fraction rich in hematopoietic stem cells and returns other blood components back to the donor circulation. This apheresis procedure generally requires administration of anticoagulants including citrate, and IV volume expansion with a duration of approximately 4-6 hours for 1-2 days (median of 1 day).

The NMDP through its research program, the Center for International Blood and Marrow Transplant Research (CIBMTR), collects data on outcomes of alloHCT donors and recipients. For donors, the data collected to date have been primarily focused on short-term outcomes. However, in 2006, the NMDP was selected by the Health Resources and Services Administration (HRSA) to operate the Bone Marrow Coordinating Center–a key component of the C.W. Bill Young Transplantation Program. Included in this responsibility is conducting long-term follow-up on donors whose collections are facilitated by the NMDP.

Both unstimulated BM and filgrastim-mobilized PBSC donors are contacted by the donor center two days post-donation and then weekly until the donor states he or she has recovered. Currently, all donors are then contacted annually for as long as possible. Unless a donor formally withdraws from long-term follow-up, attempts to contact the donor each year continue even if there is a failed attempt the previous year. Therefore, approximately 89% of donors are never completely lost to follow-up.

The reported short-term adverse effects of filgrastim mobilized PBSC donation are generally thought to be minor and acceptable by donors, given the potential benefit to the recipient (Switzer et al. 2005). However, long-term adverse effects for the donor remain poorly characterized.

Filgrastim is currently FDA-approved for use in solid tumor and acute myeloid leukemia (AML) patients receiving chemotherapy, in patients undergoing BM transplant for non-myeloid conditions, for those with severe chronic neutropenia and for mobilization of PBSC for autologous hematopoietic cell transplantation (HCT). Though indispensable for the collection of peripheral blood hematopoietic progenitors for allogeneic HCT, filgrastim is not FDA-approved for use in normal, healthy donors. To date, there is no convincing evidence that filgrastim administration results in long term sequelae in normal, healthy donors. However, there are theoretical concerns that filgrastim could be associated with malignant, thrombotic, or autoimmune disorders. There is a paucity of data in the normal, healthy population because all studies to date have addressed patients receiving filgrastim for approved indications including malignancy or marrow failure syndrome.

Hematologic Malignancies

Concern derives from an observation that granulocyte colony stimulating factor (G-CSF) may cause epigenetic changes in lymphocytes (Nagler et al. 2004; Hernandez et al. 2005); however, *in vitro*, these effects are transient, self-limiting and have indeterminate clinical significance. These observations have not been widely confirmed, but if similar changes occur in myeloid hematopoietic progenitors, there could be an increased risk of hematological malignancies such as acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) or of alterations in immune tumor surveillance. The potential influence of filgrastim on future hematological events is not easily determinable, especially in related donors where there may be both a genetic and environmental predisposition to leukemia. Studies that have assessed myeloid malignancy risk among normal donors who received filgrastim have been limited because either the sample size is too small (Cavallaro et al. 2000; Anderlini et al. 2002; Grupp et al. 2006) or the length of follow-up is too short (Ehringer et al. 2004a).

Cytogenetic abnormalities, transformation to myelodysplastic syndrome (MDS), and AML have been reported in patients who were treated with long-term daily filgrastim for severe chronic neutropenia (Neupogen Package Insert, Amgen, Sept 2007). However, such cases were confined to patients with congenital neutropenia (Kostmann syndrome) and were not seen in patients with

autoimmune neutropenia, suggesting that the underlying disease was a factor in those cases. Moreover, in a placebo-controlled study of filgrastim in AML (n=521), rates of complete remission, time to progression, and disease-free and overall survival were no different between the two groups (Heil et al. 1997).

There are three cases of hematologic malignancies reported in the medical literature occurring in previously normal related donors (Makita et al. 2004; Bennett et al. 2006). However, it is known that siblings of acute leukemia patients face a 2-5 fold increased risk of leukemia themselves compared to the general population (Pottern et al. 1991; Rauscher et al. 2002; Shpilberg et al. 1994). For this reason, related donors have been excluded from this study to minimize the risk of a significant confounding effect of an inherited predisposition to leukemia.

To date, no cases of myeloid hematologic malignancies have been reported to the NMDP in unrelated donors.

Thrombosis

Thrombosis has not been reported with filgrastim but has been reported with red blood cell growth factors and is an adverse event of interest that will be monitored in this study.

Autoimmune Disorders

Exacerbation of underlying autoimmune disease has been reported following filgrastim administration for mobilization of PBSCs prior to autologous HCT and for treatment of neutropenia in patients with multiple sclerosis (MS) (Burt et al. 2001; Openshaw et al. 2000), systemic lupus erythematosus (SLE) (Euler et al. 1997; Vasiliu et al. 2006), rheumatoid arthritis (RA) (Snowden et al. 1998), and other immune-mediated vasculidities (Farhey et al. 1995; Iking-Konert et al. 2004). However, available literature is largely limited to small case series. Among the larger studies is a report by Burt and colleagues, who surveyed 24 transplant centers to determine the outcomes of hematopoietic stem cell collection methods on disease activity in patients with autoimmune diseases undergoing autologous HCT (Burt et al. 2001). Among 56 patients who received only filgrastim to mobilize PBSC, disease flare was seen in 5 patients (2 patients with MS and 3 patients with RA). In a prospective study investigating the tolerability of filgrastim for hematopoietic stem cell mobilization in patients with severe active RA, Snowden observed disease flare in 3 out of 10 patients (Snowden et al. 1998). Openshaw and coworkers have also reported flare of MS in 4 of 10 patients receiving filgrastim for hematopoietic stem cell mobilization prior to autologous HCT (Openshaw et al. 2000). Although experience with filgrastim

in patients with existing autoimmune disease is still limited, available literature suggests that a small proportion of patients can experience disease flare during its administration.

The incidence of new onset autoimmune disease among normal donors who receive filgrastim for the facilitation of PBSC collection has not been carefully assessed. One case of autoimmune hyperthyroidism was reported after donation of filgrastim-mobilized PBSC for an unrelated recipient (Kroschinsky et al. 2004). Two cases of vasculitis have been reported based on radiographic findings and clinical symptoms, but neither of the two cases could be confirmed pathologically. MS, RA, and SLE have not been reported. A review of collected donor data by the German donor center Deutsche Knockenmark-spenderdatei (DKMS) did not find an increase in autoimmune events among 3286 healthy donors who received filgrastim before PBSC harvest (Ehringer 2004b). To date, healthy normal donors do not appear to be at increased risk of developing autoimmune disorders following filgrastim-primed hematopoietic stem cell donation. However, large studies with long-term follow-up are needed to better characterize the incidence of autoimmune diseases in healthy donors receiving filgrastim.

**Rationale**

We hypothesize that the incidence of malignant, thrombotic and autoimmune disorders after unrelated hematopoietic stem cell donation is similar between unstimulated BM and filgrastim-mobilized PBSC donors. The disorders of interest in this study, malignant, thrombotic, and autoimmune, are relatively uncommon in the general population. Given the rarity of these disorders, a prospective cohort study with recruitment and longitudinal follow-up of a large cohort of unrelated donors is the most feasible study design to determine any effect of filgrastim on their occurrence.

## 2.0 STUDY DESIGN

This is an observational study of unstimulated BM and filgrastim-mobilized PBSC donors. The primary goal is to evaluate the hypothesis that the incidence of targeted disorders, consisting of malignant, thrombotic and autoimmune disorders, after unrelated hematopoietic stem cell donation are similar between unstimulated BM and filgrastim-mobilized PBSC donors. Donors will undergo biennial surveys until study completion. Cases of targeted disorders will be reviewed by the medical monitors to confirm the veracity of the report.

The study population will be comprised of unrelated donors from the U.S. whose unstimulated BM or filgrastim-mobilized PBSC donation was facilitated by the NMDP between July 1, 1999 and approximately five years post study activation. (This also includes those donors who receive at least one injection of filgrastim during this timeframe but do not actually proceed to collection). It is anticipated that the study will enroll approximately 10,956 unstimulated BM donors and 21,172 filgrastim-mobilized PBSC donors.

## 2.1 Hypothesis and Specific Objectives

2.1.1 Hypothesis

The incidence of malignant, thrombotic and autoimmune disorders after unrelated hematopoietic stem cell donation is similar between unstimulated BM and filgrastim-mobilized PBSC donors.

2.1.2 Study Objectives

2.1.2.1 Primary Objective

To describe the long-term incidence of malignant myeloid hematologic disorders in donors who received and in those who did not receive filgrastim.

2.1.2.2 Secondary Objectives

1) To describe the long-term incidence of malignant hematologic disorders in donors who received and in those who did not receive filgrastim.

2) To describe the long-term incidence of non-hematologic malignant disorders in donors who received and in those who did not receive filgrastim.

3) To describe the long-term incidence of thrombotic events in donors who received and in those who did not receive filgrastim.

4) To describe the long-term incidence of autoimmune diseases in donors who received and in those who did not receive filgrastim.

5) To assess the drop-out rate (lost to follow-up, withdrawal of consent) of long-term donor follow-up over time.

## 2.2. Inclusion Criteria

1) Unrelated donor who donated either unstimulated BM or filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation.
2) Unrelated donor who received at least one injection of filgrastim or more, but did not donate filgrastim-mobilized PBSC between July 1, 1999 and approximately five years post study activation.
3) Donation was managed by a U.S. NMDP donor center.
4) Signed informed consent from the donor for participation in this long-term donor follow-up study.
5) Concurrent enrollment on other studies is permitted (e.g. *A Phase III Randomized Multicenter Trial Comparing G-CSF Mobilized Peripheral Blood Stem Cell with Marrow Transplantation from HLA Compatible Unrelated Donors* (BMT CTN PvM Protocol 0201) and *A Multicenter Study of Hematopoietic Stem Cell Donor Safety and Quality of Life* (RDSafe).

### 2.3. Exclusion Criteria

1) Unrelated donor who donated filgrastim-mobilized bone marrow.
2) Donation was managed by a non-U.S. donor center.
3) Donor is unable to verbally communicate in any of the following languages: English, Spanish, Mandarin Chinese, Cantonese Chinese, Vietnamese, Korean or Portuguese.

### 2.4 Donor Safety Considerations

This is an observational study to evaluate the risk of targeted disorders among normal unrelated donors who were exposed to filgrastim for PBSC donation versus those that donated BM and were not exposed to filgrastim. All donors enrolling on this study will have been evaluated according to existing NMDP Standards and Policies and Procedures and determined to be medically suitable to donate the product they were requested to donate. As an observational study, no intervention or treatment will be provided.

### 2.5 Donor Enrollment

This study will enroll unrelated donors from U.S. NMDP donor centers who donated unstimulated BM or filgrastim-mobilized PBSC between July 1, 1999 and for approximately five years post study activation.

### 2.5.1 Donors Who Donated Prior to Study Implementation

Donors who donated either unstimulated BM or filgrastim-mobilized PBSC (as well as those donors who received at least one injection of filgrastim but did not actually proceed to collection) prior to the date this study is implemented will be sent a letter prior to the anniversary date of their donation. A few days prior to this letter being mailed, the donor will be sent a postcard notifying them an invitation letter will be arriving shortly. The mailing will include a letter describing the study in detail. The donor will be asked to sign and return the enclosed consent form to participate in the study or indicate that he/she is not interested in participating. A second mailing will be sent to those donors unresponsive to the first. Approximately fifty and ten percent response rates are anticipated for the first and second mailing waves respectively.

To minimize study bias, every reasonable effort will be made to reach all donors eligible to participate. Repeated attempts utilizing all alternate contact means available to the CIBMTR Survey Research Group (SRG) will be employed to attempt to reach those donors unresponsive to the first and second mailings and those for whom no current contact information is available. This includes use of email, public resources such as government records, internet-based resources such as Facebook and MySpace, and the techniques currently used by the NMDP's Donor Call-Back Unit to obtain updated or alternate addresses and phone numbers. If it is determined that a donor has died, all efforts will be made to verify cause of death, including use of public database sources such as the National Death Index.

### 2.5.2 Donors Who Donate After Study Implementation

Donors who donate either unstimulated BM or filgrastim-mobilized PBSC (as well as those donors who receive at least one injection of filgrastim but do not actually proceed to collection) after this study has been implemented will be invited to participate in this study during the information session they attend prior to donating hematopoietic stem cells.

### 2.6 Donor Follow-up Assessments

The first follow-up assessment for donors who donated prior to this study being implemented will occur either in the first or second year of the study near the anniversary date of their donation and biennially thereafter approximately 10 years post study activation. Those donors who donate after the study is implemented will undergo their first assessment near the first anniversary of their donation and biennially thereafter until approximately 10 years post study activation.

All donor follow-up assessments will be administered by trained staff members of the CIBMTR SRG and Donor Center staff using a standardized telephone interview script. Language Line services will be utilized for non-English speaking donors who are eligible for the study. Donors will be asked if they have developed any of the targeted malignant, thrombotic or autoimmune disorders since the last follow-up assessment.

Unless a donor formally withdraws from long-term follow-up, attempts to contact the donor biennially will continue even if there is a failed attempt at the previous time point. Based on donor follow-up data previously collected by the NMDP, we anticipate that even though some donors may be lost to follow-up for a specific time point, approximately 89% of donors will never be completely lost to follow-up.

### 2.7 Targeted Disorder Reporting

In the event a donor reports a targeted malignant, thrombotic or autoimmune disorder, a designated member of the SRG team will seek consent from the donor for release of the donor's pertinent medical records. Compliance with all applicable regulations for the release of medical records will be followed. The study's medical monitor will review the medical records to verify the diagnosis. Any medical records obtained will be kept in a locked file cabinet in a key card access file room within the CIBMTR. Key card access is limited to study personnel. The medical records will be destroyed five years after the study is completed.

## 3.0 STUDY ENDPOINTS

### 3.1. Primary Endpoint

Incidence rate of malignant myeloid hematologic disorders (AML, MDS, CML, chronic myeloproliferative disorders) in normal unrelated donors who underwent PBSC mobilization with filgrastim and in those who underwent BM harvest without filgrastim.

### 3.2 Secondary Endpoints

#### 3.2.1 Malignant Hematologic Disorders

Incidence rate of malignant hematologic disorders (AML, MDS, CML, chronic myeloproliferative disorders, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphomas) in normal unrelated donors who underwent PBSC mobilization with filgrastim and in those who underwent BM harvest without filgrastim.

#### 3.2.2 Non-Hematologic Malignancies

Incidence rate of non-hematologic malignancies, as defined in the Surveillance Epidemiology and End Results (SEER) database, in normal unrelated donors who underwent PBSC mobilization with filgrastim and in those who underwent BM harvest without filgrastim.

#### 3.2.3 Thrombotic Events

Incidence rate of thrombotic events (venous and arterial) in normal unrelated donors who underwent PBSC mobilization with filgrastim and in those who underwent BM harvest without filgrastim.

#### 3.2.4 Autoimmune Disease

Incidence rate of autoimmune diseases (rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, scleroderma, vasculidities, multiple sclerosis and ITP) in normal unrelated donors who underwent PBSC mobilization with filgrastim and in those who underwent BM harvest without filgrastim.

3.2.5 Lost to Follow-Up/Consent Withdrawal

Percentage of donors who are lost to follow-up; percentage of donors who withdraw consent prior to study conclusion; percentage of donors who are either lost to follow-up or withdraw consent prior to study conclusion.

## 4.0 STATISTICAL CONSIDERATIONS

### 4.1 Reduction of Selection Bias

The study is designed to address two potential mechanisms of selection bias. The first is caused by selecting donors who are genetically predisposed to develop the targeted diseases, especially malignancies. This will be avoided by excluding related donors, where the underlying disease of the recipient may also be manifest in the donor sibling at a later point in time. In addition, by describing the incidence of targeted events in BM donors not exposed to filgrastim, a point estimate of the incidence of targeted events independent of filgrastim exposure will be generated. We assume that donors exposed and not exposed to filgrastim originate from a pool of donors not significantly dissimilar in their genetic predisposition to development of targeted disorders.

Secondly, selection bias may potentially be present due to differences between the filgrastim mobilized and the unstimulated BM groups since this allocation is not purely random. The availability of PBSC allows donation from individuals who previously could not donate due to various contraindications prohibiting marrow harvest, currently approximately 4% of unrelated donors. Likewise, for medical reasons, approximately 1% of donors can provide BM and not PBSCs. Moreover, age and health status of filgrastim mobilized PBSC donors may be different than those of unstimulated BM harvest donors, although in practice, these differences may not be substantial (Vasu et al, 2008). However, internal and external experts consider that significant differences between the two groups are still a concern. Age is particularly important as malignancies are diseases of progressing age; therefore, the analyses will include age matching. Risk factors for malignancies (i.e., co-morbid conditions, environmental exposures, and family history) may not be equally distributed between study cohorts. To reduce the potential for selection bias, background information will be collected with a structured data collection form for normal donors at the time of donation, including demographic characteristics, and a focused medical history. In addition, a donor center effect will be investigated.

### 4.2 Accrual Objective

To enroll all unstimulated BM and filgrastim-mobilized PBSC donors whose collections were facilitated by a U.S. NMDP donor center and occurred between July 1, 1999 and approximately five years post study activation. (This also includes those donors who received at least one injection of filgrastim during this timeframe but did not actually proceed to collection). It is anticipated that approximately 90% of the eligible donors projected to donate during the study accrual period will enroll on the study for a total enrollment of approximately 10,956 unstimulated BM and 21,172 filgrastim-mobilized PBSC donors.

### 4.3 Study Duration

The study duration will be approximately 10 years.

### 4.4 Primary Objective

The primary objective is to describe the long-term incidence of malignant myeloid hematologic disorders in donors who received and in those who did not receive filgrastim.

### 4.5 Sample Size and Power Considerations

Since the adoption of filgrastim-mobilized PBSC collections by NMDP in 1999, the proportion of these collections in all normal donors has continually increased (see graph below). Counter to that trend, however, is a recent CIBMTR analysis suggesting that unstimulated BM leads to better outcomes in pediatric recipients (Eapen et al. 2004). From July 1, 1999 through September 30, 2008 there were 8,506 filgrastim-mobilized PBSC donors and 7,963 unstimulated BM donors in the U.S. Based on projected increases in the number of filgrastim-mobilized PBSC and unstimulated BM collections in the U.S., we expect to have a total of 22,700 filgrastim-mobilized PBSC and 12,000 unstimulated BM unrelated donors through June 30, 2014. With follow-up through approximately September 30, 2019, this will lead to a total of 226,832 person-years of follow-up in the filgrastim-mobilized PBSC group and 159,040 person-years of follow-up in the unstimulated BM group. The anticipated accrual is detailed in the table below.

| | Tx per year | | Person-Years of follow-up | |
|---|---|---|---|---|
| Fiscal Year (Oct-Sep) | PB | BM | PB | BM |
| 1999 (from July 1) | 10 | 314 | 201 | 6319 |
| 2000 | 200 | 1207 | 3900 | 23537 |
| 2001 | 383 | 1084 | 7086 | 20054 |
| 2002 | 607 | 1090 | 10623 | 19075 |
| 2003 | 869 | 884 | 14339 | 14586 |
| 2004 | 1038 | 803 | 16089 | 12447 |
| 2005 | 1138 | 661 | 16501 | 9585 |
| 2006 | 1310 | 653 | 17685 | 8816 |
| 2007 | 1343 | 621 | 16788 | 7763 |
| 2008 | 1608 | 646 | 18492 | 7429 |
| 2009 | 1809 | 672 | 18995 | 7056 |
| 2010 | 2063 | 674 | 19599 | 6403 |
| 2011 | 2327 | 684 | 19780 | 5814 |
| 2012 | 2608 | 702 | 19560 | 5265 |
| 2013 | 2924 | 725 | 19006 | 4713 |
| 2014 (thru June 30) | 3287 | 753 | 18079 | 4142 |
| Total | 23524 | 12173 | 236719 | 163001 |

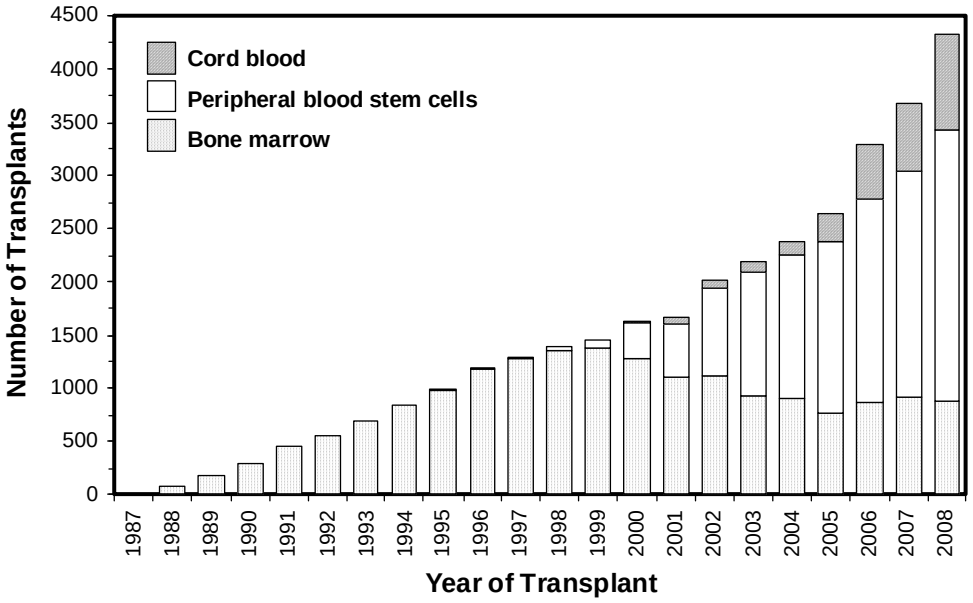


Figure 1. Graft sources for transplants facilitated by NMDP, by fiscal year 1987 through 2008. Dramatic growth in use of filgrastim-mobilized PBSC (open bars) has occurred since opening the PBSC IND protocol in 1999. In FY 2008, filgrastim-mobilized PBSC donations were 2550 compared to 882 donations of unstimulated BM.



Assuming that 90% of donors will enroll, we will enroll approximately 21,172 filgrastim-mobilized PBSC donors and 10,956 unstimulated BM donors. We assume that approximately 89% of enrolled donors will not be lost to follow-up. Assuming that we are able to capture half of the potential follow-up of the remaining enrolled donors results in an overall loss to follow-up of person-years of exposure of 15% from the initial values in the accrual table. This means we anticipate having approximately 201,211 person-years of exposure in the filgrastim-mobilized PBSC cohort and 138,551 person-years of exposure in the unstimulated BM group.

The SEER Cancer Statistics Review (2000 to 2005) calculates the incidence of a number of hematologic and non-hematologic malignancies by age in five-year time windows. Here we focus

on the primary endpoint of myeloid leukemia + MDS + myeloproliferative disorders, although we also consider power to detect effects on AML+MDS only, any type of leukemia or lymphoma, as well as melanoma. The expected incidence of acute or chronic myeloid leukemia, MDS, or myeloproliferative disease in this cohort based on the age distribution of donors is 3.5 per 100000 person-years. At the final analysis we will have 80% power to detect a relative risk of 3.7 for filgrastim mobilized PBSC donors compared to unstimulated BM donors, using a 5% two-sided significance level. This power calculation is based on Fisher's Exact test, with the n in each group equal to the person-years of exposure and p equal to the incidence rate, since the binomial distribution provides a good approximation to the Poisson distribution of the number of events in each group when the events are rare. The age-specific incidence distribution, overall incidence, and detectable relative risks for each outcome are detailed in the table below.

| Age | % of US donors | Incidence Myeloid leukemia +MDS+myeloproliferative Disease | AML/MDS only | Any leukemia or Lymphoma | Melanoma |
|---|---|---|---|---|---|
| 20-24 | 15% | 1.5 | 1.0 | 9.1 | 4.1 |
| 25-29 | 18% | 1.9 | 1.1 | 10.2 | 7.1 |
| 30-34 | 18% | 2.5 | 1.4 | 11.9 | 9.7 |
| 35-39 | 18% | 3.2 | 1.7 | 14.5 | 12.7 |
| 40-44 | 15% | 4.4 | 2.2 | 18.9 | 18.2 |
| 45-49 | 10% | 6.2 | 3.3 | 25.8 | 22.6 |
| 50-54 | 4% | 8.5 | 4.7 | 36.7 | 28.4 |
| 55-59 | 2% | 12.9 | 7.6 | 53.2 | 36.1 |
| Overall Incidence/100000 | | 3.5 | 1.9 | 15.9 | 12.7 |
| Detectable RR | | 3.7 | 5.1 | 2.0 | 2.2 |

### 4.6 Interim Analyses

Throughout the course of this study three interim assessments are planned.

**First interim analysis:. The first interim analysis will take place after completion of first-pass efforts to contact all donors with donation dates prior to the start of the study. Estimating that such contact will take about 18 months with 3 months for data cleaning and analysis, the first assessment will be completed in approximately mid-2011. Since the length of follow-up is relatively short, the result from this analysis will only serve as an interim assessment.** 2)

Second and third interim analyses: Two interim assessments (approximately 2014 and 2017) are planned for assessment of additional data collected from the prospective follow-up on all enrolled donors.

### 4.7 Final Analysis

The fourth and final assessment, to take place approximately at the end of study completion, will include all collected data. A detailed analysis will be conducted in this assessment (see Sections 4.8, 4.9).

### 4.8 Analysis of Primary Endpoint

A descriptive analysis will estimate the age-adjusted incidence rate of malignant myeloid hematologic disorders (AML, MDS, CML and chronic myeloproliferative disorders) separately for donors of filgrastim-mobilized PBSC vs. unstimulated BM. The relative risk will be estimated stratified on age of the donor, and the incidence rates will be compared using the Mantel-Haenszel test. Exact methods will be used if the number of events is small. If there are enough events, additional modeling of the incidence of each adverse event will be done using Poisson regression methods for grouped survival data, including age and other baseline demographic variables such as medical history, family history, and gender as possible covariates. Number of person-years at risk will be included as an offset term. Confidence intervals and hypothesis tests will be conducted using likelihood ratio-based methods. Results of interim analyses will be adjusted using group sequential methods.

### 4.9 Analysis of Secondary Endpoints

Descriptive analyses will estimate the age-adjusted incidence rate of hematologic and non-hematologic malignancies, thrombotic events and autoimmune diseases separately for donors of filgrastim-mobilized PBSC vs. unstimulated BM. The relative risk will be estimated stratified on age of the donor, and the incidence rates will be compared using the Mantel-Haenszel test. Exact methods will be used if the number of events is small. If there are enough events, additional modeling of the incidence of each adverse event will be done using Poisson regression methods for grouped survival data, including age and other baseline demographic variables such as

medical history, family history, and gender as possible covariates. Number of person-years at risk will be included as an offset term. Confidence intervals and hypothesis tests will be conducted using likelihood ratio-based methods. Results of interim analyses will be adjusted using group sequential methods.

Additionally, the percentage of donors lost to follow-up during the course of the study, the percentage withdrawing consent prior to study conclusion, and the percentage of donors either lost to follow-up or withdrawing consent prior to study conclusion will be calculated.

**Appendix A**

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**Appendix B**

## LONG-TERM DONOR FOLLOW-UP STUDY SCHEMA