Healthy donor hematopoietic stem cell mobilization with biosimilar granulocyte-colony-stimulating factor: safety, efficacy, and graft performance

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BACKGROUND: Biosimilar granulocyte-colony-stimulating factors (G-CSFs) have been available in the European Union since 2008, and Sandoz’ biosimilar filgrastim was approved in the United States in March 2015 for all of the reference product’s indications except acute radiation syndrome. Biosimilar G-CSFs have been largely embraced by the medical community, except for some reservations about healthy-donor stem cell mobilization, for which use outside of clinical studies was cautioned against by some members of the scientific community.

STUDY DESIGN AND METHODS: In a two-center safety surveillance study (National Clinical Trial NCT01766934), 245 healthy volunteer stem cell donors were enrolled. Of 244 donors who began mobilization with twice-daily Sandoz biosimilar filgrastim, 242 received a full (n = 241) or partial (n = 1) course of G-CSF and underwent apheresis. Efficacy and safety were assessed and are reported here.

RESULTS: Biosimilar filgrastim was accompanied by the typical G-CSF class-related adverse effects of expected frequency and severity. Median mobilization for CD34-positive stem cells was 97/μL (range, 20-347/μL); after one apheresis (91%) or two aphereses (9%) from all but three donors (1.2%), cell doses in excess of the typical 4×10^6 CD34-positive cells/kg of the recipient had been collected (range, 3-52×10^6/kg). Biochemical and hematologic alterations were consistent with previous reports; all had normalized by the first follow-up 1 month after mobilization. Stem cell products engrafted with typical probability and kinetics for G-CSF-mobilized stem cell products.

CONCLUSION: These data support the use of biosimilar filgrastim for healthy-donor stem cell mobilization as safe and effective.

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Biologics are copies of biologicals whose patent protection has expired. To account for the complexity of biologicals manufacturing, biosimilars are regulated differently from generics (copies of small-molecule drugs) through a specific regulatory process. To receive biosimilar status, evidence of physicochemical, pharmacological, and clinical similarity must be provided, including comparative trials for at least one

ABBREVIATIONS: G-CSF = granulocyte-colony-stimulating factor; GVHD = graft-versus-host disease.

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“sensitive” indication for which the reference product is licensed. Once regulators are convinced of the evidence provided, the marketing authorization will be issued for all licensed indications of the reference product (“extrapolation”). Proposed benefits of biosimilars include reduced health care expenditure and, at least in less affluent countries, improved access.\(^1\)\(^\text{–}^4\)

Compared with many biologicals, granulocyte-colony-stimulating factor (G-CSF) is a relatively simple molecule and thus, biosimilarity is easy to ascertain. Biochemical near-identity of this small, single-chain, nonglycosylated peptide can be demonstrated at very high resolution. G-CSF exerts all effects, desired or not, through canonical G-CSF receptor signaling, hence in vitro receptor on-off kinetics and studies of downstream kinase activities, all of which were shown to be highly similar between reference product and biosimilar G-CSF; provide precise measures of biological activity. Moreover, the concept of pharmacokinetics can be applied to G-CSF; and pharmacodynamic effects (in healthy individuals) are rapid, quantifiable, and methodologically simple to read out. For all biosimilar G-CSFs, high similarity with the reference product was demonstrated for pharmacokinetics and pharmacodynamic effects.\(^5\)\(^\text{–}^8\) Consequently, biosimilar G-CSFs have been largely embraced by the medical community, have assumed significant market penetration, and have improved access to G-CSF in some countries, including first-world countries like the United Kingdom.\(^3\)\(^,^9\)\(^,^10\) Reservations remain, however, about healthy volunteer donor mobilization with biosimilar G-CSF; and use outside of clinical studies was cautioned against by several professional groups, including the World Marrow Donor Association.\(^11\)\(^\text{–}^14\) Although the reasons why biosimilar G-CSF might be associated with a different safety profile than reference product G-CSF; which is considered adequately safe,\(^15\)\(^\text{–}^23\) were not spelled out, it is certainly true that maximal caution must be exercised when dealing with donors; and the frequently named advantages of biosimilars, that is, cost and access, are of little relevance in the context of stem cell transplantation. In this two-center, 10-year, noninterventional safety surveillance study, 244 healthy volunteer stem cell donors consented to 5 or 6 days of mobilization by twice-daily, self-administered injections of Sandoz’ biosimilar filgrastim, followed by stem cell apheresis. Acute safety and efficacy, as well as the in vivo performance of biosimilar G-CSF mobilized grafts, were assessed and are reported here.

**MATERIALS AND METHODS**

Study EP06-501 is a noninterventional study according to the German Medicinal Products Act that was approved by the ethics committees at both centers (approval no. 385/10), and was sent for notification to the Federal Institute for Drugs and Medicinal Devices. The study is registered as “Non-interventional, Long-term Safety Data Collection of Zarzio/Filgrastim HEXAL in Stem Cell Donors (SMART)” (clinicaltrials.gov identifier NCT01766934). Inclusion criteria were those defining eligibility for G-CSF mobilized, unrelated donor stem cell donation according to World Marrow Donor Association and German National Bone Marrow Donor Registry recommendations and applicable national laws and guidelines. For specifics on the process of donor evaluation and clearance, see Brauninger and colleagues.\(^24\) Donors provided written informed consent to participate in this study; nonconsenting donors were mobilized by the same protocol, donated stem cells using the same technology, and were offered participation in the identical regular donor follow-up provided by the German Stem Cell Donor Registry.

This two-center trial enrolled participants at German Red Cross Blood Service Baden-Württemberg-Hessen stem cell donor locales in Frankfurt (Center 1) and Ulm (Center 2). Mobilization was performed using the Sandoz biosimilar filgrastim (marketed as filgrastim Hexal in Germany, as Zarzio [filgrastim-sndz] in the United States, and as Zarzio in all other countries) at doses not exceeding 10 μg/kg·day (dosed to the nearest full prefilled syringe containing 30 or 48 million IU/300 or 480 μg of filgrastim) by self-injection in two divided subcutaneous doses, according to clinical standards.\(^20\)\(^\text{–}^29\) Donors from Center 1 applied the first injection on the morning of Day −4 and collected after the ninth injection, and donors from Center 2 started on the evening of Day −4 and consequently collected after 8 doses. Two to 4 hours after the eighth (or ninth) dose, apheresis was initiated on Day zero via peripheral venous access using conventional apheresis equipment (Spectra Optia MNC; Terumo BCT, Lakewood, CO) as reported.\(^30\)\(^\text{–}^32\) On the basis of the concentration of circulating cells that were CD34-positive (CD34\(^+\)), empirical algorithms (H.B., unpublished results) were used to predict the process volume required to collect the requested dose of CD34\(^+\) cells with 99.5% probability, and aphereses were terminated either when the required volume had been processed or after a maximal apheresis duration of 300 minutes. If, after the first apheresis, the commonly accepted standard dose of 4 × 10\(^6\) CD34\(^+\) cells/kg of the recipient had not been collected, then two additional doses of G-CSF were dispensed, and a second apheresis was performed on Day +1. No more than two apheresis and no more than 11 doses of G-CSF were routinely given (two donors received 12 doses; see below) according to standard operating procedures of the apheresis centers, backed by the Hemotherapy Guidelines of the German Medical Association,\(^33\) irrespective of the collected total stem cell dose.

Before each apheresis, bone pain, which was the most frequent adverse effect of G-CSF; was assessed using a visual analog scale from 0 to 10 to quantify the average and maximal pain, and a mannequin was used to document the affected site(s). A laboratory panel (see below)
was analyzed at the time of donor assessment; before apheresis; and at 1, 6, and 12 months after apheresis and will be analyzed annually thereafter for a total of 10 years. At the same time points, open questionnaires were used to document adverse events (AEs); these were mailed, but telephone follow-up was allowed to clarify comments if needed. Follow-up instructions were mailed at the indicated times; donors who did not reply were actively contacted by telephone. The donor’s wish to withdraw from active follow-up ended participation in this study. The definition of a severe AE (SAE) followed the Good Clinical Practice definition. Relatedness/unrelatedness of an AE to G-CSF treatment was determined by physicians from the participating donor centers based on whether similar AEs had previously been observed or could theoretically be compatible with the mechanism of action of G-CSF.

Mobilization efficiency (CD34⁺ cells/µL blood) and CD34⁺ cell dose in the apheresis product were determined using single-platform flow cytometry in accordance with the European Directorate for the Quality of Medicines and Health Care, as reported. Standard automated laboratory equipment in accredited laboratories was used for the assessment of all other laboratory parameters. Engraftment data were derived from Day +100 reports from the transplant centers as part of ongoing product quality review. Despite being obligated to Day-100 reporting according to the Joint Accreditation Committee of International Society for Cellular Therapy and European Society for Blood and Marrow Transplantation, reports were obtained for only 144 of 241 transplants provided. Of note, these engraftment data were not collected within this safety surveillance study and were not audited by the sponsor; the data were extracted from the German Stem Cell Donor Registry recipient outcome database.

Statistics
Donor characteristics at study entry were descriptively summarized using frequencies and percentages for categorical variables and mean, standard deviation, median, minimum, and maximum values for continuous variables. For categorical comparisons, the Wilcoxon or Fisher exact test was used, as appropriate. The following laboratory parameters were analyzed with plots at screening, mobilization, and safety follow-up visits: alkaline phosphatase (AP), aspartate aminotransferase/serum glutamic-oxaloacetic transaminase, basophils, eosinophils, γ-glutamyl transferase, hemoglobin, hematocrit, lactate dehydrogenase (LDH), lymphocytes, monocytes, neutrophils, platelets, red blood cells (RBCs), uric acid, and white blood cells (WBCs). In addition, at the mobilization visit, the linear relationship between the laboratory parameters and CD34⁺ cells in blood, as well as between demographic data and CD34⁺ cells in blood, were assessed with Pearson correlation.

Using a data-driven approach with data from the screening and apheresis visits, classification tree modeling was attempted with recursive partitioning to identify decision rules for predicting mobilizing status. Donor mobilization status was defined according to the clinical observation that, historically, one apheresis had sufficed in all donors who mobilized in excess of 60/µL CD34⁺ cells, irrespective of donor-recipient weight ratio, to extract an optimal dose of CD34⁺ cells for the recipient. Thus, for the purpose of these (failed) analyses, a donor with CD34⁺ cells in ≥60/µL blood directly before apheresis was classified as a good mobilizer.

AEs were summarized using System Organ Class and Preferred Term according to The Medical Dictionary for Regulatory Activities, with numbers and percentages of donors who reported AEs that occurred before mobilization, during the mobilization period (the time between the first G-CSF injection and 30 days thereafter), and after the mobilization period (>30 days after the first filgrastim administration). Statistical analyses were performed using SAS for Windows Statistical Analysis Software, version 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS
Between June 2011 and August 2014, 245 donors (194 in Center 1 and 51 in Center 2) consented to participate in this study, of which 244 received at least one dose of the Sandoz biosimilar filgrastim (population for safety results). All donors were Caucasian, 25.4% were women, the median age was 34 years (range, 19-60 years), and the median body mass index was 26 kg/m² (range, 18.5-40 kg/m²). Two donors discontinued mobilization after receiving one or seven doses of G-CSF, respectively, because of recipient-related issues that rendered transplantation impossible, and no apheresis was performed. Two hundred forty-one donors completed the course of mobilization as per self-reported outcome (population for efficacy results), confirmed consumption of the designated number of filgrastim syringes, and characteristic laboratory alterations. One donor received only six of nine planned G-CSF injections because of needle-induced syncope and subsequent dose reduction but went on to apheresis. Despite identical rules for daily dosing (see Materials and Methods), donors in Center 1 received slightly smaller individual doses of G-CSF (nonsignificant) than in Center 2, so that, despite the nine versus eight doses administered in Center 1 versus 2, respectively, the cumulative G-CSF dose received before the first apheresis was no different (Table 1). Of the 242 donors who thus proceeded to apheresis collection, 91% underwent one apheresis, and 9% underwent two aphereses. The CD34⁺ yield was analyzed for the 241 donors who received a full course of G-CSF. Follow-up was attempted for all 244...
donors, and 212 donors (87%) completed their first safety follow-up visit 30 days after mobilization.

Safety observations during the mobilization period

According to the protocol definition, the “mobilization period” started on the first day of G-CSF administration and continued until 30 days thereafter. All 244 donors who received any G-CSF experienced at least one AE; and, in 241 of those donors (98.8%), at least one of the AEs was considered to be related to G-CSF by the investigators. Most G-CSF-related AEs emerged during the mobilization phase; 11 donors experienced G-CSF-related AEs, which occurred postmobilization during the follow-up period. Table 2 summarizes all AEs.

As expected for a G-CSF product, the most frequent drug-related AE was bone pain, which was reported by 93.9% of the donors. Average bone pain was scored (median) as 3/10 (range, 0-9), and the median maximum severity was scored as 6/10 (range, 1-10) on a visual analog scale. Paracetamol (acetaminophen), to be taken as needed, was dispensed for analgesia. Seventy percent of donors took at least one medication during mobilization for bone pain (69% took paracetaol); in 83% of those donors, this medication adequately controlled the pain. Pain was most frequently reported in the spine and pelvis (70-80% of donors) or the head/skull (55% of donors) (Fig. S1, available as supporting information in the online version of this paper).

During the mobilization period, four donors reported six events classified as SAEs. Four events in three donors that were considered to be related to G-CSF—presyncope, chest pain, and chest pain with dyspnea—were classified as SAEs solely because the donors were admitted to hospital for overnight surveillance (uneventful) or for the exclusion of myocardial infarction or pulmonary embolus (both ruled out). The three donors recovered from SAEs without any sequelae. Two other SAEs that were not suspected to be related to G-CSF were proctalgia (n = 1) and an anal abscess that required in-patient surgical drainage (n = 1) in the same donor.

Some blood values were changed as expected, in keeping with the known

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**TABLE 1. Granulocyte-colony-stimulating factor dosing by donor center**

| Variable | Center 1, N = 192 | Center 2, N = 49 | Total, N = 241* |
|----------|------------------|-----------------|-----------------|
| Average G-CSF dose per injection, μg/kg | Mean ± SD | Minimum-Maximum | Median | 25th-75th percentile |
| | | | | |
| | 4.35 ± 0.33 | 3.17-5.26 | 4.35 | 4.05-4.58 |
| Mean ± SD | 4.71 ± 0.46 | 3.25-5.42 | 4.70 | 4.41-5.13 |
| Minimum-Maximum | 3.17-5.42 | 4.38 | 4.13-4.63 |
| Total G-CSF dose before first apheresis, μg/kg | Mean ± SD | Minimum-Maximum | Median | 25th-75th percentile |
| | | | | |
| | 39.17 ± 2.96 | 28.50-47.37 | 39.13 | 36.49-41.20 |
| Mean ± SD | 37.68 ± 3.71 | 26.00-43.33 | 37.59 | 35.31-41.05 |
| Minimum-Maximum | 38.89 ± 3.16 | 26.00-47.37 | 38.86 | 36.38-41.20 |

*Only the 241 donors who received a full course of granulocyte-colony-stimulating factor (G-CSF) were considered for this analysis. SD = standard deviation.

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**TABLE 2. Adverse events during mobilization and follow-up periods**

| Type of adverse event (AE) | Mobilization period, N = 244* | Follow-up period, N = 221† | Total, N = 244 |
|---------------------------|-----------------------------|---------------------------|----------------|
| Any AE | Any serious AE (SAE) | 243 (99.6) [97.7-100.0] | 241 (98.8) [96.4-99.7] | 44 (1.6) [0.4-4.1] | 11 (5.0) [2.5-8.7] | 244 (100.0) [98.5-100.0] |
| AEs related to G-CSF | 3 (1.2) [0.3-3.6] | 2 (0.9) [0.1-3.2] | 5 (2.0) [0.7-4.7] |
| AEs related to apheresis | 1 (0.4) [0.0-2.3] | Not applicable | 1 (0.4) [0.0-2.3] |
| Deaths | 0 (0.0) [0.0-1.5] | 0 (0.0) [0.0-1.7] | 0 (0.0) [0.0-1.5] |

*Adverse events (AEs) that started between the first administration of granulocyte-colony-stimulating factor (G-CSF) and 30 days thereafter were regarded as events that occurred “during” the mobilization period.
†These were AEs that started >30 days after the first G-CSF application until data cutoff (December 31, 2015). CI = confidence interval; SAEs = severe adverse events.
mechanism of action of G-CSF. Thus, LDH (presumably from disintegrating neutrophils) and AP (presumably from bone, but isoenzymes were not discriminated), were transiently elevated above the upper limit of normal in most donors. Aspartate aminotransferase, c-glutamyl transferase, and uric acid levels also were higher before apheresis than during screening (p < 0.0001) but remained within normal limits in the vast majority of donors (Fig. 1). On average, total WBC concentrations at apheresis were elevated eightfold over baseline to 47.2 × 10⁹/L (range, 6.1-131.7 × 10⁹/L); all WBC lineages contributed (p < 0.0001), lymphocytes with an elevation to less than threefold and neutrophils by approximately tenfold (Fig. 1). A systematic, statistically significant (p < 0.0001) but quantitatively minor and clinically insignificant reduction was observed for platelet count and hemoglobin concentration on the apheresis day (Fig. 1); platelet and RBC indices were unchanged (not shown).

None of the AEs that were connected with elevated blood values persisted at the time of the first follow-up 1 month after the apheresis. The observed pattern and intensity of drug-related AEs were in line with the expected side effects of G-CSF treatment. ¹⁸,²⁰,²²,³⁵,³⁹

**Efficacy of mobilization**

Mobilization of a median of 97/µL (range, 20-347/µL) CD34+ cells was achieved. Mobilization was better in Center 1 (after nine doses) compared with Center 2 (after eight doses) at 100/µL (range, 27-347/µL) versus 74/µL (range, 20-262/µL), respectively, and the difference reached statistical significance (p = 0.0049; Wilcoxon test).

After one (91%) or two (9%) aphereses, a median dose of 7.9 × 10⁶ (range, 3.52 × 10⁶) CD34+ cells/kg/recipient was collected (Center 1: median, 8.6 × 10⁶; range, 4.52; Center 2: median, 6.9 × 10⁶; range, 3.17-10⁶). A second apheresis was performed for 8.3% donors in Center 1 and for 12.2% in Center 2 (Fisher exact test for difference: not significant).

Three of 242 donors (1.2%) failed to achieve the typical per-kg dose of 4 × 10⁶ CD34+ cells. Those three donors had achieved relatively lower mobilization, and the recipients of two also had a considerably higher body weight than the donors, yielding grafts of 3.0 × 10⁹/kg (donor with 23 CD34+ cells/µL; two aphereses), 3.7 × 10⁹/kg, or 3.9 × 10⁹/kg (donors with 44-45 CD34+ cells/µL, each undergoing only one apheresis) of the recipient, respectively, with appropriate yield for the mobilization efficiency. In the latter two donors, the decision to forgo a second apheresis was taken in agreement with the transplant center. Mobilization data and harvested cell doses are depicted in Table 3.

All collected apheresis products were sent to the collaborating transplant centers for immediate transplantation. Confirmation of graft transfusion was received for 144 transplants; some parameters of graft function were also reported by most of them. Thus, for 130 of 133 patients (98%), transplant centers confirmed primary engraftment, with median neutrophil engraftment (neutrophils >500 × 10⁹/L on 2 consecutive days) on Day 17 (range, 7-43 days), median RBC engraftment (freedom from RBC transfusion requirements) on Day 20 (range, 1-65 days), and median platelet engraftment on Day 14 (range, 1-46 days). Eighty-two percent of the recipients were alive on Day +100, and 56% had experienced any degree of graft-versus-host disease (GVHD). Data about conditioning regimens, GVHD prophylaxis, or the severity of GVHD were not available.

**Correlation of demographic data and laboratory values with mobilization efficacy**

Laboratory values from screening and preceding apheresis (mobilized blood) as well as demographic data were tested for linear correlation (Pearson) with mobilization efficiency (CD34+ cells/µL), with the aim of “predicting” good and poor mobilizers. None of the laboratory parameters collected at screening, although several of those collected immediately before apheresis, were positively correlated with CD34+ cells/µL (total WBC and all WBC lineages individually, as well as LDH, p < 0.0001; AP, p = 0.004; RBC, p = 0.035). Similarly, girth (BMI; p = 0.0019) and age (p = 0.035) were correlated positively and negatively, respectively, with mobilization efficiency. Mobilization efficiency was independent of sex (Wilcoxon p value 0.24).

**Preliminary data on extended safety**

Although this is a 10-year safety follow-up study, at the time of data cutoff for this publication (i.e., December 31, 2015), the mean follow-up period was 433.3 days (range, 2-1528 days). Laboratory values had normalized at the first follow-up. Multiple SAEs were reported during follow-up, each once, including umbilical hernia, cholecystitis, road traffic accident with multiple fractures of the upper extremity, pregnancy, and nephrolithiasis, all of which were deemed unrelated to G-CSF.

No hematological or solid malignancies have been observed to date. One case of benign breast neoplasm and one benign thyroid neoplasm, which completely resolved after surgical removal of the cold nodule, were reported. In one donor, sarcoidosis was diagnosed but did not require medical treatment.

AEs that were regarded as related to apheresis were observed in 77 donors (31.6%). These events were usually mild or moderate and mainly were associated with asymptomatic hypokalemia (21.3% of donors). In one case, asymptomatic hypocalcemia was reported.

The SAEs reported in this study were infrequent (15 donors; 6.1%) and resolved or resolved with sequelae in
Fig. 1. Laboratory parameters at the time of donor evaluation (screening), after granulocyte-colony-stimulating factor (G-CSF) treatment just before the first apheresis and during follow-up (FU) (1 mo, 6–8 mo, 1 y, etc.). For white blood cell (WBC) counts, gray shading indicates the different leukocyte species; the height of each bar indicates the mean value, and whiskers denote the standard error of the mean WBC count. For all other laboratory values, box plots indicate the median (center line) and the 25th to 75th percentile (boxes), and whiskers extend from lowest to highest datum still within 1.5 interquartile range of the lower/upper quartile. Outliers are marked with dots, and diamonds indicate mean values. All laboratory values were statistically significantly different from baseline/screening only in the blood sample immediately before apheresis, as indicated by asterisks (p < 0.0001; paired t test, corrected for multiple testing) but not at any of the follow-up times. ASAT/SGOT = aspartate aminotransferase/serum glutamic-oxaloacetic transaminase; GGT = γ-glutamyl transferase; LDH = lactate dehydrogenase.
most cases; two SAEs in two patients were reported as ongoing at the time of data cutoff (sarcoidosis and tendon rupture, respectively). Apart from the patient with sarcoidosis (relationship unclear), all possibly or definitely drug-related SAEs, reported in five donors (2.0%) overall, had completely resolved.

AEs associated with G-CSF are rarely dose-limiting; an AE requiring premature discontinuation of the mobilization occurred in only one donor who, because of serious “presyncope,” discontinued G-CSF. The donor recovered completely and underwent successful apheresis as planned without any complications.

**DISCUSSION**

The European Medicines Agency and, more recently, the US Food and Drug Administration reviewed and approved Sandoz’ biosimilar filgrastim based on the totality of the evidence indicating biosimilarity. Sandoz performed the current noninterventional postapproval study, the results of which are in alignment with the European and US regulators’ verdict of biosimilarity. The biosimilar status implies that no novel insight about the active compound should be gained in such a study, and indeed this is the prevailing picture. In total, 242 healthy volunteer donors were mobilized with biosimilar G-CSF according to routine schedule and underwent stem cell apheresis. With respect to demographics, they were representative of a German stem cell donor population—exclusively Caucasian, predominantly in the third and fourth decade of life, overwhelmingly male, and slightly overweight.19,20 The reported acute AEs were entirely in keeping with reported data for the reference product in type, incidence, and severity.19,20,22,35,39 If none of the reported more severe acute AEs of filgrastim were observed here (activation of autoimmune disease, pulmonary hemorrhage, acute respiratory distress-like syndrome, splenic rupture, etc.), then this presumably is attributable both to very careful donor selection24 and to the relatively small cohort size. Laboratory values were influenced by G-CSF in the expected fashion, both qualitatively and quantitative-ly,19,20 but changes were transient in that, at the first follow-up, all values had returned to premobilization levels, including WBCs. The efficacy of mobilization of CD34+ cells and WBCs was similar to that reported for a cohort treated with the reference product at the same dose and dosing schedule.20 Better mobilization in this study than reported in several other trials is likely attributable to higher doses of the mobilizing agent19 and/or use of a split-dose as opposed to once-daily administration.25,27,28 Our data demonstrate a remarkably large, statistically significant difference of 25% in mobilization efficiency between donors who received eight (Center 2) versus nine (Center 1) doses of G-CSF. This difference is probably clinically meaningful, because the probability of requiring a second apheresis to meet a dose was increased by one-half (although the increase was not statistically signif-icant). The actual individual per-kg dose dispensed in

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**TABLE 3. Stem cell mobilization and harvest**

| Variable | Donors with one apheresis, N = 219 | Donors with two aphereses, N = 22 | All donors, N = 241* |
|----------|-----------------------------------|-----------------------------------|---------------------|
| CD34+ cell concentration/μL in blood before first apheresis | Mean ± SD | Median (range) | Mean ± SD | Median (range) | Mean ± SD | Median (range) |
| CD34+ cell apheresis total yield after first apheresis, ×10⁶ | | | | | | |
| Mean ± SD | 684 ± 289 | 46 ± 18 | 104 ± 56 |
| Median (range) | 100 (28-347) | 44 (20-84) | 97 (20-347) |
| CD34+ cells/kg recipient BW after first apheresis, ×10⁶/kg | | | | | | |
| Mean ± SD | 9.5 ± 5.0 | 3.6 ± 0.7 | 8.9 ± 5.1 |
| Median (range) | 8.6 (3.7-51.9) | 3.6 (1.7-4.9) | 7.9 (1.7-51.9) |
| CD34+ cells/kg donor BW after first apheresis, ×10⁶/kg | | | | | | |
| Mean ± SD | 8.1 ± 3.3 | 4.1 ± 1.2 | 7.7 ± 3.4 |
| Median (range) | 7.4 (2.8-21.3) | 4.1 (2.4-7.2) | 7.2 (2.4-21.3) |
| CD34+ cell apheresis yield; cumulative, ×10⁶ | | | | | | |
| Mean ± SD | 684 ± 289 | 501 ± 151 | 667 ± 284 |
| Median (range) | 643 (245-1656) | 478 (258-765) | 619 (245-1656) |
| CD34+ cells/kg recipient BW cumulative, ×10⁶/kg | | | | | | |
| Mean ± SD | 9.5 ± 5.0 | 5.6 ± 1.1 | 9.1 ± 4.9 |
| Median (range) | 8.6 (3.7-51.9) | 5.7 (3.7-4.9) | 7.9 (3.7-51.9) |
| CD34+ cells/kg donor BW cumulative, ×10⁶/kg | | | | | | |
| Mean ± SD | 8.1 ± 3.3 | 6.4 ± 1.8 | 7.9 ± 3.3 |
| Median (range) | 7.4 (2.8-21.3) | 6.1 (3.7-10.1) | 7.3 (2.8-21.3) |

*Because recipient weight was independent from any donor variable, per-kg CD34-positive (CD34+1) cell doses also were calculated relative to donor body weight (BW) to facilitate estimation of the quality of stem cell collection.

*Only those donors who received a full course of G-CSF are presented in this table.

SD = standard deviation; BW = body weight.
Center 2, if anything, was higher than in Center 1, and the total amount of G-CSF received per kg was almost the same despite the receipt of one less injection (Table 1) (the differences were not statistically significant). Thus, the markedly better mobilization in Center 1 was not because of differences in absolute G-CSF dosing but, instead, seems to indicate that, 84 hours after the first dose, peak mobilization is not yet reached. At least quarterly laboratory comparability exercises exclude systematic differences in stem cell enumeration as possible confounders. Conclusions of two recent studies exploring mobilization and apheresis or mobilization only after 4 versus 5 days of G-CSF administration confirm a very relevant increase in circulating CD34+ cells between Days 4 and 5, in agreement with our current observation.15,40 Engraftment probability and velocity reproduce data in published reports.31,42 Postapheresis follow-up, although it currently does not extend beyond 4 years and is available only for a fraction of the cohort, was nonyielding, in agreement with expectations raised by the aggregate favorable impression of G-CSF safety in donors.18,19,23

The vastly divergent mobilization response in donors often is remarked upon20,29,43,44 and was also apparent in this cohort. On occasion, insufficient mobilization results in the generation of smaller than desirable stem cell transplants. Therefore, it would be useful to be able to predict mobilization efficiency on the basis of data that are available during donor assessment, that is, under homeostatic conditions. Moreover, correlations between clinical data and mobilization might suggest leads about the currently elusive genetics underlying mobilization efficiency. Therefore, we used various statistical tools to try to derive predictive algorithms from the data available at donor assessment and immediately before apheresis (i.e., under the influence of G-CSF). Alas, significant correlative relations between the number of mobilized CD34+ cells/μL and baseline donor variables other than the very modest effects of girth and age were not apparent in our study. Only after G-CSF treatment (immediately before apheresis) were correlative relations observed with all mature leukocyte subsets and for LDH. Too late to be truly predictive anyway, the strength of the correlation was insufficient to provide a meaningful (i.e., sufficiently narrow) prediction of circulating CDC34+ cells. Previous exercises to the same end by other groups have been equally disappointing.20,29,43-46 In agreement with published reports, body mass and age are positively or negatively correlated with mobilization efficiency, respectively; whereas, in contrast to some reports, but in agreement with our previous work, men and women mobilize equally well.19,20,44,45

Although it is broadly accepted by hematologists and oncologists for prescription to patients, the use of biosimilar G-CSF in healthy donors has been approached cautiously.11-14 Vague statements about the “potential risks” of biosimilar G-CSF (presumably risks that are different, more severe, or more frequent than those with the reference product G-CSFs) have been made, although available clinical data have not suggested a differential risk profile. Specifically with respect to stem cell mobilization, the acute and long-term safety of G-CSF is constantly re-assessed. Statements supporting a sufficiently good safety profile for healthy donor mobilization to remain ethically acceptable were renewed just recently.18-20,22,23 A frequently mentioned risk of biologicals is neutralizing antibody induction. Although it is not human-analog, filgrastim, on which all short-acting, biosimilar G-CSFs are modeled, has never been found to induce antifunctional antibodies, nor do the compound or the treatment schedule used for mobilization possess any of the risk factors associated with such antibodies.67 Because all effects of G-CSF are mediated through interactions with its only receptor and canonical downstream signaling, evidence of near-identical receptor on-off-kinetics and down-stream signaling cascades5 should alleviate fears of unexpected or excessive nonimmunological AEs. Therefore, the reasons for the guarded approach of some professional organizations to biosimilar G-CSF in volunteer donors currently remain unclear.

It is noteworthy that the indication “stem cell mobilization in healthy volunteers” was not obtained by extrapolation; instead, this very pharmacological effect (mobilization of CD34+ cells) was measured for all biosimilar G-CSFs, albeit in rather small cohorts within Phase I studies, to establish similarity in pharmacokinetics/pharmacodynamic effects for the registration of these products.5-7 Hence, preliminary evidence for efficacy in donor mobilization was already available before the start of this postapproval study.

This is the largest donor cohort reported to date that was mobilized with biosimilar G-CSF (Sandoz’ filgrastim) in the framework of a formal, long-term safety surveillance study. Both the safety profile and the efficacy of stem cell mobilization and graft function were demonstrated. Long-term safety will be monitored for 10 years.

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CONFLICT OF INTEREST

AS, SG, and NK are employees, and PS is a former employee, of Hexal AG, sponsor of the study. The clinical sites receive money from the sponsor to offset costs of study-related activities. HB serves on the speakers’ bureau and as a scientific adviser to Hexal AG for questions pertaining to biosimilar G-CSF. None of the other authors have any declarations to make with respect to these studies.

REFERENCES

1. Bonig H, Becker PS, Schwebig A, et al. Biosimilar granulocyte-colony-stimulating factor for healthy donor stem cell mobilization: need we be afraid? Transfusion 2015; 55:430-9.
2. Schulz M, Bonig H. Update on biosimilars of granulocyte colony-stimulating factor—when no news is good news. Curr Opin Hematol 2016;23:61-6.
3. Sun D, Andayani TM, Altyar A, et al. Potential cost savings from chemotherapy-induced febrile neutropenia with biosimilar filgrastim and expanded access to targeted antineoplastic treatment across the European Union G5 countries: a simulation study. Clin Ther 2015;37:842-57.
4. Welte K. G-CSF: filgrastim, lenograstim and biosimilars. Expert Opin Biol Ther 2014;14:983-93.
5. Gascon P, Fuhr U, Sorgel F, et al. Development of a new G-CSF product based on biosimilarity assessment. Ann Oncol 2010;21:1419-29.
6. Lubena H, Sveikata A, Gumbrevicius G, et al. Bioequivalence of two recombinant granulocyte colony-stimulating factor products after subcutaneous injection in healthy volunteers. Int J Clin Pharmacol Ther 2009;47:275-82.
7. Lubena H, Bias P, Maly AK, et al. Pharmacokinetic and pharmacodynamic profile of new biosimilar filgrastim XM02 equivalent to marketed filgrastim Neupogen: single-blind, randomized, crossover trial. BioDrugs 2009;23:43-51.
8. Soergel F, Lerch H, Lauber T. Physicochemical and biologic comparability of a biosimilar granulocyte colony-stimulating factor with its reference product. BioDrugs 2010;24:347-57.
9. Gascón P, Tesch H, Verpoort K, et al. Clinical experience with Zarzio® in Europe: what have we learned? Support Care Cancer 2013;21:2925-32.
10. Bocquet F, Paubel P, Fusier I, et al. Biosimilar granulocyte colony-stimulating factor uptakes in the EU-5 markets: a descriptive analysis. Appl Health Econ Health Policy 2014;12:315-26.
11. Japan Society for Hematopoietic Cell Transplantation. Position statement of the Japan Society for Hematopoietic Cell Transplantation regarding the use of biosimilar granulocyte-colony stimulating factors for the mobilization of hematopoietic stem cells in healthy donors. 2013 April [cited 2016 Jun 30]. Available from: http://www.jsht.com/english.
12. Barosi G, Bosi A, Abbraccchio MP, et al. Key concepts and critical issues on epoetin and filgrastim biosimilars. A position paper from the Italian Society of Hematology, Italian Society of Experimental Hematology, and Italian Group for Bone Marrow Transplantation. Haematologica 2011;96:937-42.
13. Shaw BE, Confer DL, Hwang WY, et al. Concerns about the use of biosimilar granulocyte colony-stimulating factors for the mobilization of stem cells in normal donors: position of the World Marrow Donor Association. Haematologica 2011;96:942.
14. Gastl G, Geissler D, Geissler K, et al. ASHO position paper on biosimilars. Mag Eur Med Oncol 2009;4:232-3.
15. Anderlini P, Korbling M, Dale D, et al. Allogeneic blood stem cell transplantation: considerations for donors. Blood 1997;90:903-8.
16. Anderlini P, Przepiorka D, Korbling M, et al. Blood stem cell procurement: donor safety issues. Bone Marrow Transplant 1998;2153:535-9.
17. Anderlini P, Rizzo JD, Nugent ML, et al. Peripheral blood stem cell donation: an analysis from the International Bone Marrow Transplant Registry (IBMTR) and European Group for Blood and Marrow Transplant (EBMT) databases. Bone Marrow Transplant 2001;27:689-92.
18. Anderlini P, Chan FA, Champlin RE, et al. Long-term follow-up of normal peripheral blood progenitor cell donors treated with filgrastim: no evidence of increased risk of leukemia development. Bone Marrow Transplant 2002;30:661-3.
19. Holig K, Kramer M, Kroschinsky F, et al. Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single-center experience in 3928 donors. Blood 2009;114:3757-63.
20. Mueller MM, Bialleck H, Bomke B, et al. Safety and efficacy of healthy volunteer stem cell mobilization with filgrastim G-CSF and mobilized stem cell apheresis: results of a prospective longitudinal 5-year follow-up study. Vox Sang 2013;104:46-54.
21. Pulsipher MA, Chitpakdithai P, Miller JP, et al. Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the National Marrow Donor Program. Blood 2009;113:3604-11.
22. Pulsipher MA, Chitpakdithai P, Logan BR, et al. Acute toxicities of unrelated bone marrow versus peripheral blood stem cell donation: results of a prospective trial from the National Marrow Donor Program. Blood 2013;121:197-206.
23. Shaw BE, Confer DL, Hwang W, et al. A review of the genetic and long-term effects of G-CSF injections in healthy donors: a reassuring lack of evidence for the development of haematological malignancies. Bone Marrow Transplant 2015;50:334-40.
24. Brauning S, Thurausch K, Luxembourg B, et al. Deferrals of volunteer stem cell donors referred for evaluation for matched-unrelated stem cell donation. Bone Marrow Transplant 2014;49:1419-25.
25. Arbona C, Prosper E, Benet I, et al. Comparison between once a day vs twice a day G-CSF for mobilization of peripheral blood progenitor cells (PBPC) in normal donors for allogeneic PBPC transplantation. Bone Marrow Transplant 1998;22:39-45.
26. Ings SJ, Balsa C, Leverett D, et al. Peripheral blood stem cell yield in 400 normal donors mobilised with granulocyte colony-
stimulating factor (G-CSF): impact of age, sex, donor weight and type of G-CSF used. Br J Haematol 2006;134:517-25.

27. Kroger N, Renges H, Kruger W, et al. A randomized comparison of once versus twice daily recombinant human granulocyte colony-stimulating factor (filgrastim) for stem cell mobilization in healthy donors for allogeneic transplantation. Br J Haematol 2000;111:761-5.

28. Kröger N, Sonnenberg S, Cortes-Dericks L, et al. Kinetics of G-CSF and CD34+ cell mobilization after once or twice daily stimulation with rHu granulocyte-stimulating factor (lenograstim) in healthy volunteers: an intraindividual crossover study. Transfusion 2004;44:104-10.

29. Schulz M, Karpova D, Spohn G, et al. Variant rs1801157 in the 3'UTR of SDF-1ss does not explain variability of healthy-donor G-CSF responsiveness. PLoS One 2015;10:e0121859.

30. Brauninger S, Bialleck H, Thorausch K, et al. Mobilized allogeneic peripheral stem/progenitor cell apheresis with Spectra Optia v.5.0, a novel, automatic interface-controlled apheresis system: results from the first feasibility trial. Vox Sang 2011;101:237-46.

31. Brauninger S, Bialleck H, Thorausch K, et al. Allogeneic donor peripheral blood "stem cell" apheresis: prospective comparison of two apheresis systems. Transfusion 2012;52:1137-45.

32. Flommersfeld S, Sohlibach K, Jaques G, et al. Collection of peripheral blood progenitor cells on Day 4 is feasible and effective while reducing granulocyte-colony-stimulating factor exposure to healthy donors. Transfusion 2015;55:1269-74.

33. National Medical Council. [Guidelines for collection of blood and blood components and for the application of blood products (hemotherapy)]. Köln (Germany): Deutscher Ärztverlag: 2008.

34. Daubers K, Becker D, Odendahl M, et al. Enumeration of viable CD34+ cells by flow cytometry in blood, bone marrow and cord blood: results of a study of the novel BD stem cell enumeration kit. Cytotherapy 2011;13:449-58.

35. Anderlini P, Przepiorka D, Seong C, et al. Clinical toxicity and laboratory effects of granulocyte-colony-stimulating factor (filgrastim) mobilization and blood stem cell apheresis from normal donors, and analysis of charges for the procedures. Transfusion 1996;36:590-5.

36. Anderlini P, Lauppe J, Przepiorka D, et al. Peripheral blood stem cell apheresis in normal donors: feasibility and yield of second collections. Br J Haematol 1997;96:415-7.

37. Bonig H, Papayannopoulou T. Mobilization of hematopoietic stem/progenitor cells: general principles and molecular mechanisms. Methods Mol Biol 2012;904:1-14.

38. Bonig H, Papayannopoulou T. Hematopoietic stem cell mobilization: updated conceptual renditions. Leukemia 2013;27:24-31.

39. Amgen, Inc. Neupogen UK summary of product characteristics. 2015 [cited 2016 Mar 18]; Available from: https://www.medicines.org.uk/emc/medicine/23292.

40. Winkler IG, Wirzinska E, Barbier V, et al. Mobilization of hematopoietic stem cells with highest self-renewal by G-CSF precedes clonogenic cell mobilization peak. Exp Hematol 2016;44:303-14.

41. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med 2012;367:1487-96.

42. Ringden O, Labopin M, Beelen DW, et al. Bone marrow or peripheral blood stem cell transplantation from unrelated donors in adult patients with acute myeloid leukaemia, an Acute Leukaemia Working Party analysis in 2262 patients. J Intern Med 2012;272:472-83.

43. Hsu JW, Wingard JR, Logan BR, et al. Race and ethnicity influences collection of granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells from unrelated donors, a Center for International Blood and Marrow Transplant Research analysis. Biol Blood Marrow Transplant 2015;21:165-71.

44. Lenk J, Bornhauser M, Kramer M, et al. Sex and body mass index but not CXCL12 801 G/A polymorphism determine the efficacy of hematopoietic cell mobilization: a study in healthy volunteer donors. Biol Blood Marrow Transplant 2013;19:1517-21.

45. Anderlini P, Przepiorka D, Seong C, et al. Factors affecting mobilization of CD34+ cells in normal donors treated with filgrastim. Transfusion 2015;55:2855-63.

46. Schellekens H, Casadevall N. Immunogenicity of recombinant human proteins: causes and consequences. J Neurol 2004;251:114-9.

47. An additional Supporting Information may be found in the online version of this article at the publisher’s website:

**Fig. S1.** Anatomical sites affected by G-CSF induced bone pain and relative frequency. Darker shades of red indicate increasingly frequent mentioning of pain localization.