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A pilot study evaluating the safety and CD34+ cell mobilizing activity of escalating doses of plerixafor in healthy volunteers

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Summary

This study evaluated the safety and CD34+ cell mobilizing activity of escalating doses of plerixafor in healthy volunteers. Three cohorts of six subjects received two different doses of plerixafor separated by at least 2 weeks to allow for adequate pharmacodynamic wash-out. The following dosing cohorts were evaluated: 0.24 and 0.32 mg/kg (Cohort 1); 0.32 and 0.40 mg/kg (Cohort 2); and 0.40 and 0.48 mg/kg (Cohort 3). Circulating CD34+ cells were measured 0, 2, 4, 6, 8, 10, 12, 14, 18 and 24 h after each dose. Blood colony-forming units were measured at baseline and 6 h after each dose. Common adverse events were diarrhoea, injection site erythema, perioral numbness, sinus tachycardia, headache, nausea, abdominal distention and injection site pain. No dose limiting toxicities occurred. When higher doses of plerixafor were administered, there was a trend towards higher peak CD34+ counts and CD34+ area under the curves, although these differences did not achieve statistical significance, perhaps due to intra-subject variability. Together, these data show that the higher doses of plerixafor evaluated in this study are reasonably safe and suggest that a larger study should be performed to definitively answer whether increased numbers of CD34+ cell are mobilized with higher doses of plerixafor.

Keywords

stem cell mobilization/homing; plerixafor; CD34; BMT; clinical research

Peripheral-blood progenitor cells (PBPC) are the preferred source of haematopoietic stem cells (HSCs) for autologous and allogeneic transplantation because PBPCs engraft faster than bone marrow-derived progenitor cells and are easier to collect (Larochelle *et al*, 2006). Granulocyte-colony stimulating factor (G-CSF) successfully mobilizes PBPCs in most healthy donors. However, prior chemotherapy treatment sometimes leads to inadequate autologous PBPC mobilization in patients with haematological malignancies (Visani *et al*, 1999; Devine *et al*, 2004).

On December 15, 2008, plerixafor (Mozobil® , formerly known as AMD3100) was granted US Food and Drug Administration (FDA) approval as a HSC mobilizing drug, in

combination with G-CSF for collection and subsequent autologous transplantation in patients with non-Hodgkin lymphoma and multiple myeloma. Plerixafor inhibits the binding of stromal cell-derived factor-1 (SDF-1) to its cognate receptor CXCR4 resulting in the mobilization of CD34⁺ HSCs into the peripheral circulation (Aiuti *et al*, 1997; Liles *et al*, 2003; Flomenberg *et al*, 2005). Plerixafor is currently investigational for PBPC mobilization in the allogeneic setting. One report described successful mobilization in 20 of 25 donors with sustained engraftment of allogeneic PBPCs in all 20 patients who received plerixafor-mobilized PBPCs (Devine *et al*, 2008). However, the long term outcomes of patients receiving plerixafor-mobilized allografts are not known and there may be immunological differences between G-CSF- and plerixafor-mobilized grafts (Smith *et al*, 2007). Additionally, the optimal dose of plerixafor for PBPC mobilization in healthy allogeneic stem cell donors remains unknown. In the study by Devine *et al* (2008), the plerixafor-mobilized allo-grafts contained a median 2.9×10^6 CD34⁺ cells/kg recipient weight. However, 33% of 24 donors failed to mobilize the required 2.0×10^6 CD34⁺ cells/kg recipient weight necessary for transplantation following one dose of plerixafor and one apheresis procedure (Devine *et al*, 2008). Additionally, the CD34⁺ doses were lower than the 4.0×10^6 CD34⁺ cells/kg shown to be associated with improved survival in a retrospective study of G-CSF mobilized allogeneic transplants (Singh *et al*, 2007).

Healthy volunteers tolerated single doses up to 0.32 mg/kg of plerixafor without experiencing severe adverse events (AEs) (Liles *et al*, 2003; Lack *et al*, 2005). Following subcutaneous (s.c.) plerixafor injection, white blood cell (WBC) counts peaked at 6–10 h and returned to baseline levels by approximately 24 h (Liles *et al*, 2003). Additionally, plerixafor has a short half-life (Liles *et al*, 2003) and plerixafor administration on 3 consecutive days resulted in a consistent, reversible increase in peripheral blood CD34⁺ cells (Hubel *et al*, 2004), indicating a rapid pharmacodynamic wash-out effect. Because of the small number of subjects in the original dose escalation studies and the inter-subject variability associated with CD34⁺ cell mobilization, additional investigations are warranted to determine the optimal mobilizing dose of plerixafor. In a non-human primate model, rhesus macaques received a single dose of up to 1 mg/kg of plerixafor, which was well tolerated and mobilized CD34⁺ cell numbers comparable to G-CSF (Laroche *et al*, 2006). This three-cohort, dose-escalation, pilot study was designed to determine the safety and CD34⁺ cell mobilizing activity of up to 0.48 mg/kg plerixafor in healthy volunteers.

Methods

All subjects provided informed consent and received investigational study drug on a National Heart, Lung and Blood Institute Institutional Review Board (IRB)-approved research protocol designed to study the safety and CD34⁺ cell mobilizing activity of escalating doses of s.c. plerixafor. The enrollment period was from June 14, 2006 until May 7, 2008. Volunteers 18 and 50 years of age who had normal renal, hepatic and haematological function were eligible. Exclusion criteria included cerebrovascular disease, cardiac disease, a Framingham 10-year coronary risk of >10%, positive human immunodeficiency virus status, a history of hepatitis B or C, a history of cancer in the past 5 years, or a history of autoimmune disease.

This three-cohort, dose-escalation, pilot study investigated doses of plerixafor up to 0.48 mg/kg in healthy volunteers and was designed so that each subject received two different doses of plerixafor. The two dose per-subject design allowed for the exploration of dose-response relationships for both safety and activity that minimized inter-subject variability because each subject served as their own control. The protocol mandated that further enrollment be prohibited (pending IRB and Data and Safety Monitoring Board review) for any occurrence of a Grade 4 treatment-related AE or any Grade 3 AE known to be related to

plerixafor (for example, Grade 3 thrombocytopenia, gastrointestinal symptoms and neuropathy).

Healthy volunteers (Fig 1) received the following s.c. doses of plerixafor: 0.24 and 0.32 mg/kg (Cohort 1); 0.32 and 0.40 mg/kg (Cohort 2); and 0.40 and 0.48 mg/kg (Cohort 3). Subjects received the higher dose at least 14 days after the first to allow adequate wash-out of plerixafor. The absolute number of circulating CD34+ cells was measured by standard flow cytometry techniques after each dose of plerixafor at the following times: 0, 2, 4, 6, 8, 10, 12, 14, 18 and 24 h. The samples were evaluated for CD34+ cell content using the International Society of Hematotherapy and Graft Engineering guidelines (Sutherland *et al*, 1996). Fluorochromes used were CD45 APC-Cy7 and CD34 APC (BD Biosciences, San Jose, CA, USA), using the BD FACSCanto instrument (BD Biosciences). Analysis was performed using the FACSDiva software (BD Biosciences).

Adverse reactions were recorded, graded and summarized using National Cancer Institute Common Terminology Criteria for Adverse Events, version 3 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae_v3.pdf). Each cohort consisted of a planned enrollment of six subjects. If a subject did not complete both doses of plerixafor and did not experience DLT, that subject was replaced. Dose escalation was allowed if protocol-specified stopping rules for toxicity were not met. In addition to inpatient monitoring during the first 24 h after each injection, subjects returned for an assessment 1 week after each dose. Complete blood counts and serum chemistry panels were obtained on all subjects after each dose of plerixafor and during each follow-up visit at 1 week. Subjects in the 0.40 and 0.48 mg/kg dose cohorts were monitored by telemetry for 24 h following each dose. Additionally, following a protocol amendment, subjects in Cohort 3 underwent electrocardiography (EKG) monitoring with QTc measurements at baseline, 8, 12 and 24 h after each dose.

To assess colony forming units (CFU), peripheral blood mononuclear cells were inoculated at 1×10^5 cells/ml in three different methylcellulose culture medias (MethoCult H4230; Stem Cell Technologies, Vancouver, Canada), supplemented with 5 u/ml of recombinant human (rHu) erythropoietin (Epo; Amgen, Thousand Oaks, CA, USA), 10 ng/ml rHu granulocyte-macrophage colony stimulating factor (GM-CSF; Sandoz, East Hanover, NJ, USA), 10 ng/ml rHu interleukin-3 and 100 ng/ml rHu stem cell factor (SCF; R&D Systems, Minneapolis, MN, USA). Plated cells were incubated at 37°C with 5% CO₂ for 10–14 days. Colonies were then counted with pre-plerixafor dosing colonies compared to post-plerixafor dosing colonies.

Blood samples for pharmacokinetic (PK) analyses were collected for six subjects in Cohort 2 who received a dose of 0.40 mg/kg and six subjects in Cohort 3 who received a dose of 0.48 mg/kg. Samples for PK analysis were collected prior to dosing and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h post-dose. Plasma plerixafor concentrations were determined using a validated liquid chromatography-mass spectrometry/mass spectrometry method (Eruofins AvTech, Kalamazoo, MI, USA). Pharmacokinetic parameters [performed by the Clinical Pharmacology Department at Genzyme Corporation using WinNonlin Professional, version 5.2 (Pharsight Corp., Mount View, CA, USA)] were determined from non-compartmental methods using nominal times of blood collection. Pre-dose concentrations below the lower limit of quantitation were set to zero for the purposes of the analysis.

The study was primarily designed to determine the safety of escalating doses of plerixafor. The safety analysis included data from all subjects. An exploratory analysis of CD34+ cell mobilization was conducted to evaluate whether preliminary data suggested higher doses of plerixafor increased CD34+ cell mobilization as a rationale for a potentially larger study.

The analyses of CD34+ cell mobilization included data from subjects who received two successive doses of plerixafor. Summary statistics were analysed for demographics, peak CD34+ mobilization, intra-subject differences in CD34+ cell mobilization, CD34+ cell AUC and intra-subject differences in CD34+ cell AUC. For the primary analyses of CD34+ cell mobilization, peak CD34+ cell numbers and CD34+ cell area under the curve (AUC) values for 24 h following plerixafor injection (beginning at 2 h) were calculated for each subject at each dose level. The Kruskal–Wallis test statistic was used to compare the peak CD34+ cell numbers and AUCs at the three-first-dose levels and the three-second-dose levels. The Kruskal–Wallis test was used to compare the differences in peak CD34+ cell counts and AUC at the higher and lower dose levels in the successive cohorts. Linear regression was used to model peak CD34+ cell AUC at the second dose level as a function of peak CD34+ cell AUC at the first dose level, dose, age and gender.

Results

Subject characteristics

A total of 21 subjects participated in the study and 18 subjects received both doses of plerixafor to complete all three study cohorts. One subject was withdrawn prior to the first dose due to premature ventricular contractions found on a baseline EKG. Two subjects in Cohort 3 who received plerixafor at the 0.40 mg/kg dose withdrew prior to the second dose of study drug. One experienced Grade 1 decreased haemoglobin and one patient withdrew due to personal choice after experiencing Grade 2 events including a vasovagal episode, headache and nausea. Baseline demographic characteristics for the 18 subjects who received both doses of plerixafor are shown in Table I.

Safety

The per-subject incidence rate of AEs was analysed by dose level. No subject experienced an AE that was Grade 3 in severity. Commonly reported AEs included diarrhoea, injection site erythema, perioral numbness, bloating sensation, fatigue and headache. Most AEs resolved prior to discharge (i.e. lasted <24 h). Sinus tachycardia (all Grade 1) was frequently observed in subjects monitored by telemetry at the 0.40 and 0.48 mg/kg dosing cohorts. Grade 2 abdominal distention/bloating was reported by four subjects. However, any 'symptomatic' bloating is considered Grade 2 by CTCAE version 3; all cases observed during this study were considered mild and transient. The following additional Grade 2 AEs were reported: diarrhoea ($n = 2$ (one subject experienced Grade 2 diarrhoea at 0.32 and 0.40 mg/kg)); depression ($n = 1$, 0.40 mg/kg); allergic reaction ($n = 1$, 0.40 mg/kg); vasovagal episode ($n = 1$, 0.40 mg/kg); vomiting ($n = 1$, 0.40 mg/kg); headache ($n = 1$, 0.40 mg/kg); and fatigue ($n = 1$, 0.40 mg/kg). One Grade 2 event of an elevated creatinine kinase elevation was considered unlikely to be related to plerixafor because the measurement occurred after a subject performed strenuous weight lifting and resolved with the next laboratory measurement. All Grade 2 AEs resolved without incident.

Table II shows the per-subject incidence of AEs that were considered at least possibly related to plerixafor. AEs with a clear alternative explanation were considered to be unlikely related or unrelated to plerixafor. The overall numbers of subjects per cohort were limited; however, the per-subject incidence of AEs commonly known to occur following the administration of plerixafor were similar in each cohort, including diarrhoea, bloating, cramping, injection site erythema/induration, headache and perioral numbness. Some AEs were more common in the higher dose cohorts including vomiting, dyspepsia, dizziness and dyspnea. Because telemetry was not performed in the lower (0.24 and 0.32 mg/kg) dose cohorts, a direct dose comparison cannot be made for cardiac events. Grade 2 events were

more common in the 0.40 mg/kg cohort, with all but one non-bloating event [diarrhoea ($n = 1$ at 0.32 mg/kg)] occurring at the 0.40 mg/kg dose level.

QTc assessment

No subject was diagnosed with Grade 2 QTc prolongation during conduction of the study following any dose of plerixafor. Only one subject experienced a post-dose QTc interval of >450 ms (466 ms at 12 h); however, this subject had a baseline QTc interval of 457 ms. Changes in QTc intervals from the pre-dose QTc intervals were also assessed. One subject in Cohort 2 developed a QTc prolongation of >30 ms at 12 h following 0.32 mg/kg dose of plerixafor, although the absolute QTc interval remained under 450 ms.

Pharmacokinetic data

Pharmacokinetic data were obtained from six subjects at the 0.40 mg/kg dose of plerixafor and six subjects at the 0.48 mg/kg dose of plerixafor. Peak plasma concentrations of plerixafor occurred approximately 30–60 min after each dose. Mean apparent volume of distribution was 377 ml/kg [standard deviation (SD) 39] at the 0.40 mg/kg dose and 419 ml/kg (SD 66) at the 0.48 mg/kg dose. Mean half-life was similar at both doses: 5.1 h (SD 1.0) at 0.40 mg/kg and 5.5 h (SD 0.6) at 0.48 mg/kg. These values were similar to the half-lives reported in previous studies evaluating the 0.24 mg/kg dose (Hubel *et al*, 2004; Stewart *et al*, 2009). Values of $C_{\max}$ and AUC determined for the 0.40 and 0.48 mg/kg doses were both proportionally higher than those observed at plerixafor doses of 0.24 or 0.32 mg/kg in previous studies (Hubel *et al*, 2004). Figure 2 shows the mean plerixafor plasma concentrations over time observed following the 0.40 and 0.48 mg/kg doses. Mean $C_{\max}$ was 1368 ng/ml (SD 169) at 0.40 mg/kg and 1687 ng/ml (SD 151) at 0.48 mg/kg. Mean $AUC_{0-\infty}$ was 7849 h ng/ml (SD 1568) at the 0.40 mg/kg dose and 9224 h ng/ml (SD 1231) at the 0.48 mg/kg dose.

CD34+ cell mobilization

Summary statistics for CD34+ mobilization including the peak CD34+ cell number and the CD34+ cell AUC over 24 h are shown in Table III. The differences in CD34+ cell counts between dosing cohorts were not significant ($P > 0.2$) although the CD34+ counts (both CD34+ cell peak and AUC) appeared to be increased with the higher dose (second dose) compared to the lower dose (first dose) in all three cohorts. The peak CD34+ cell number was achieved in most subjects with the higher plerixafor dose although 5 out of 18 subjects experienced a higher CD34+ count following the lower dose and two additional subjects experienced no changes in peak CD34+ counts following the higher dose (Fig 3).

Table IV shows the mean and median intra-subject differences in peak CD34+ cell counts and CD34+ cell AUCs between doses. The increases seen in peak CD34+ cell counts, CD34+ AUCs, or times of peak CD34+ cells over the two dose levels in all three cohorts did not achieve statistical significance. Among all donors treated, the median CD34+ cell peak was 0.042×10^9 cells/l following the 0.48 mg/kg plerixafor dose *versus* 0.023×10^9 cells/l at the 0.24 mg/kg dose ($n = 6$ for both doses). Using linear regression in an exploratory analysis, peak CD34+ cell count at the second dose was influenced by the peak CD34+ cell count at the first dose. Additionally, being female increased the peak CD34+ count at the second dose by almost 0.007×10^9 CD34+ cells/l.

Peaks in CD34+ cell numbers tended to occur later in the higher dose cohorts. The median time to peak CD34+ count was 8, 10, 10 and 11 h at the 0.24, 0.32, 0.40 and 0.48 mg/kg doses, respectively. The difference in time to peak CD34+ mobilization appeared more pronounced in the paired per-subject analysis (Table IV). Furthermore, the pharmacodynamic effects of plerixafor mobilizing CD34+ cells into the circulation appeared

to last longer at the higher plerixafor doses; at the 24 h time-point, the median CD34+ cell number in donors who received a dose of 0.48 mg/kg of plerixafor was 0.026×10^9 cells/l (range 0.011–0.033) compared to 0.0085×10^9 cells/l (range 0.003–0.019) in donors after the 0.24 mg/kg dose. Nevertheless, CD34+ counts were decreasing from peak levels in all subjects at all dose levels at the 24-h time-point.

Figure 3 shows the peak CD34+ cell count for each subject following each dose of plerixafor (for subjects who received both plerixafor doses). Some subjects demonstrated increased CD34+ counts following the higher dose of plerixafor; however, some subjects including two in the third cohort demonstrated a lower CD34+ count following the second plerixafor dose. Such data suggest that for smaller dose escalation increments, intra-subject variability in CD34+ counts following plerixafor administration may make interpretation of the dose response difficult.

Figure 4 shows each subject's CD34+ counts over time and the CD34+ AUC by individual cohorts following each plerixafor dose. The time until CD34+ cells peaked tended to occur later and the CD34+ cell counts at the 24-h time-point was higher with the higher plerixafor dose cohorts.

Colony forming unit (CFU) assay results

No dose-effect relationship was observed in erythroid colonies following plerixafor exposure in healthy volunteers. Figure 5 shows the mean number of erythroid CFU colonies per cohort following each dose of plerixafor. Compared to baseline, there was a small increase in erythroid colony counts following plerixafor administration at most dose levels; however, substantial inter-subject variation was observed in regards to erythroid CFU mobilization.

In contrast to erythroid colonies, there was a larger effect observed following plerixafor exposure in regards to increasing the number of granulocyte-macrophage (GM)-CFU colonies. Plerixafor administration mobilized GM progenitor cells, although there was no predictable dose-response relationship observed between dose and fold increase in GM-CFU colonies. Figure 6 shows the mean number of GM-CFU colonies per cohort following each dose of plerixafor.

Discussion

Plerixafor is US FDA approved in combination with G-CSF to mobilize haematopoietic stem cells for peripheral autologous stem cell collection and subsequent autologous transplant in patients with non-Hodgkin lymphoma (NHL) and multiple myeloma (Brave *et al*, 2010). Plerixafor, when administered as a single agent at a dose of 0.24 mg/kg, rapidly mobilizes CD34+ progenitor cells into the circulation. This study was designed to evaluate the safety and CD34+ cell mobilizing capacity of plerixafor when administered as a single agent in escalating doses up to a dose of 0.48 mg/kg. In an attempt to minimize inter-subject variability and because of the short half-life and rapid wash-out effect of plerixafor, we administered two different escalating doses of plerixafor to each subject, evaluating the peak CD34+ cell count and CD34+ cell AUC achieved after each dose.

This study showed that higher doses of plerixafor given subcutaneously were tolerated by healthy volunteers without Grade 3 AEs, with AEs being similar to those reported with the conventional 0.24 mg/kg dose of plerixafor. Nevertheless, Grade 2 AEs were more common in the 0.40 mg/kg cohort and non-serious cardiac effects were observed (i.e. sinus tachycardia) at the higher doses, especially when standing or walking. It could not be determined whether the non-serious cardiac effects were dose-related as telemetry

recordings were only conducted at the 0.40 and 0.48 mg/kg doses. Two subjects who received 0.40 mg/kg did not receive the 0.48 mg/kg dose of plerixafor; one of whom elected to discontinue study participation following a Grade 2 vasovagal episode. Safety measures, including adequate hydration and instructing plerixafor recipients to remain recumbent during the first few hours after dosing, should be considered in future studies evaluating higher doses of plerixafor.

The maximum tolerated dose of plerixafor that optimizes CD34+ cell mobilization in humans has not yet been determined. In humans, a prior phase I study of limited sample size showed no increase in CD34+ cell mobilization when the dose of plerixafor was increased from 0.24 to 0.32 mg/kg (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022311s000_ClinPharmR.pdf, accessed on 21 June 2010). At our institution, an ongoing trial comparing haematopoietic stem cell mobilization with a single injection of 0.24 mg/kg of plerixafor compared to five daily doses of G-CSF has shown preliminary evidence that the CD34+ cell yield following a matched volume apheresis in healthy donors is lower with plerixafor compared to G-CSF (Takahashi *et al*, 2005). More importantly, in this study, the median number of CD34+ cells collected after a single 18–25 l apheresis following the administration of 0.24 mg/kg of plerixafor was only 2.03×10^6 CD34+ cells/kg, lower than the 4×10^6 CD34+ cells/kg dose previously reported to optimize outcomes following allogeneic cell transplantation (with total body irradiation) using G-CSF mobilized peripheral blood stem cells (Singh *et al*, 2007) and when CD34+ cells are administered in the unrelated setting (Pulsipher *et al*, 2009).

Furthermore, in the study by Devine *et al* (2008), which evaluated single-agent plerixafor at a dose of 0.24 mg/kg to mobilize stem cell donors for allogeneic transplantation, apheresis products contained a median 2.9×10^6 CD34+ cells/kg recipient weight; however 1/3 of donors failed to mobilize the required 2.0×10^6 CD34+ cells/kg recipient weight necessary for transplantation following one dose of plerixafor and one apheresis procedure (Devine *et al*, 2008). It has been established that the combined administration of G-CSF with plerixafor has a potent synergistic effect on CD34+ cell mobilization. However, combined G-CSF/plerixafor administration requires that multiple daily doses of G-CSF be administered (i.e. typically 5 days) and is associated with the typical G-CSF toxicities which can be debilitating. In non-human primates, a single dose of up to 1 mg/kg of plerixafor was well-tolerated and mobilized CD34+ cell numbers comparable to that observed with 5 days of G-CSF administration. Therefore, establishing a dose of plerixafor that maximizes CD34+ cell mobilization in humans potentially could lead to a mobilization strategy where a single dose of plerixafor mobilizes sufficient numbers of CD34+ cells to require that only a single apheresis procedure be performed on the donor.

Although not the primary endpoint of this study, we did not observe sufficient evidence to conclude that increasing the dose of plerixafor above the conventional 0.24 mg/kg dose results in enhanced mobilization of CD34+ cells in healthy volunteers. We chose to study peak CD34+ counts because 1) Peak CD34+ counts have been shown to correlate with apheresis yields following G-CSF (Brissot *et al*, 2009) and 2) Lower peak CD34+ counts (i.e. between 0.02 and 0.030×10^9 cells/l) appear to correlate with sub-optimal apheresis collections following G-CSF (Moncada *et al*, 2003; Pastore *et al*, 2004; Vasu *et al*, 2008). Increases in the peak CD34+ cell counts and CD34+ AUCs were observed when higher doses of plerixafor were administered. However, these differences did not reach statistical significance, perhaps due to the small number of donors enrolled into each dose cohort and intra-subject variability. In the paired analysis, CD34+ cell counts appeared to peak later following the 0.40 and 0.48 mg/kg doses of plerixafor, although the unpaired analysis did not show a delay to CD34 cell count peak with higher plerixafor doses. CD34+ cell numbers in the higher dose cohorts were still trending downwards and had not returned to baseline 24

h after plerixafor administration. Among all donors, the median CD34+ cell peak was 0.042×10^9 cells/l following the 0.48 mg/kg plerixafor dose *versus* 0.023×10^9 cells/l at 0.24 mg/kg dose, although again these differences did not achieve statistical significance. A larger adequately powered study comparing two doses (i.e. 0.40 or 0.48 mg/kg vs. 0.24 mg/kg) where donors are randomized in terms of the dose order is necessary to determine if a higher plerixafor dose will result in increased peripheral CD34+ cell counts.

An additional dosing consideration is whether the plerixafor weight-based dosing regimen provides lower drug exposure for patients weighing <85 kg. Genzyme has agreed to a US post-marketing commitment (PMC) to design, conduct and submit a clinical trial to evaluate weight-based and flat dosing schedules in lower weight NHL patients (<http://www.access-data.fda.gov/scripts/cder/drugsatfda/index.cfm>, accessed on 5 March 2010). This PMC was based on a population PK analysis conducted by the FDA that identified a decreased response rate in patients with NHL who weighed <85 kg. In the current study, three of four subjects who had a $> 0.005 \times 10^9$ /l increase in peak CD34+ counts from the first to the second dose weighed <85 kg; however, no conclusions can be made based on this data as there were also lower weight subjects (4 of 10) who did not experience an increase in peak CD34+ cell counts from the first to the second dose. Additionally, the number of subjects was too small in this study to make any further conclusions based on weight. Stratification based on weight may be useful when designing new dose-finding studies, especially when conducting a paired dosing design as was utilized in this study.

An additional consideration that must be taken into account in future dose-findings studies is the observation that peaks in CD34+ cell counts may be delayed when higher doses of plerixafor are administered subcutaneously. Should higher doses of plerixafor prove to have better CD34+ cell mobilizing effects, the CD34+ AUCs presented in this analysis would suggest apheresis procedures following single-agent s.c. plerixafor may need to be initiated at 8–12 h rather than 4–6 h following plerixafor administration. Additionally, analyses of CD34+ cell AUCs may need to incorporate time points after 24 h to capture the full pharmacodynamic effect of plerixafor. Alternatively, some investigators are exploring whether intravenous (i.v.) plerixafor may result in a shorter time to peak CD34+ counts compared to s.c. dosing (Rettig *et al*, 2007).

Finally, if higher doses of plerixafor are found to induce higher peripheral CD34+ cell counts, additional studies will be necessary to determine the optimal CD34+ cell count for a plerixafor-mobilized allograft and whether plerixafor-mobilized allografts offer any advantages or disadvantages compared to G-CSF-mobilized grafts. Because CD34+ counts have been used as a surrogate marker for true long-term pluripotent haematopoietic stem cells, it is feasible that 'CD34+ cells' may represent different cell populations in donors mobilized with G-CSF or plerixafor. A study conducted in rhesus macaques showed that plerixafor-mobilized cell products contained more B-, T- and mast cell precursors, and G-CSF-mobilized cell products contained more neutrophil and mononuclear precursors (Donahue *et al*, 2009). Furthermore, in the setting of an allogeneic transplant, a number of transplant outcomes, such as engraftment, graft-*versus*-tumour effects and graft-*versus*-host disease depend on the allograft content of CD4 and CD8+ T-cells, regulatory T-cells and T-cell cytokine polarization status. In humans, investigators have also found that cell products mobilized with plerixafor contain similar numbers of CD3+ T-cells and higher numbers of B-cells compared to G-CSF-mobilized products. Furthermore, in contrast to G-CSF, plerixafor mobilization does not appear to alter the phenotype and cytokine polarization of T-lymphocytes (Smith *et al*, 2007). Therefore, long term comparative data will be necessary to determine whether these differences will impact transplant outcomes, such as GVHD, relapse or even survival, following PBPC transplantation using different mobilization strategies.

Prior to adequate and well controlled trials demonstrating that higher doses of plerixafor can indeed be both safely administered, and that higher doses are more effective in mobilizing CD34+ cells, we do not recommend administering higher doses of plerixafor to patients or healthy volunteers donating PBPCs for allogeneic stem cell transplants outside the context of a clinical study with adequate IRB oversight and informed consent. However, based on these preliminary data, we believe a larger study should be performed to definitively answer whether increased numbers of CD34+ cell are mobilized with higher doses of plerixafor.

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Fig 1.
Dosing and assessment schedule.

Fig 2.
Plot of mean plerixafor plasma concentrations by time on a semi-log scale.

Fig 3.
Per-subject peak CD34⁺ cell count following each dose of plerixafor by cohort.

Fig 4.
Per-subject CD34⁺ cell mobilization following plerixafor administration over time.

Fig 5.

Mean erythroid CFU counts before and after plerixafor exposure by cohort. MNCs, mononuclear cells.

Fig 6.
Mean GM-CFU counts before and after Plerixafor Exposure by Cohort.

Table I

Demographic data for pharmacodynamic analysis population.

	Cohort 1	Cohort 2	Cohort 3	Overall
<i>N</i> subjects	6	6	6	18
Dose (mg/kg)	0.24, 0.32	0.32, 0.40	0.40, 0.48	
Median interval between doses, days (range)	16 (14–27)	21 (14–42)	33 (23–77)	23.5 (14–77)
Median weight, kg (range)	79 (53–101)	72 (57–93)	77 (59–95)	73 (53–101)
Median age, years (range)	30 (18.7–49.1)	36.4 (27.6–48.8)	41.1 (24.2–50.8)	38.3 (18.7–50.8)
Female (%)	33%	66.7%	16.7%	38.9%
White (%)	83.3%	66.7%	50%	67%
African American (%)	0	33.3%	33.3%	22%
Asian (%)	16.7%	0	16.7%	11%

Table II

Per-subject incidence of adverse events by cohort.

Adverse event	0-24 mg/kg N = 6		0-32 mg/kg N = 12		0-40 mg/kg N = 14		0-48 mg/kg N = 6		All doses N = 20*	
	N	%	N	%	N	%	N	%	N	%
Cardiac										
Sinus tachycardia ^{†‡}	–	–	–	–	12	86	3	50	13	65
Sinus bradycardia ^{†‡}	–	–	–	–	1	7	4	67	5	25
Sinus arrhythmia	0	0	1	8	1	7	1	17	3	15
Premature ventricular contraction ^{†‡}	–	–	–	–	2	14	0	0	2	10
Premature atrial contraction	0	0	0	0	1	7	1	17	2	10
Gastroenterology										
Diarrhoea	3	50	5	42	7	50	3	50	15	75
Nausea	1	17	4	33	3	21	0	0	7	35
Abdominal distention/bloating	1	17	4	33	3	21	1	17	6	30
Cramping	1	17	1	8	2	14	0	0	3	15
Vomiting	0	0	1	8	3	21	0	0	3	15
Dyspepsia	0	0	0	0	3	21	0	0	3	15
Flatulence (gas)	0	0	1	8	1	7	1	17	3	15
Early satiety	0	0	0	0	2	14	0	0	2	10
Injection related										
Injection site erythema	3	50	6	50	7	50	2	33	14	70
Injection site pain	1	17	4	33	1	7	0	0	6	30
Injection site induration	0	0	2	17	3	21	0	0	4	20
Constitutional/General/Musculoskeletal										
Headache	1	17	4	33	6	43	2	33	10	50
Fatigue	1	17	2	17	4	29	0	0	5	25
Dizziness	0	0	0	0	2	14	1	17	3	15
Vivid dreams	0	0	0	0	2	14	0	0	2	10
Neurology/Ocular/Psychiatric										
Other-perioral numbness	3	50	7	58	7	50	3	50	13	65

Adverse event	0-24 mg/kg N = 6		0-32 mg/kg N = 12		0-40 mg/kg N = 14		0-48 mg/kg N = 6		All doses N = 20*	
	N	%	N	%	N	%	N	%	N	%
Watery eye	1	17	1	8	2	14	0	0	4	20
Other										
Platelets high	0	0	2	17	1	7	1	17	4	20
Dyspnea	0	0	0	0	3	21	1	17	3	15
Bilirubin, high	0	0	2	17	1	7	0	0	3	15

* Numbers may differ from the sum across cohorts because some subjects experienced an AE following two different doses of plerixafor. All possibly related adverse events occurring in more than one subject are included except for Grade 1 electrolyte abnormalities.

† Continuous telemetry assessed at the 0-40 and 0-48 mg/kg doses.

‡ Premature ventricular contraction (PVC) was recorded in nursing notes in two subjects; however, PVCs could not be confirmed by cardiology review of the telemetry strips.

Table III

Summary statistics (median, range) for CD34+ cells ($\times 10^9/l$), CD34+ AUC2–24 (h CD34 $\times 10^9/l$) and time to peak CD34+ count.

	Dose 1		Dose 2	
Cohort 1*				
Plerixafor Dose (mg/kg)	0–24		0–32	
Peak CD34+	0.023	(0.012–0.049)	0.028	(0.011–0.057)
CD34+ AUC	0.361	(0.185–0.852)	0.415	(0.186–0.918)
Time to peak CD34+ (h)	8	(8–14)	10	(8–12)
Cohort 2				
Plerixafor dose (mg/kg)	0.32		0.40	
Peak CD34+	0.026	(0.015–0.049)	0.032	(0.013–0.041)
CD34+ AUC	0.428	(0.261–0.887)	0.497	(0.258–0.726)
Time to peak CD34+ (h)	9	(6–14)	10	(10–18)
Cohort 3				
Plerixafor dose (mg/kg)	0.40*		0.48	
Peak CD34+	0.035	(0.016–0.057)	0.042	(0.020–0.047)
CD34+ AUC	0.611	(0.276–1.083)	0.708	(0.364–0.845)
Time to peak CD34+ (h)	8	(6–12)	11	(8–12)

* One subject in cohort 1 received a total dose of 23.6 mg instead of 21 mg due to a weight data entry error.

Table IV

Summary statistics (median, range) for peak CD34+ differences ($\times 10^9/l$), CD34+ AUC₂₋₂₄ ($h \text{ CD34} \times 10^9/l$) differences and differences in times of peak CD34+ cells by cohort.

	Cohort 1 (<i>n</i> = 6)	Cohort 2 (<i>n</i> = 6)	Cohort 3 (<i>n</i> = 6)
Plerixafor doses mg/kg	0.24, 0.32	0.32, 0.40	0.40, 0.48
Median per-subject peak CD34+ cell number difference between doses	+0.0035 (−0.001 to 0.008)	+0.002 (−0.008 to 0.013)	+0.0015 (−0.013 to 0.016)
Median per-subject CD34+ AUC ₂₋₂₄ difference between doses	+0.0495 (0.001 to 0.074)	+0.048 (−0.161 to 0.239)	+0.043 (−0.270 to 0.344)
Difference in hours of peak CD34+ levels between doses	+2 (−4 to 2)	+2 (0 to 4)	+2 (−2 to 6)