European Medicines Agency

Doc.Ref.:EMEA/CHMP/303556/2009

CHMP ASSESSMENT REPORT

FOR

Mozobil

International Nonproprietary Name: plerixafor

Procedure No. EMEA/H/C/001030

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Genzyme Europe B.V. submitted on 5 June 2008 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Mozobil, which was designated as an orphan medicinal product EU/3/04/227 on 20 October 2004. Mozobil was designated as an orphan medicinal product in the following indication: treatment to mobilise progenitor cells prior to stem cell transplantation. The calculated prevalence of this condition was less than 0.6 per 10,000 EU population.

The applicant applied for the following indication: Mozobil is indicated to enhance mobilisation of haematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with lymphoma and multiple myeloma (see section 4.2).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

Protocol Assistance
The applicant received Protocol Assistance from the CHMP on 17 March 2005 and on 16 September 2005. The Protocol Assistance pertained to the clinical aspects of the dossier.

Licensing status:
Mozobil has been given a Marketing Authorisation in the USA on 18 December 2008.

The Rapporteur and Co-Rapporteur appointed by the CHMP were:
Rapporteur: **Barbara van Zwieten-Boot** Co-Rapporteur: **Bengt Ljungberg**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 5 June 2008.
- The procedure started on 25 June 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 17 September 2008 The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 22 September 2008.
- During the meeting on 20-23 October 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 23 October 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 15 December 2008.
- A clarification meeting with the Rapporteurs on the CHMP consolidated List of Questions was held on 15 January 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 3 February 2009.
- During the CHMP meeting on 16-19 February 2009 the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP list of outstanding issues on 20 March 2009.
- The Rapporteurs circulated an updated Joint Assessment Report on the applicant’s responses to the list of outstanding issues to all CHMP members on 6 April 2009
- During a SAG Oncology on 8 April 2009, experts were convened to address questions raised by the CHMP.
- A clarification meeting with the Rapporteurs on the CHMP list of outstanding issues was held on 14 April 2009.

- The applicant submitted further responses to the CHMP list of outstanding issues and further to the SAG Oncology meeting on 17 April 2009.
- A further clarification meeting with the Rapporteurs on the CHMP list of outstanding issues was held on 6 May 2009.
- The applicant submitted further responses to the CHMP list of outstanding issues on 12 May 2009.
- The Rapporteurs circulated an updated Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members 20 May 2009 and a further update on 25 May 2009.
- During the meeting on 26-29 May 2009 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Mozobil on 29 May 2009. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 27 May 2009.

2 SCIENTIFIC DISCUSSION

3.1 Introduction

Haematopoietic Progenitor Stem Cell Transplantation in NHL and MM

Patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) may be treated with high-dose chemotherapy, which is myelosuppressive or myeloablative and requires reinfusion of HSCs to repopulate the bone marrow and regenerate trilineage blood cells (red blood cells, platelets, neutrophils). The HSCs used for reinfusion (transplantation) can be harvested from the bone marrow, cord blood or peripheral blood, and can be autologous (from the patient) or allogeneic (from a donor).

A number of factors such as disease type, ability to harvest cells from the patient, and donor availability contribute to the decision of autologous versus allogeneic transplantation. Since the 1980s, autologous transplantation of HSCs has become a widely used strategy for haematologic and immunologic recovery subsequent to high-dose chemotherapy for haematological malignancies, with approximately 80% of autologous transplants for the indications of MM, NHL.

In the case of autologous transplantation, the use of peripheral blood as a HSC source is preferred to bone marrow due to the ease of harvesting and less likelihood of tumour cell contamination (Lopez, 1997, Blood; Gribben, 1994, Blood).

During steady-state homeostasis the levels of HSCs in the peripheral circulation are low with a CD34+ cell count of ≤5 cells/μL representing <0.05% of white blood cells (WBCs).Therefore, the cells have to be "mobilized" from the bone marrow, where they normally reside, into the peripheral blood. Mobilization of cells from the bone marrow into the peripheral blood can be accomplished by treatment with chemotherapy, cytokines (usually G-CSF which is the most frequently used cytokine for HSC mobilization), or chemokines, either alone or in combination. The minimum number of CD34+ cells required for successful autologous transplantation is considered to be 2 × 106 cells/kg (Gazitt, 1999, J Hematotherapy; Tricot, 1995, Blood; Bender, 1992, J Hematotherapy), although higher target cell counts of 5 × 106 cells/kg result in earlier cell engraftment than transplantation with lower cell doses (Shpall, 1998, Biol Blood Marrow Transplant). Peripheral blood CD34+ (PB CD34+) cell count has been shown to correlate positively with apheresis yield (Schwella, 1996, J Clin Oncol; Haas, 1994, Blood).Timing to peak mobilization of HSCs into the peripheral blood depends on the mobilization regimen used. Peak mobilization after G-CSF alone usually occurs 4 to 5 days after initiation of G-CSF (Körbling, 2004, Clinical Bone Marrow and Blood Stem Cell Transplantation), while peak mobilization following chemotherapy-based regimens is more variable and generally occurs 7 to 14 days after last dose of chemotherapy. Due to the difficulty in predicting accurately the peak response of chemotherapy-based mobilization, daily blood samples need to be taken to measure the PB CD34+ count, particularly if chemotherapy is used alone for mobilization, in order to assess whether apheresis should be performed.

A significant proportion of patients may not be able to mobilize a sufficient or target number of cells for transplantation(s) with current HSC mobilization regimens, including cytokines with or without chemotherapy. These patients require multiple mobilizations, thus increasing associated costs and the potential of disease progression between the first and subsequent mobilization attempts. Furthermore, while potentially effective for HSC mobilization and treatment of the underlying malignancy, chemotherapy is associated with multiple risks such as febrile neutropenia, infection, and bleeding which require treatment and may require hospitalization and could be avoided if another effective mobilization regimen were used (Filshie, 2002, Current Pharmaceutical Design).

The Medicinal Product

Plerixafor (Mozobil) is a small-molecule bicyclam derivative that reversibly antagonizes the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal cell-derived factor-1α (SDF-1α, also known as CXCL12). The mechanism of action of plerixafor involves interruption of the CXCR4/SDF-1α interaction resulting in mobilization of haematopoietic stem cells (HSCs) positive for cell surface glycoprotein CD34 (CD34+ cells) to the peripheral blood where they can be collected for HSC transplantation.

Plerixafor is a bicyclam derivative, a selective reversible antagonist of the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal cell-derived factor-1α (SDF-1α), also known as CXCL12. Plerixafor-induced leukocytosis and elevations in circulating haematopoietic progenitor cell levels are thought to result from a disruption of CXCR4 binding to its cognate ligand, resulting in the appearance of both mature and pluripotent haematopoietic stem and progenitor cells in the systemic circulation. CD34+ cells mobilised by plerixafor are functional and capable of engraftment with long-term repopulating capacity. The proposed indication for Mozobil is as follows Mozobil is indicated in combination with G-CSF to enhance mobilisation of haematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with lymphoma and multiple myeloma whose cells mobilise poorly (see section 4.2).The recommended dose of Mozobil is 0.24 mg/kg body weight/day. It should be administered by subcutaneous injection 6 to 11 hours prior to initiation of apheresis following 4 day pre-treatment with G-CSF. In clinical trials, Mozobil has been commonly used for 2 to 4 (and up to 7) consecutive days. Based on increasing exposure with increasing body weight, the plerixafor dose should not exceed 40 mg/day. In pivotal clinical studies supporting the use of Mozobil, all patients received daily morning doses of 10 μg/kg G-CSF for 4 consecutive days prior to the first dose of plerixafor and on each morning prior to apheresis.

3.2 Quality aspects

Introduction

Composition

Mozobil is a sterile, preservative-free, clear, colourless to pale yellow, isotonic, solution for subcutaneous injection containing plerixafor as the active substance in a concentration of 20 mg/ml. Each vial is filled to deliver 1.2 ml of solution containing 24.0 mg of plerixafor. The vials are overfilled with 0.25 ± 0.10 ml to a target volume of 1.45 ml in order to allow the withdrawal of the labelled volume, 1.2 ml.Other ingredients include sodium chloride, water for injections, hydrochloric acid and/or if needed for the pH adjustment also sodium hydroxide, if needed for pH adjustment.

The product is packed in single-use, 2 ml, Type I clear glass vials, stoppered with grey chlorobutyl rubber stoppers and sealed with aluminium cap seals.

Active Substance

The chemical name of plerixafor is 1,1′-[1,4-phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane. It has eight basic amine functional groups, which show four pKa values in the 8.5 to 11.5 range and four pKa values at less than 2.4. The active substance is a white to off-white

crystalline solid, slightly soluble in water and saline, freely soluble in alcohols, glycol, and aqueous solutions of less than pH 10.

Plerixafor is an achiral molecule, isolated as a single crystalline form, which is highly hygroscopic. The available data show that no stereo-isomers are found and no evidence of polymorphism has been observed by x-ray powder diffraction.

The chemical structure of plerixafor has been confirmed through a combination of elemental analysis, Fourier transform infrared spectroscopy, ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, distortionless enhancement by polarisation transfer NMR, mass spectroscopy and ultraviolet spectroscopy. In all cases the obtained spectra support the proposed structure of plerixafor.

- Manufacture

Plerixafor is synthesised from the starting materials in the following four steps; Cyclam protection; alkylition, where protected cyclam units are linked together; de-protection and purification. The synthetic process has been sufficiently described, the critical steps have been identified and appropriate in-process controls have been set. The analytical methods used for in-process testing are suitable and set specifications are adequately ensuring the quality and consistency of the manufacturing process.

There are four process related impurities that result from undesired reactions during the manufacture of plerixafor. Another observed impurity is a by-product generated during the de-protection reaction. Based on the maximal daily plerixafor dose the impurity limits do not exceed the limits determined in the relevant guidelines (ICH Q3A, Q3C and EMEA/CHMP/SWP/4446/2000) and are supported by the results of toxicological studies. The solvents used have been shown to be efficiently removed during the purification and drying operations.

Batch analysis data from multiple plerixafor batches (development, stability and clinical) have been provided. The data show that in all cases all process control parameters were within the acceptance limits and that the synthetic process is robust and can reproducibly produce an active substance that will comply with the pre-defined specifications.

- Specification

The active substance specification includes tests for appearance, colour of solution (Ph.Eur), identification (FTIR, HPLC), related impurities (HPLC), residue on ignition (Ph. Eur), heavy metals (Ph. Eur), water content (Karl-Fischer), organic volatile impurities (GC), microbial limit test (Ph. Eur) and bacterial endotoxins (Ph.Eur).

- Stability

Stability studies have been performed in accordance with ICH requirements. Samples from multiple batches have been stored at long term conditions (25 ± 2°C / 60 ± 5% RH) for up to 36 months and at accelerated conditions (40 ± 2°C / 75 ± 5%RH) for up to 9 months.

The parameters tested were appearance, identification, assay, specified and unspecified impurities, , water content, bacterial endotoxins and microbiological counts. In all cases the data were within the predefined acceptance criteria.

In addition forced degradation studies were performed to identify potential degradation products of plerixafor. The conditions studied included elevated temperature, elevated temperature and humidity, elevated temperature and strong acid, elevated temperature and strong base, oxidation, and light. Very minimal degradation was observed in most of the conditions studied. Increased levels of degradation have only been observed under severe stress conditions.

The results from photostability studies also show that plerixafor is not photosensitive and does not require special protection against light exposure. However, the current packaging which is necessary as a moisture barrier also prevents exposure to light.

In conclusion, the stability studies results provided support the proposed retest period and storage conditions.

Medicinal Product

- Pharmaceutical Development

Significant changes during the development of Mozobil have included: change in route of administration (IV to subcutaneous due to change of indication), change of concentration with concomitant changes in excipient concentrations as well as change to fill volume (due to finalization of dose), and change in container closure system from ampoule to vial. The manufacturing process and formulation of pivotal clinical batches and the intended product could be considered essentially similar

The excipients and diluents used in formulation are commonly used compendial materials described in Ph. Eur. Their compatibility (chemical or physical) with the active substance has been demonstrated with long-term stability studies.

Plerixafor injection is a sterile product, terminally sterilised by a validated moist heat sterilisation cycle in an autoclave. Since the product is intended to be administered in a single dose format no preservative is added to the formulation.

The effectiveness of the container closure system to provide an integral barrier that prevents microbial contamination of the product, has been demonstrated with container closure integrity tests. Also a detailed evaluation of leachables/extractable testing has been provided that showed no major risk of leachables during the manufacture of the finished product.

- Manufacture of the Product

The manufacturing process is a standard process for these kinds of formulations and consists of the following steps: compounding; filtration; vial filling; terminal sterilisation and analysis.The process is operated under nitrogen atmosphere until just prior to the filtration step.

The manufacturing process has been adequately validated according to relevant European guidelines. Process validation data on the product has been presented for three full-scale batches. All critical process parameters have been identified and controlled by appropriate in process controls. A validated maximum holding time limit between the end of bulk filtration and terminal sterilisation has been set. The validation report demonstrates that the process is reproducible and consistently provides a finished product that complies with the in-process and finished product specifications.

- Product Specification

The specification for the finished product at release and shelf life includes tests for appearance, identification (HPLC, FTIR), assay and degradation products (HPLC), pH (Ph. Eur.), osmolality (Ph. Eur.), volume in container (Ph. Eur.), particulate matter (Ph. Eur.), sterility (Ph. Eur.) and bacterial endotoxins.

All tests included in the specification have been satisfactorily described and validated/qualified. Batch analysis data from the proposed production site have been provided on multiple full-scaled batches demonstrating compliance with the release specification.

- Stability of the Product

Stability studies were carried out on multiple production scale batches according to the ICH requirements. The batches were produced in accordance with the proposed manufacturing method and were presented in the proposed commercial packaging. The studies were ongoing, but data were presented for samples stored at 25°C/60% RH for 12 months, at 30°/65% RH for 9 months and 40°C/75% RH for 9 months.

Supporting stability data were also available for multiple batches with a slightly larger fill volume (1.7 ml) presented in the proposed commercial vial and stored for more than 24 months at 25°C/60% RH.

The parameters tested were clarity and colour (visual), identification, assay, impurities, pH, osmolality and particulate matter. In all cases the results were within the specifications. No degradation products or significant trends were observed. Therefore the stability studies results support the proposed shelf life for the commercially packaged product under the conditions specified in the SPC.

Discussion on chemical, pharmaceutical and biological aspects.

The quality of Mozobil is adequately established. In general, satisfactory chemical and pharmaceutical documentation has been submitted for marketing authorization. There are no major deviations from EU and ICH requirements.

The active substance is well characterised and documented. The excipients are commonly used in these types of formulations and comply with Ph. Eur. requirements. The packaging material is commonly used and well documented. The manufacturing process of the finished product is a terminal sterilisation process that fulfils the Ph.Eur. requirements. Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life.

3.3 Non-clinical aspects

Introduction

The non-clinical studies submitted by the applicant included primary pharmacodynamics, secondary pharmacodynamics, safety pharmacology programme, pharmacokinetics, single dose toxicity studies, repeat dose toxicity studies, genotoxicity, carcinogenicity, reproductive toxicity, toxicokinetic data, local tolerance and an ecotoxicity/environmental risk assessment.

Pharmacology

- Primary pharmacodynamics

Both in vivo and in vitro primary pharmacology studies were submitted. In vitro pharmacology studies were designed to evaluate the activity of plerixafor as a selective inhibitor of the chemokine receptor CXCR4. In vivo primary pharmacodynamic studies were designed to identify whether plerixafor was capable of mobilising HSC and HPC cells in multiple species, and that those mobilised cells were able to re-engraft in the bone marrow leading to long-term, durable reconstitution of haematopoiesis.

A series of in vitro studies showed the activity of plerixafor at the human CXCR4 receptor. Using the CCRF-CEM cell line which endogenously expresses CXCR4, plerixafor was shown to potently inhibit (i.e., nM range) SDF-1 α ligand binding to CXCR4, and SDF-1α-mediated calcium flux, G-protein activation, and chemotaxis.

Receptor selectivity was confirmed by demonstrating lack of inhibition of ligand-induced calcium flux and/or ligand binding for a series of chemokine receptors (CXCR1, CXCR2, CXCR3, CXCR7, CCR1, CCR2b, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, BLT1) either in cells naturally expressing the receptors or in transfected cell lines expressing recombinant receptor.

The molecular interactions of plerixafor with the CXCR4 chemokine receptor were investigated using receptor site directed mutagenesis studies. Receptor mutagenesis identified Asp171 and Asp262 as being essential for the ability of plerixafor to block the binding of the receptor antibody, 125I-12G5 anti-CXCR4, and SDF-1.

The ability of plerixafor to mobilise haematopoietic progenitor cells (HPC) and HSC from the bone marrow to blood was shown in mice. The administration of a single SC injection of plerixafor induced a rapid and dose-dependent mobilisation of progenitor cells from the bone marrow with peak

mobilisation occurring at 1 hour post dosing. Repeat daily dosing (3 days) with plerixafor gave consistent HPC mobilisation after each dose, indicating that there was no desensitisation with repeated administration. Plerixafor was also shown to be a potent HPC mobiliser in strains where G-CSF is a poor HPC mobiliser. Plerixafor was shown to augment G-CSF-induced mobilisation. The bone marrow was confirmed as the site of action of plerixafor in a study in mice in which the femoral bone marrow was directly perfused in situ with plerixafor, followed by quantification of stem and progenitor cells in the perfusate.

The repopulating potential of plerixafor-, G-CSF-, and plerixafor plus G-CSF-mobilised murine low density mononuclear cells (LDMC) obtained from C57BL/6 mice in lethally irradiated B6BoyJ mice and of human CD34+ cells in a myeloablated non-obese diabetic severe combined immunodeficiency disease (NOD/SCID) mouse repopulation assay, was shown.

Furthermore, the ability of plerixafor to mobilise CD34+ stem cells which, following autologous or allogeneic transplantation, result in timely and durable engraftment and reconstitution of the bone marrow has been shown in myeloablated dog and monkey models.
In general, the primary pharmacodynamic data are considered sufficient to support the indication, but a question is asked on the comparability of the HSC and HPC mobilised by plerixafor combined with G-CSF, since some studies suggest that there may differences in characteristics of mobilised cells.

- Secondary pharmacodynamics

Several exploratory studies were conducted to investigate the effect of CXCR4 inhibition, using plerixafor as a CXCR4 antagonist, in models of disease where the SDF 1/CXCR4 axis has been implicated in disease pathogenesis, different from the indication of the present application. These studies provided some insight in potential effects of plerixafor in CXCR4 related diseases. They included: 1) antiviral activity against T-tropic, CXCR-4-using strains of HIV, 2) mobilisation of pluripotent stem cells to sites of damage to induce tissue repair; 3) the anti-inflammatory effect resulting from inhibition of the chemo-attractant effect of SDF-1; and 4) anticancer activity. The anticancer studies were conducted with plerixafor in various tumour models: Plerixafor in vitro was shown to (a) inhibit SDF-1 induced migration or (b) have antiproliferative effects in a number of tumour cell lines. (c) Plerixafor was also shown to inhibit tumour growth in in vivo models of non-Hodgkin's lymphoma (NHL), glioblastoma and medulloblastoma. (d) Treatment with plerixafor enhanced the anti-tumour effect of cytarabine (AraC) in a transgenic mouse model of acute promyelocytic leukaemia (APL)

- Safety pharmacology programme

Central nervous system: Radioligand binding studies with a range of CNS receptors revealed evidence of moderate affinity of plerixafor for α1- and α2-adrenergic and D1-dopaminergic receptors (pKi values of 5.5-6.9). Based on these *in vitro* data, *in vivo* inhibition of noradrenergic and dopaminergic receptors cannot be excluded. Three behavioural/CNS primary observation tests were provided, one in mice and two in rats. All three studies indicate behavioural/CNS effects shortly after administration of doses in the range 5-20 mg/kg.

Respiratory system: A single subcutaneous injection of plerixafor at doses of 2 - 20 mg/kg in male rats caused a dose related decrease of tidal volume and respiration rate.

Cardiovascular system: In vitro data: A hERG channel assay revealed no evidence of significant inhibition of I_{kr}. In an enzyme assay testing the affinity of plerixafor for angiotensin converting enzyme, an IC_{50} value of (probably) 2.5 μM was found, which is only little above the reported therapeutic concentration in humans. Significant agonistic activity was found for neuropeptide Y_2 and Y_3 receptors at 6 and 18 μM and moderate agonistic activity for neuropeptide Y_1 receptors at 18 μM. Dose dependent antagonism of angiotensin-II induced contractions in primary rat aortic smooth muscle cell cultures was found at 2 – 20 μg/ml.

Cardiovascular system. In vivo studies: In anesthetised rats, at 10 mg/kg i.v. a dramatic fall of arterial blood pressure, heart rate, +dP/dt, -dP/dt and cardiac output occurred, and systemic vascular resistance increased. At 1 mg/kg i.v. smaller effects on cardiovascular parameters occurred. Subcutaneous administration of 20 mg/kg induced moderate decrease of mean arterial blood pressure, heart rate, +dP/dt, -dP/dt and cardiac output. Visual inspection of the ECG showed that the P wave became flat, negative, or undetectable in 4 of 5 animals after treatment. Intravenous infusion of 5-10 mg/kg/hr plerixafor in dogs resulted in increased heart rates, possibly due to stress. No ECG changes were seen in dogs.

Endocrine system: In a subcutaneous rat study increased levels of prolactin and corticosterone were found 1 hour post dose.

- Pharmacodynamic drug interactions

No specific pharmacodynamic drug interaction studies were provided.

Pharmacokinetics

The absorption, distribution, metabolism, and excretion of plerixafor have been evaluated in mouse, rat, and dog. Nine in vitro studies and ten in vivo studies were completed to describe the pharmacokinetics of plerixafor. Absorption studies of plerixafor were performed in mouse (SC only), rat and dog following SC, IV and PO administration. In vitro plasma protein binding and red cell partitioning was evaluated for rat, dog and human. Tissue distribution using [^{14}C]-plerixafor was performed in rat after SC, PO and IV administration. The metabolism of plerixafor was evaluated in in vitro studies in microsomes, hepatocytes, and whole blood. In vivo metabolism was evaluated following SC and IV administration to rat and dog. The excretion of radioactivity was evaluated following SC and IV administration of [^{14}C]-plerixafor to rat and dog.

The source of active pharmaceutical ingredient used in these studies included both the plerixafor octahydrochloride salt and the plerixafor free base. After pH adjustment, equivalent formulated test articles are produced from either the salt or the free base. All reported doses and concentrations were normalised to their free base equivalent.

- Methods of analysis

Quantitation of plerixafor in plasma was performed by high performance liquid chromatography (HPLC) coupled with various methods of detection including ultraviolet (HPLC-UV), electrochemical detection (HPLC-ECD), and mass spectrometry (HPLC-MS). [^{14}C]-plerixafor in plasma and urine was quantitated by HPLC coupled with detection by liquid scintillation counting (HPLC-LSC) or reverse isotope dilution (LC-RID). Radioactivity in plasma, urine, faeces, intestinal content, carcass, and cage washes was determined by liquid scintillation counting (LSC), and radioactivity in tissues was determined by quantitative whole body autoradiography (QWBA). Concentrations of [^{14}C]-plerixafor or total radioactivity in fluids and tissues are expressed as ng-eq/ml or ng-eq/g, where ng-eq represents the equivalent quantity of plerixafor free base represented by the measured radioactivity in decays per minute.

- Absorption

Plasma pharmacokinetics following single SC doses of plerixafor were consistent across mouse, rat, dog, and human. Absorption following SC administration to mouse, rat and dog was rapid and considered to be complete (F = 1). Plerixafor exhibited low plasma clearance for all species investigated, namely 564, 255-365, and 114-144 ml/h/kg in mouse, rat and dog respectively. The apparent volume of distribution of plerixafor is moderate across all tested species (0.3-0.6 l/kg) and human. Consistent with scaling, the elimination t1/2 ranged from 0.75, 0.90-1.16, 1.56-1.93 hours in mouse, rat and dog, respectively, to 4.83 hours in human. Exposure to plerixafor following SC administration to rat and dog was dose proportional within the evaluated ranges 0.3 to 12.1 mg/kg for

rats and 0.25 to 4 mg/kg for dogs. No significant accumulation of plerixafor in plasma was observed following 7 once daily doses to rat. Plerixafor exhibited low oral bioavailability in rat (<1%).

In the repeated dose studies in rat and dog, the T_{max} did not change with dose or duration or administration. Overall, a dose-proportional increase in C_{max} and AUC was observed with increasing dose. A less than proportional increase in AUC in male rats and a greater than proportional increase in C_{max} for male and female dogs were noted in the 4-week studies with once daily dosing schedule. There were apparent gender differences noted based on C_{max} and AUC of plerixafor. In the 4-week once-daily dose studies, the mean C_{max} was slightly higher in male rats than females, and the mean C_{max} and AUC were slightly higher in female dogs than in males. No sex differences were observed in the 4-week twice-daily dose studies. The prolonged administration (4-weeks) of plerixafor on a once- or twice-daily dosing schedule produced increase in C_{max} in rats over the 4 week period (~6–117 % increase) with the once- and twice-daily dosing. An increase in C_{max} was seen in dogs (~ 6-76 % increase) with once-daily dosing. A similar increase was usually also seen with AUC; with the exception, the 4-week dog study with twice-daily dosing actually showed a decrease in these parameters with prolonged dosing. An increase in t1/2, considered possibly related to decreased clearance from plasma, was observed with prolonged daily dosing in the 4-week rat and dog studies with twice-daily dosing.

- Distribution

Tissue distribution studies conducted in rats following SC, PO and IV administration showed that plerixafor distributed readily into the majority of tissues evaluated, with the exception of brain, muscle, pancreas, renal fat, salivary gland, spinal cord and testis. The highest tissue concentrations of drug-derived material were observed between 0.5-4 hours after administration in kidney, liver, cartilage, spleen and blood vessel walls. Elimination of plerixafor from most tissues occurred between 4 and 24 hours, however retention of drug-derived material in bone marrow, cartilage, spleen, liver, and kidney tissues was noted at up to 144 hours post SC administration. After 168 hours following SC administration to rat and dog up to 30 % of drug-derived material remained in the body. Accumulation of drug-derived material in rat was observed following 7 days of once daily 1 mg/kg SC doses in certain tissues (kidney, liver, cartilage, bone marrow and spleen), however no significant accumulation of plerixafor in plasma occurred, as measured by AUC or C_{max}. Tissue distribution was similar in male and female rat following SC administration.

Plasma protein binding

Binding of plerixafor to plasma proteins was moderate for rat, dog, and human. The percentage of plerixafor bound to protein ranged from 33% to 54% in rat plasma, from 34% to 46% in dog plasma, and from 37% to 58% in human plasma *(see also Clinical Pharmacokinetics)*.

Blood-to-plasma ratio

An in vitro study (GT-249-PK-4) was conducted to assess the potential for plerixafor to partition into red blood cells. [^{14}C]-plerixafor was incubated in rat, dog, and human whole blood for 2 hours after which the red blood cell partition coefficient was determined. Partition coefficients in human whole blood at an incubation concentration of 0.1 µM were 0.20 and 0.18, for male and female respectively. Partition coefficients at an incubation concentration of 1.0 µM were 0.20 and 0.11, for male and female respectively..

Placental transfer in rabbits

The mean fetal tissue concentrations in a rabbit embryofoetal developmental dose finding study (# 6045k) were 111, 510 and 1287 ng/g, and the ratios fetal tissue versus maternal plasma levels at 6 hours after application were 0.62, 1.97 and 1.55 after administrations of 0.6, 1.8 and 6.1 mg/kg/day, respectively.

- Metabolism

Plerixafor was metabolically stable and not subject to hepatic metabolism in *in vitro* metabolism studies conducted with rat, dog and human liver microsomes and hepatocytes. Furthermore, plerixafor

was also found to be stable in rat, dog and human whole blood. In vivo studies conducted in rat and dog showed that the non-parent radiolabelled components present in plasma and urine were Cu^{2+} complexes with plerixafor. The 1:1 and 2:1 ratios of Cu^{2+}: plerixafor that were observed are consistent with plerixafor's two potential chelating sites, the two cyclam rings.

- Excretion

The primary route of elimination in rat and dog was renal excretion. Following SC and IV administration in rat and dog the majority of the radioactivity (63-72 % of the dose) was excreted in the urine within 48 hours. Elimination in the faeces accounted for < 12 % of total radioactivity in rat and dog. Radioactivity remaining in the carcass after 7 days after SC administration accounted for 16.1-27.5% and 27.4% of the dose for rat and dog respectively. Excretion into breast milk was not investigated.

- Pharmacokinetic drug interactions

Concentrations of plerixafor that inhibit enzyme activity by 50% (IC50) for all isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, CYP2A6, CYP2B6, CYP 2C8, and CYP2E1) were >100 µM with and without preincubation, indicating that plerixafor is neither a direct nor a mechanism-based inhibitor of the major CYP enzymes. Plerixafor did not exhibit inhibitory activity in vitro towards the major drug metabolizing cytochrome P450 (CYP450) enzymes. In *in vitro* studies with human hepatocytes, plerixafor doses did not induce CYP1A2, CYP2B6, and CYP3A4 enzymes.

- Other pharmacokinetic studies

No other pharmacokinetic studies were performed.

Toxicology

Two different forms of test article were used in the conduct of the toxicity studies, plerixafor octahydrochloride salt and plerixafor free base. Plerixafor octahydrochloride was used in the initial battery of toxicity studies, whereas plerixafor free base was used in later toxicity studies conducted in support of marketing application as well as in clinical trials. The vehicles for the plerixafor dosing solutions were aqueous phosphate buffer and pH adjusted saline for studies with plerixafor octahydrochloride and plerixafor free base, respectively. At the neutral pH used for the dosing formulations, plerixafor from both sources would be protonated to the +4 state. Thus, the two different test article forms used in these studies are considered identical.

- Single dose toxicity

Lethality was found after intravenous doses of 5 mg/kg and higher in both species and after subcutaneous doses of 14 mg/kg (mice) or 40 mg/kg (rats) and higher. Clinical signs in surviving animals of both species after both routes of administration included sedation, spasms, dyspnoea, ventral recumbency. These signs lasted for about 1 – 2 hours post dose. In one rat study blood and urinary chemistry were studied at 30 min – 4 hrs post dose. Observed significant changes were: decreased total protein and albumin (doses of 2-30 mg/kg), transient increase of blood magnesium and inorganic phosphorus and decrease of blood pH at 30-40 mg/kg and decreased urinary sodium, potassium and pH at 30-40 mg/kg. A small decrease in plasma Ca was found which was considered biologically not relevant. These changes could not be related to the observed clinical signs.

- Repeat dose toxicity (with toxicokinetics)

The table (Table 1) below summarizes the repeat dose toxicity studies submitted.

Species (Strain)	**Study no / Duration / GLP status**	**No / sex / group**	**Doses (mg/kg)**	**Noteworthy Findings**

Rat (WIST (SPF))	GT-249-TX-4 14 days Non-GLP	4F	9.49 QD	9.49: Copper and zinc - Urinary excretion greatly increased at all times, concentrations in urine less than that of plerixafor, plasma concentrations not affected; Calcium and magnesium – Both show stable decrease in plasma concentrations, urine calcium concentrations progressively increased to two fold over control by study end, urine calcium concentrations higher than plerixafor, magnesium levels in urine not affected.
Rat (WIST (SPF))	189DFR-tox 16 days Non-GLP	4M 4F	0 3 QD 6 QD 9 QD 12 QD	≥ 3: Sedation and piloerection; ↑ blood iron, ↑ urine calcium; ↑spleen size, ↑ extramedullary haematopoiesis and megakaryocytes in the liver and spleen, Histiocyte aggregates and/or focal lymphoid hyperplasia, birefringent deposits, dermatitis, inflammation, haemorrhage, necrosis and muscle degeneration and regeneration at injection sites ≥ 6: Recumbency, twitching, and laboured respiration; ↓ blood calcium and magnesium; ↑ spleen weights ≥ 9: Mortality; ↓ rectal temperature, ↓ body weight gain, ↓ haemoglobin and/or RBC, ↑ WBC, ↑ reticulocytes, lymphoid depletion 12: ↑ blood phosphorus; hemorrhagic fluid in abdomen
Rat (Wistar)	CTBR 89342 Up to 7 days (5 days for 6 BID and a single dose for 36 and 50) Non-GLP	2M 2F	1.5 BID 6 BID 12 BID 24 BID 36 50	≥ 24 BID: Mortality; ↓ activity, cold to touch, head tilt, partly closed eyes, non-sustained convulsion, uncoordination and tremors ≥ 36: Mortality; weakness, recumbency, shallow and irregular breathing, skin pallor, and vocalization
Rat (SD)	ITR 1663 14 days Non-GLP	5F	0 2 BID 6 BID 12 QD	2 and 6 BID: No findings 12 QD: No findings
Rat (WIST (SPF))	428R-tox 4 weeks GLP	10M/10F + 6M/6F control and high dose for 35 days recovery	0 0.6 QD 1.9 QD 7.6 QD	≥0.6: Liver, extramedullary haematopoiesis. Subcutaneous haemorrhage and inflammation at injection site. ≥1.9: ↑ Monocytes (M), ↑ urine calcium (M) 7.6: Ventral recumbency, twitching, and rales (15 min – 1 hour post-dose. ↑ Monocytes, eosinophls, basophils, lymphocytes. ↑ Urine calcium and magnesium. ↑ Spleen weight
Rat (WIST (SPF))	432R 4 weeks GLP	10M/10F + 6M/6F for 49 days recovery	0 11.4 QD 15.2 QD	≥11.4: ↓ Body weight gain, food consumption. Ventral recumbency, laboured respiration, hyperexcitation (15 min – 1 hour post-dose). ↑lymphocytes, moncytes, neutrophils, eosinophils and basophils. ↓serum magnesium. ↑ urine calcium, magnesium. ↓ Thymus weight ↑ Spleen weight. Lymphoid atrophy in thymus. Hematopoiesis in liver and spleen. Increased incidence and/or severity of subcutaneous haemorrhage at the injection site. ↓Bone mineral content, bone volume. 15.2: Mortality (13/20 M and 10/20 F), from week 1 to week 4. Recovery from all treatment-related microscopic findings.
Rat (Wistar)	CTBR 89289 4 weeks GLP	10M/10F + 5M/5F for 14 days recovery	0 0.3 BID 0.6 BID 2 BID 12 BID	≥2: ↑ WBC (primarily neutrophils). ↑Urine calcium. ↓Thymus weight. ↑Liver weight 12: Increased respiratory rate, abnormal breathing sound, increased vocalisation. ↓Body weight, food consumption. (M) ↑ ALT ↓Serum magnesium. ↑Spleen weight ↑ Kidney weight (M). Increased incidence of dark areas at injection sites.

				At recovery slightly elevated WBC counts in high dose animals.
Dog (Beagle)	LSR 94/ SPM030 /0883 Days 1-3, 8 and 16 Non-GLP	1M/1F	4.0 QD (Day 1-3) 5.0 QD (Day 16) 6.0 QD (Day 8)	≥ 4.0: Transient increase pulse rate and reduction blood pressure; slight increase in rectal temperature at 0.5 hr and decrease at 2 hours (not measured at higher dose levels) ≥ 5.0: Hypoactivity, salivation, ataxia, hunched posture, tremors prostration, retching, emesis and excessive drinking, recovery seen at 1 hour post dose; slight reduction pulse pressure and marked increase in pulse rate 6.0: Ears cold to touch, pale gums/ears,vomiting
Dog (Beagle)	189DFD-tox 15 days Non-GLP	1M/1F	0 2 QD 4 QD 6 QD	≥ 2: Diarrhoea, emesis; ↑ heart rate by 50-100 % at 1 h post dose; 2-3 fold increases in CSF calcium levels; ↑ serum aldosterone; ↑ extramedullary hematopoiesis in the liver ≥ 4: Salivation, tremor, ataxia, impaired balance, sedation, and mydriasis; ↓ body weight gains. 6: Mortality (sacrifice); abnormal posture, increased defecation, twitches, weak legs, lateral recumbency, apathy, pale oral mucous membrane, ptosis and protrusion of the nictitating membrane; ↓ food consumption; ↓ rectal temperature; ↑ serum ACTH and cortisol; moderate fibrinoid necrosis of the myocardial blood vessel wall with minimal necrosis and fibrosis in adjacent myocardium.
Dog (Beagle)	CTBR 89349 Up to 7 days (a single dose for 9 BID)	1M/1F	6 BID 9 BID	≥ 6 BID: Uncoordination, limited use of limbs, tremors, ↓ activity; ↑ WBC 9 BID: Mortality, severe salivation, tremors/convulsions, difficult breathing; ↑ heart rate
Dog (Beagle)	. LSR 94/SPM028 /0891-tox 4 weeks GLP	3M/3F +2M/2F for 14 days recovery	0 0.25 QD 1 QD 4 QD	≥ 1: ↑Pulse rate, ↓Body weight gain, food consumption. Local reactions at injection sites 4: Hypoactivity, diarrhoea No findings after recovery
Dog (Beagle)	CTBR 89349 4 weeks GLP	3M/3F +1M/1F for 14 days recovery	0 0.15 BID 0.75 BID 4 BID	≥ 0.75: Thin appearance, ↓Body weight gain, food consumption. ↑WBC (primarily neutrophils). ↓Plasma magnesium. ↑Urine calcium. No findings at recovery

The table below (Table 2) summarizes the toxicokinetic repeat dose studies with plerixafor in rats and dogs.

Study ID	**Timepoint**	**Dose (mg/kg)**	**AUC (µg.h/ml)**		**Cmax (µg/ml)**		**Ratio animal/human***	
			♂	♀	♂	♀	AUC	Cmax
428R-tk Rat 4-week	day 30	0.6 QD 1.9 QD 7.6 QD	18.19 30.36 63.50	6.51 22.39 62.66	2.73 7.55 26.61	1.87 6.25 27.07	3.9 8.3 20.0	2.7 8.1 31.6
432R Rat 4-week	day 29	11.4 QD 15.2 QD	134.80 -	110.90 161.70	47.80 -	47.00 54.60	39 51	56 64
CTBR 89289 Rat 4-week	day 28	0.3 BID 12 BID	0.82 45.54	1.06 39.36	0.42 23.97	0.50 19.81	0.3 42.4	0.5 21.9

LSR 94/ SPM028/0891-tk Dog 4-week	week 4	0.25 QD 1 QD 4 QD	2.55 6.92 25.80	1.50 7.61 39.14	0.29 1.24 6.09	0.19 1.30 12.14	0.6 2.3 10.3	0.3 1.5 10.7
CTBR 89290 Dog 4-week	day 28	0.15 BID 0.75 BID 4 BID	0.58 4.47 25.71	0.65 4.93 31.81	0.17 1.07 8.26	0.25 1.30 9.26	0.2 1.5 28.8	0.2 1.4 10.3

*Human exposure after a single dose of 0.24 mg/kg (Study 010237): AUC: 3.16 µg.h/ml; Cmax: 0.85 µg/ml.

- Interspecies comparison

All preclinical species exhibited linear pharmacokinetics with dose-proportional exposures as measured by the C_{max} and AUC values. After SC administration of plerixafor, C_{max} in plasma occurred at 0.25-0.5 h in all preclinical species and at 0.65 h in human. The elimination half-life following SC administration was 0.75 h in mice, 0.90 h in rats, 1.58 h in dogs and 4.83 h in humans. The longer half-life in humans correlates with the lower clearance (59 ml/h/kg). Apparent volumes of distribution were moderate in all species (322-612 ml/kg).

Table 3. Pharmacokinetic parameters following single SC administration of plerixafor to mouse, rat, dog and human

Parameter	Mouse	Rat	Dog	Human
Dose (mg/kg)	5.0	1.0	0.25	0.24
C_{max} (ng-eq/ml)	10,951	1550	568	847
C_{max}/D (ng-eq/ml)	2190	1550	2324	3529
t_{max} (h)	0.25	0.5	0.5	0.65
t_{last} (h)	6	7	10	24
$t_{1/2}$ (h)	0.75	0.90	1.58	4.83
AUC_{0-t} (ng-eq·h/ml)	8823	2720	1738	3159
AUC_{0-t}/D (ng-eq·h/ml)	2001	2720	6952	13,162
V_z (ml/kg)	612[a]	477	322	454
Cl (ml/h/kg)	564[a]	365	141	59
Study No.	AOM0069	9608.0258	9608.0256	010237

[a] F = 1 assumed for mouse

- Genotoxicity

The table below summarizes the genotoxicity studies submitted.

Table 4. Overview of genotoxicity studies

Type of test/study ID/GLP	Test system	Concentrations/ Concentration range/ Metabolising system	Results Positive/negative/equivocal
Gene mutations in bacteria/ Study mb-15-94 (GLP)	Salmonella typhimurium, strains TA1535, TA97a, TA98, TA100, TA102	0, 8, 40, 200, 312.5, 625, 1000, 1250, 2500, 5000 µg/plate, +/- S9	Negative
Chromosomal aberrations in vitro/ Study z48/ (GLP)	V79 CHO-cells	- S9: 0, 1000, 3000, 5000 µg/ml (1st exp), 0, 250, 1000, 3000, 5000 µg/ml (2nd and 3rd exp) + S9: 0, 1000, 3000, 5000 µg/ml (2 experiments)	Negative
Chromosomal aberrations in vivo/ctbr-960379/ (GLP)	Rat micronucleus test, 5 rats/sex/dose level/sampling time point	0, 6.25, 12,5 and 25 mg/kg SC, sampling at 24 hrs for all dose levels and in addition at 48 hrs for only the 0 and 25 mg/kg dose.	Negative.

- Carcinogenicity

A justification for the absence of carcinogenicity studies was submitted, with reference to ICH S1A Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals, November 1995.

- Reproduction Toxicity

The table below summarizes the developmental toxicity studies submitted.

Table 5. Overview of developmental toxicity studies

Study type/ Study ID / GLP	Species; Number Female/ group	Route & dose	Dosing period	Major findings	NOAEL (mg/kg &AUC ng.h/ml)
Embryo-fœtal development/ range-finding/ ctbr-900518/non-GLP	Rat, 6 females/group	SC: 0, 1, 5, 15, 30 mg/kg/day	GD 6-17	- 30 mg/kg: all rats died/were euthanised. - 15 mg/kg: Lower body weights, body weight gains, gravid uterus weights, food intake. More early, middle and total resorptions and post implantation loss, less live fetuses. Lower fetal weight. One fetus with omphalocele. - 5 mg/kg: Lower fetal weight.	Not established (too few animals)
Embryo-fœtal development/ctbr-900519/GLP	Rat, 22 females/group + 6 toxkin rats/drug group	SC: 0, 0.5, 3, 15 mg/kg/day	GD 6-17	- 15 mg/kg: ↓ body weight, body weight gain (also after correction for fewer/smaller fetuses). ↓ food intake. ↑ early resorptions, total resorptions, post implantation loss. Middle and late resorptions not stat sign different from control but above historical control. 1 dead fetus. ↓ live fetuses, ↓ weight gravid uterus. ↓ fetal weight, ↑ incidence litters and fetuses with major malformations and multiple malformations. ↑ overall incidence of litters and fetuses with external and visceral malformations, minor skeletal anomalies and common skeletal variants. ↑ dark discoloration of adrenal glands. - 3 mg/kg: ↓ food intake. 1 fetus with multiple, major malformations (total No fetuses: 280).	F0: 0.5 mg/kg F1: 0.5 mg/kg. AUC = 3154 (GD6) – 2102 (GD 17)
Embryo-fœtal development/ range-finding/6045k/non-GLP	Rabbit, 6 females/group	SC: 0, 1, 3, 10 pler oct mg/kg /day = 0, 0.6, 1.8, 6.1 mg pler/day	GD 6-18	- 6.1 mg/kg: 3 dams died. Clinical tox signs. ↑ pre- and post implantation loss. ↓ litter size. ↑ incidence malformed fetuses (1/3): head and toe malformations. - 1.8 mg/kg: 1 dam died. Clinical tox signs. Increased incidence malformed fetuses (3/33): aplasia of toes (n=1), head malformations (n=2)	Not established (too few animals), but confirms positive findings in rat study and suggests that rabbit may be more sensitive than the rat.

Fertility and early embryonic development

No studies have been conducted to evaluate the effects of plerixafor treatment on male or female fertility in rats and dogs. A justification was provided to this effect.

Embryo-Fœtal development

Two studies in rats and one study in rabbits were conducted to evaluate the effects of plerixafor on embryo-fœtal development. In one of the rat studies, no clinical signs were reported at ≤ 15 mg/kg. Clinical signs at 30 mg/kg included, but were not limited to: decreased activity (5/6), dehydration (5/6), eyes partly closed (5/6), tremors (5/6), chewing action (1/6), uncoordinated (5/6), abnormal gait (4/6), lying on side (3/6), abnormal breathing sounds (4/6), laboured breathing (2/6) and weakness (2/6). The cause of the early deaths or the necessity for preterminal euthanasia could not be elucidated based on the gross pathological findings.

In the second rat study, the most prevalent major malformation in the 15 mg/kg group was hydrocephaly (9/21 litters). In addition, the following major malformations of head, eyes, heart, abdomen, limbs and tail were found: anophthalmia, microphthalmia, cyst at parietal/frontal bones, globular heart, dilatation of ascending aorta, interventricular septal defect, dilatation of pulmonary truncus, stenosis of descending aorta, ringed aorta, anal atresia, intestinal stenosis, acaudia, microcaudia, brachdactyly and omphalocele. One fetus in the 3 mg/kg group had omphalocele, fused

kidneys, anal atresia, microcaudia, multiple fusions and anomalies in the thoracic vertebrae, and absence of lumbar vertebrae (centra and arches).

The increased incidence of dark discoloration of adrenal glands was considered incidental, but occurred in 5 fetuses in 5 litters vs 0 in all other groups and was not described in historical control data. Fused kidneys, absence of lumbar vertebrae were not reported either in the historical control data.

In the rabbit study, the number of assessable animals at the different dose levels was: 3, 6, 4 and 2 in the 0, 0.6, 1.8 and 6.1 mg/kg dose groups respectively. Clinical toxicity signs at 1.8 mg/kg/day were: weak limbs, lateral position and slowed breathing about 1 hr post dose on day 15 post insemination (p.i.) in one dam, and hyperactivity 15-30 min post dose on day 15 p.i. in another dam. At 6.1 mg/kg/day, beginning from day p.i. on, clinical signs occurred in all dams between 10 min and 5 hrs post dose, mostly disappearing within 2 hrs: sedation, prone or lateral position, reduced muscle tone or muscle contractions, weak limbs, miosis, exophthalmus, negative cornea reflex, cyanosis and slowed or shallow breathing. In the 1.8 mg/kg/day group, 1 dam died, in the 6.1 mg/kg/day group 3 dams died.
Head malformations at 6.1 mg/kg: flatened telencephalon, aplasia of eye anlagen, jaw dysplasia, flatened snout, aplasia of nasal pits.
Head malformations at 1.8 mg/kg (in 2 fetuses in the same litter): flatened tel- and/or mesencephalon, rhombencepahlon bulged, dysplastic face (flatened, vesicular evagination), aplasia of eye anlagen, jaw dysplasia. A third fetus (not known whether this was from the same or a different litter) had aplasia of 3 toes of one hindlimb.

No GLP embryo-fetal developmental study in rabbits was conducted, and the applicant provided a justification to this effect.

Prenatal and postnatal development, including maternal function

No studies on prenatal and postnatal developmental toxicity of plerixafor were submitted. A justification was provided to this effect.

Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

A non-GLP single and repeated-dose range finding study was conducted in 5 juvenile male Yorkshire pigs, done to find a safe single dose and a safe dose for administration during 7 consecutive days, to characterise plasma kinetics, white blood cell mobilisation and to compare toxicity in pigs to human, rat and dog.

Two of the animals received twice a single dose with a washout period of minimally 48 hrs between the doses, the others only one single dose. Tested doses were 2 and 8 mg/kg (animal A), 4 and 12 mg/kg (animal B), 1 and 6 mg/kg (in 2 different pigs). The 5th animal received 4.75 mg/kg at 4 consecutive days. The animal which received 12 mg/kg died about 20 min after the dose. Although no clear cause for its death was found, it was considered test article related. Post-mortem a plasma concentration of 57 µM was found. The animal dosed 8 mg/kg showed clinical signs between 1 and 2 hrs post-dose (lateral recumbence, "looked uncomfortable"), but recovered. At doses of 4 and 2 mg/kg observed clinical signs were loose feces, and slight shaking (but this might be related to temperature difference due to separation from herd). No clinical signs were found at 1 and 6 mg/kg or after repeated administration of 4.75 mg/kg. Pharmacokinetics after doses of 1, 2, 4, 6 and 8 mg/kg showed an approximately dose proportional increase of $AUC_{0\text{-}24hr}$ of 10.15 – 75.40 hr.µM, a less than dose proportional increase of C_{max} of 5.02 – 23.59 µM, a t_{max} of 0.5 hr, and an elimination half life in the range 3.51 – 4.41 hr. Two distinct phases of increase of white blood cell counts (WBC) were found, peaking near 4 and 12 hrs post-dose respectively. At 24 hrs WBC were back at baseline. No clear difference in effect was found between 1, 2 and 4 mg/kg, or between 6 and 8 mg/kg. The effect (area under curve of WBC) of 6 and 8 mg/kg clearly exceeded that of the three lower doses.

A final juvenile toxicity study designed specifically to evaluate the potential toxicity of plerixafor in juvenile animals was not conducted. A justification to this effect was provided.

- Toxicokinetic data

Toxicokinetic data is presented in Table 1(see repeat dose studies).

- Local tolerance

A non-GLP study compared the irritation caused by two formulations of plerixafor at intradermal administration in the New Zealand White Rabbit. Three animals were used for the test of each formulation. Formulation A (HCl base) was tested in 1 male and 2 females, at 6 concentrations in the range 1.5 – 50.1 mg/ml, all concentrations were injected at different sites in each animal. Formulation B (Citric acid base), was planned to be tested at 1.5 - 124.7 mg/ml in 1 males and 2 females. However, after the first dose one female died due to toxicity, and was replaced by a male. Due to this mortality and severe clinical signs in the animals receiving formulation B, a ≥ 6 hr interval was kept between the first dose and the remainder of the doses. All animals received in addition saline and cottonseed oil injections at 6 other sites (as neg and pos control). The scoring system described in the ISO 10993 part 10 Test Guideline was followed

With this system formulation A with a plerixafor concentration of 1.5 mg/ml was negligible irritant, and this formulation with plerixafor concentrations of 3 – 50.1 mg/ml was slightly irritant. Formulation B with plerixafor concentrations of 1.5 – 12 mg/ml was negligible irritant, and this formulation with plerixafor concentrations of 25 – 124.7 mg/ml was slightly irritant. Formulation B was slightly less irritant than formulation A. The degree of irritation by both formulations was also positively correlated with the plerixafor concentration.

- Other toxicity studies

A GLP study examined the effect of plerixafor on human whole blood haemolysis. No haemolytic or flocculating effect of plerixafor was found at the tested concentration. However, the tested concentration was below the observed human Cmax of ≈ 0.8 μg/ml.

A non-GLP single dose haematology study was conducted in 9 male rats, all receiving a subcutaneous dose of 10 mg plerixafor/kg. Blood was sampled in 3 rats per time point at 9 time points ranging from 0 up to 24 hrs post dose. No clinical abnormalities, or effects on body weight were observed after dosing.

A non-GLP study examined effects of plerixafor (octahydrochloride) on metal homeostasis during once daily subcutaneous administration of 4 female Wistar rats with 15 mg plerixafor octahydrochloride corresponding to 9.49 mg plerixafor/kg for 14 days, because of known metal binding characteristics of azamacrocycles. Urinary excretion of Cu and Zn was increased, However, Ca levels (far above those of plerixafor) increased and continued to increase during the treatment period to a level of about twice the initial level. This increase could not be explained by binding to plerixafor. Plasma levels of Zn, Cu and Fe were not affected by plerixafor administration, indicating that in the rat homeostasis is maintained in spite of increased excretion . Plasma levels of Ca and Mg decreased with 31% and 55% respectively after repeated plerixafor administration, but reached a steady level during steady state levels of plerixafor. The decrease in plasma Ca level did not mirror the increasing level in urine. Urinary Mg was not affected by plerixafor. No major toxicity was observed upon short term daily dosing.

Immunotoxicity

A non-GLP study examined the effect of subcutaneous administration of 0, 8 and 20 mg/kg plerixafor on 4 consecutive days on the *in vivo* antibody response to sheep erythrocytes in 5 rats/dose group. Plerixafor did not affect the number of plaques formed per spleen, whilst cyclosporin completely inhibited the response. A justification was provided for not conducting additional immunotoxicity studies, citing ICH S8 Immunotoxicity Studies for Human Pharmaceuticals, September 2005.

Studies on Impurities.

No pharmacology or toxicity studies with individual impurities were submitted. All specified and unspecified impurities were qualified based on their determined levels in the various batches of plerixafor and plerixafor octahydrochloride used in nonclinical safety studies.

Ecotoxicity/environmental risk assessment

The Applicant submitted an expert report, based on the Environmental Risk Assessment (ERA) Guideline (EMEA/CHMP/SWP/4447/00). The applicant calculated PECsurfacewater values using a refined Fpen based on the number of autologous transplantation procedures that are performed per year. The PECsurfacewater based on a daily dose of 20.6 mg.inh-1.d-1 and a refined Fpen of 0.0000251 is 0.00026 μg/L, which is below the action limit of 0.01 μg/L. Therefore plerixafor is unlikely to represent a risk for the environment following its prescribed usage in patients. The Phase I assessment was considered completed; a Phase II assessment did not need to be performed.

Discussion on the non-clinical aspects

In general, the primary pharmacodynamic data were considered sufficient to support the indication. A series of *in vitro* studies looked at the activity of plerixafor at the human CXCR4 receptor, whilst the ability of plerixafor to mobilise haematopoietic progenitor cells (HPC) and HSC from the bone marrow to blood was shown in mice. With regards to secondary pharmacodynamics, studies with plerixafor to evaluate the effect of CXCR4 inhibition in models were provided for a number of CXCR4-related diseases, different from the indication of the present application. Safety pharmacology studies in the central nervous system, respiratory system, cardiovascular system and endocrine system were also provided. Taking into account the affinity of plerixafor for NA and DA receptors, and the passage of the blood-brain barrier, it cannot be excluded that plerixafor directly induces CNS effects.

No data on pharmacodynamic interactions other than with G-CSF were provided, given the administration schedule of plerixafor. The pharmacokinetics of plerixafor were studied adequately, especially the subcutaneous administration route in rat and dog. Five single dose studies in mice and rats were provided, one in each species using the intravenous route and one study in mice and two in rats using the subcutaneous route. Repeated dose toxicity studies up to a duration of 4 weeks, with recovery phases, were carried out in rats and dogs. Safety pharmacology results suggest that at the systemic concentrations tested in the laboratory animals, also effects on noradrenergic, dopaminergic, 5-HT receptors and on enzymes and receptors involved in regulations of blood pressure may be induced. Effects mediated by these receptors and enzymes might play a role in the acute clinical toxicity of plerixafor, however this possibility was insufficiently investigated.

A sufficient package of genotoxicity tests (bacterial gene mutation test, CHO chromosomal aberration test, rat micronucleus test) revealed no evidence of genotoxic potential. No conventional carcinogenicity studies were submitted given that there is no evidence for genotoxicity of plerixafor, and plerixafor will only be used for a short period. A dose-finding and a full embryo-foetal developmental toxicity study were done in rats and a dose-finding study in rabbits. No fertility or pre/postnatal developmental toxicity studies were provided in this indication and the known adverse effects on development. Additional immunotoxicity studies with subcutaneous doses of 8 and 20 mg/kg plerixafor did not significantly affect the *in vivo* antibody response of rats to sheep erythrocytes, whilst cyclosporin (positive control) completely inhibited the response. The Applicant provided acceptable justification for not conducting additional immunotoxicity studies. Sufficient justification was also provided for specification limits regarding drug substance related impurities.

An adequate environmental risk assessment was provided. The applicant calculated PECsurfacewater values using a refined Fpen based on the number of autologous transplantation procedures that are performed per year. Plerixafor is unlikely to represent a risk for the environment following its prescribed usage in patients , therefore a Phase II assessment does not need to be performed.

During the assessment, the CHMP raised the following main concerns with regards to the non-clinical data submitted as part of this dossier.

1. A general receptor screen testing the affinity of plerixafor for other receptors, unrelated to CXCR4 was not carried out initially by the Applicant. Given the multiple effects of plerixafor found in the toxicity and safety pharmacology studies and the existing evidence of affinity for α1- and α2-adrenergic and D1-dopaminergic receptors, inhibition of angiotensin converting enzyme and agonistic actions on neuropeptide Y receptors, the CHMP considered it possible that a general receptor screen would reveal activity on other receptors and enzymes. The analysis of the results of a general receptor screen study along with any subsequent SPC update will be performed as a follow-up measure.

2. Given that plerixafor is recommended to be combined with G-CSF., the CHMP noted that no safety studies had addressed this combination and requested that the Applicant discuss this issue further. The Applicant provided a discussion of the safety of the combination (plerixafor and G-CSF) based on clinical data from patients treated with both compounds (see Clinical Safety Discussion).

3. The CHMP noted that the potential phototoxicity of plerixafor had not been addressed. Plerixafor is given by sub-cutaneous injection and distribution data showed it was retained at site of injection. The Applicant conducted a scan of the light absorbance of plerixafor in the 290 to 700 nm range. The visible spectrum (290 to 700 nm) of plerixafor injection was obtained and indicated that there is no significant absorbance in the visible range (data not shown). This is consistent with the structure of plerixafor as it does not contain a strong chromophore. In accordance with the Note for Guidance on Photosafety Testing (CPMP/SWP/398/02), the CHMP therefore concluded that phototoxicity studies were not required.

4. The Applicant was asked to discuss to which extent acute toxicity signs reported may be due to effects on receptors other than CXCR4. Although plerixafor does affect blood Ca and Mg levels, the changes were not entirely consistent through all studies. Therefore, the CHMP questioned that the acute and short lasting clinical toxicity signs could be explained by these changes in blood chemistry. Instead, these effects could be due to noradrenergic, dopaminergic, 5-HT receptors and enzymes and receptors involved in regulations of blood pressure. The Applicant was asked to include the results from the general receptor screen when answering this question. The analysis of the results of this screen along with any subsequent SPC update will be performed as a follow-up measure

5. The CHMP raised concerns over the potential of short-term plerixafor treatment in stimulating the dissemination of tumours. The Applicant provided an extensive discussion on the possible mobilisation of tumour cells by plerixafor. The review emphasized that tumour cell mobilisation and metastasizing of pre-existing tumours are complicated processes in which many factors are involved, and therefore difficult to predict from the existing data However, too little relevant scientific data exists be reassured that Mozobil could not potentially increase tumour cell mobilisation and thereby increase the risk of formation of new tumours. Therefore this uncertainty was considered in the final clinical assessment of the risk benefit balance for the patient.

6 From the absence of studies submitted, the CHMP noted that the risk of plerixafor administration on fertility remained unknown. The Applicant did not deem conventional fertility studies necessary, arguing that patients treated for the indication of plerixafor would also be exposed to other conditions with adverse effects on fertility after plerixafor treatment such as irradiation. However, the data on elimination of plerixafor showed that after one week an appreciable quantity of this very stable compound is present in the body. Therefore, the Applicant provided an assessment based on data of period needed to eliminate the compound completely from the body. The CHMP concluded that in clinical practice, no adverse effects on reproduction were expected from plerixafor, and did not deem it necessary to ask for studies on the potential risk of adverse effects on male or female fertility or embryo foetal development after completion of the treatment.

7. The CHMP raised questions with regards to the information provided in the environmental risk assessment as the phase I risk assessment could not be completed. The Applicant subsequently provided all data neccessary for the risk assessment. Based on the result of the Phase I and PBT assessments, the CHMP concluded that Mozobil is unlikely to represent a risk for the environment following its prescribed usage in patients.

8. The CHMP requested nonclinical studies to evaluate tumour cell mobilisation. The Applicant expressed its commitment to assess available tumour cell mobilization lymphoma and/or multiple myeloma animal models that could provide meaningful data comparing different mobilisation regimens for tumour cell mobilization. Pending the completion of this assessment (by end of October 2009), the Applicant committed to provide a comprehensive overview of the currently available multiple myeloma and Non-Hodgkin lymphoma animal models and discuss their relevance as a post-approval follow-up measure. The CHMP was in agreement with regards to the importance of discussing the relevance of these models, as animal studies may not always be applicable to the human situation. In addition, to provide more data about extensive immunophenotyping of the tumour cells mobilised after G-CSF and G-CSF plus Mozobil and functional cell studies of tumour cells after binding with Mozobil was considered helpful but not required.

3.4 Clinical aspects

Introduction

Nine exploratory clinical trials including pharmacokinetic and pharmacodynamic data on plerixafor alone and in conjunction with G-CSF in healthy volunteers, oncology patients including patients with Non-Hodgkin's lymphoma (NHL), multiple myeloma (MM) and Hodgkin's disease (HD) and one compassionate use study CUP001 in oncology patients have been submitted .

Table 6 Clinical pharmacology studies following subcutaneously administration of a single dose of plerixafor with or without treatment with G-CSF 10 µg/kg.

Study	*population*	*Study type*	*Number of subjects*	*Dose plerixafor (µg/kg)*	*Coadministration of G-CSF 10 µg/kg QD*
-98-01#$	Healthy	PK	13	10, 20, 40, 80, 160	
06-H-0156	Healthy	PK	6	400	no
-1002	Healthy	PK /PD	24 (18 PK, 23PD)	40, 80, 160, 240	no
-1003	Healthy	PD	31 (25PD)	160	No
				160	Yes
				240	Yes
-1004	NHL, MM	PD	21 (20 PD)	160, 240, 320	No
-1005	Healthy	PD	10 (6 PK, 6PD)	320	No
-C201*	NHL, MM	PK/PD	23 (13 PK, 4PD)	240	Yes
-2106*	HD	PK/PD	22 (9 PK,4 PD)	240	Yes
-1101	Renal impaired	PK	23	240	No
-CUP001	Oncology	PK	7 (5 PK)	160, 240	Yes

Pharmacokinetic data in studies -98-01 are provided for informational use only due to audit issues of the testing laboratory

**Ongoing studies*

$ prerixafor was administered intravenously at doses 10, 20, 40, 80 µg/kg,subcutaneously at doses 80 and 160 µg/kg and per oral at 80 and 160 µg/kg

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

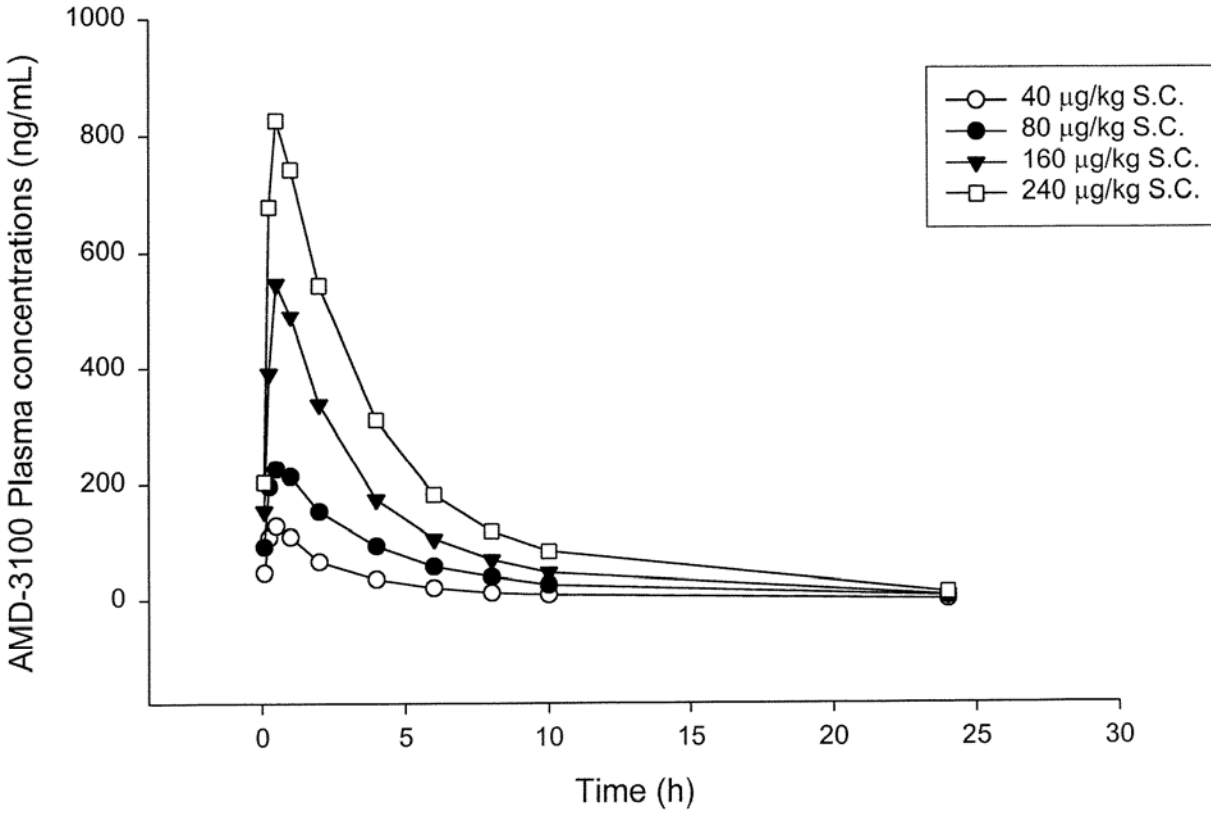
The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

- Absorption

The pharmacokinetics of plerixafor were characterized by a rapid absorption, with the time to maximum plasma concentration (t_{max}) occurring at approximately 0.5 to 1 hour after SC administration (figure 1).

Figure 1. Average plerixafor concentration-time curves following subcutaneous administration of four dose levels in 18 healthy volunteers in Study-1002.



- Bioavailability

An open-label, single-arm, single-dose, dose-escalation study designed to determine the safety, pharmacokinetics, bioavailability, and tolerability of single-dose plerixafor (IV, SC, and/or PO) in healthy subjects was conducted. The results from this study led to selection of the subcutaneous administration route for subsequent studies in healthy volunteers and the patient population.

Exposures and half-lives at 40 and 80 µg/kg between IV and SC administration were comparable. Oral absorption was not detectable at doses up to 160 µg/kg. Based on similar exposures and half lives between the at 40 and 80 µg/kg between IV and SC administration and the greater convenience of SC dosing, SC injection was chosen as the route of administration for subsequent studies.

- Bioequivalence

Four different formulations of Mozobil were used in the clinical setting. Initial clinical trial material used in the Phase 1 as well as early Phase 2 trials was produced as a 10 mg/ml formulation supplied in 1 ml or 5 ml (in study AMD3100-2001 only) ampoules. For later Phase 2 and the Phase 3 trials, the formulation was 20 mg/ml formulation filled either to 1.7 or 1.2 ml in a 2 ml vial with slight variations in the amount of sodium chloride present in the solution. Four studies (AMD3100-2104, -2106, -2108, and -C201) and the Compassionate Use Program (AMD3100-CUP001) used both the 10 mg/ml (1 ml ampoule) and 20 mg/ml formulations.

No human bioequivalence studies have been performed to compare the 10 mg/mL versus the 20 mg/mL formulations.

- Distribution

Binding in human plasma was 52% at 1 µg/ml, 58% at 3 µg/ml, and 37% 10 µg/ml. There was a decrease in the percentage of protein binding for plerixafor in all species at the highest concentration tested (10 µg/ml), indicative of saturation (study AOM0036).

Partition into red blood cells was investigated (see non-clinical pharmacokinetics).

The apparent volume of distribution was approximately 30L. This was in concordance with the distribution volume estimated by population PK analysis: volume of distribution for central compartment was 4 L and for peripheral compartment 21L.

- Elimination

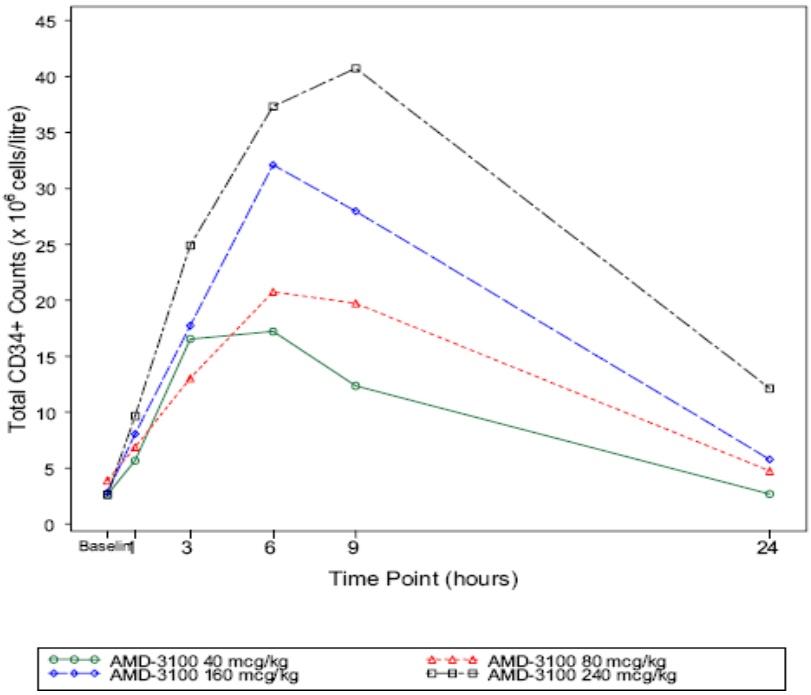
No formal mass balance study was conducted in humans. In study 1101, the effects of impaired renal function on the pharmacokinetics of 240 µg/kg s.c. dose of plerixafor was assessed. In healthy subject with normal renal function, 71% of the applied dose was excreted as intact plerixafor in the urine within 24 h of administration. The $t_{½}$ following s.c. administration of plerixafor was approximately 3-5 hours and apparent plasma clearance (CL/F) ranged from 3.7 to 5.7 L/h in healthy volunteers. Using popPK analysis population estimate of CL/F was determined to be 4.4 L/h. Renal clearance in healthy subjects was 3.1 L/h indicating that ~25% of plerixafor is eliminated by another (non-renal) elimination route

- Dose proportionality and time dependencies

Pharmacokinetics of plerixafor were studied over the dosing range of 40 to 400 µg/kg after a single SC administration in normal healthy volunteers in two studies.. In patients with renal impaired function the mean elimination half-life was increased up to 16 hours and accumulation is likely to occur.

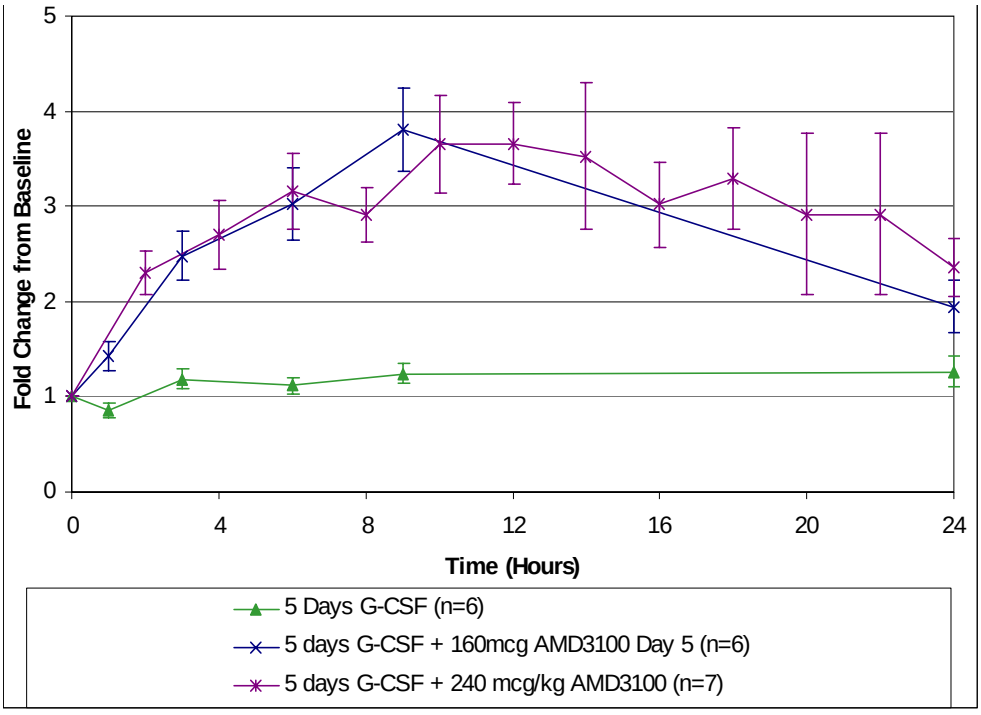
Study 1002 also determined the increase in CD34+ cell counts as pharmacodynamic response to plerixafor. Single-dose administration of plerixafor injection produced increases in mean absolute PB CD34+ cell counts in all dose groups (40 µg/kg N=3; 80 µg/kg N=10; 160 µg/kg N=5; 240 µg/kg N=5). The changes from baseline were dose dependent (see below) not only for CD34+ cells, but also for colony forming units, total WBC, neutrophils, lymphocytes, monocytes and basophils. Peak absolute responses were observed at 6 to 9 hours post-dose for all dose levels with an absolute maximal PB CD34+ cell count of ~40 cells/µL. Values returned to approximately baseline levels at 24 hours post-dose, with the exception of the 240-µg/kg dose.

Figure 2 Dose response of mean absolute PB CD34+ Cell Counts in healthy subjects after a single subcutaneous dose of plerixafor in study 1002.



A further comparison was performed in patients treated with 5 days of G-CSF alone, plerixafor 160 µg/kg given with G-CSF on Day 5, and plerixafor 240 µg/kg given with G-CSF on Day 5. The greatest pharmacodynamic response, measured by either mean change in absolute CD34+ levels or fold-increase in PB CD34+ cells occurred when plerixafor 160 µg/kg was given with G-CSF on Day 5. After a 4-day regimen of G-CSF, similar and mean fold-increases in CD34+ levels (see Figure below) were noted when 160-µg/kg and 240-µg/kg plerixafor were administered with G-CSF on Day 5. The peak response in the 240-µg/kg dose level was from 10 to 14 hours post-dose. At this dose level, plerixafor increased absolute PB CD34+ cells counts (following G-CSF administration) from a baseline cell count of ~50 cells/µL to a peak cell count of 200 cells/µL .

Figure 3 Mean Fold-Change in PB CD34+ Cell Counts From Baseline in Healthy Subjects After an Injection of Plerixafor (160 µg/kg or 240 µg/kg) After 5 Days of G-CSF in Study 1003



Time dependency

No studies investigating the pharmacokinetics of plerixafor following repeated dosing have been submitted.

Intra- and inter-individual variability
By using population PK analysis, an interindividual variability for Cl/F was estimated at 22%.

Target population
Pharmacokinetic characteristics of plerixafor were consistent across healthy subjects and oncology patients (i.e., NHL, MM, and HD).

- Special Populations

The effect of renal function on PK of plerixafor was examined in subjects with mild, moderate and severe renal dysfunction. Plerixafor was rapidly absorbed in all subjects and mean Cmax values were not affected by renal impairment. Plasma clearance of plerixafor decreased with decreasing renal function, which resulted in higher exposure of 22%, 51% and 70% in mild, moderate and severe renal impaired subjects compared to the control group. The apparent elimination half-life was prolonged with decreasing renal function; the mean t1/2 in the control, mild, moderate and severe groups was 4.9, 7.8, 12.1, and 15.8 hours, respectively. Based on these results, patients with creatinine clearance 20-50 ml/min should have their dose of plerixafor reduced by one-third to 0.16 mg/kg/day (see section 5.2 of the SPC). Clinical data with this dose adjustment are limited. There is insufficient clinical experience to make alternative posology recommendations for patients with a creatinine clearance <20 ml/min, as well as to make posology recommendations for patients on haemodialysis. Based on increasing exposure with increasing body weight the dose should not exceed 27 mg/day if the creatinine clearance is lower than 50 ml/min.

In a population PK analysis, creatinine clearance was the most influential covariate on clearance. The second most influential covariate was total body weight on central volume of distribution. Age was a significant covariate on peripheral distribution volume. Gender and race did not affect the pharmacokinetics of plerixafor.

The experience in paediatric patients is limited. The safety and efficacy of Mozobil in paediatric patients have not been established in controlled clinical studies. No dose modifications are necessary in elderly patients with normal renal function. Dose adjustment in elderly patients with creatinine clearance ≤ 50 ml/min is recommended. In general, care should be taken in dose selection for elderly patients due to the greater frequency of decreased renal function with advanced age.

- Pharmacokinetic interaction studies

In clinical studies, plerixafor has primarily been investigated in conjunction with G-CSF 10 µg/kg as a first-line mobilization therapy regimen. PK of plerixafor appeared not to be different in presence of G-CSF. No further drug-drug interaction studies were conducted.

- Pharmacokinetics using human biomaterials

No pharmacokinetic studies using human biomaterials were submitted.

Pharmacodynamics

- Mechanism of action

No clinical mechanism of action studies were submitted.

- Primary and Secondary pharmacology

The pharmacodynamic effect of plerixafor was studied both in healthy subjects and oncology patients, as single agent and as an additive mobilizing agent when administered with G-CSF.

Primary pharmacology

AMD3100-1002

This was a phase 1 study of the safety, pharmacokinetic and haematological activity of one dose of plerixafor administered by subcutaneous injection to healthy volunteers**.** Twenty-three subjects received a single sc dose of plerixafor injection: 40 µg/kg (3 subjects), 80 µg/kg (10 subjects), 160 µg/kg (5 subjects) and 240 µg/kg (5 subjects). Three subjects received serial sc doses of 80 µg/kg for 3 consecutive days; two of these subjects had previously received a single dose of 80 µg/kg.

Single-dose administration of plerixafor injection produced dose-dependent increases in circulating CD34+ cells, CFUs, total WBCs, neutrophils, lymphocytes, monocytes and basophils. For CD34+ cells, peak responses were observed at 6 to 9 hours post-dose for all doses. Values returned to approximately baseline levels at 24 hours post-dose with the exception of the 240 µg/kg dose. A higher, later and broader peak of CD34+ cells was seen for plerixafor 240 µg/kg than for lower doses. CD34+ cell increases were similar each day when 80 µg/kg plerixafor was given for 3 consecutive days to 3 healthy volunteers, suggesting that CD34+ cells were able to re-home after each dose.

AMD3100-1005

This was a phase I study of the safety and hematological activity of one dose of plerixafor administered by subcutaneous injection at a dose of 240 µg/kg or 320 µg/kg to healthy volunteers. Four subjects received 240 µg/kg, and six subjects received 320 µg/kg

Figure 4. Mean PB CD34+ Cell Count in SubjectsWho Received 320 µg/kg Plerixafor Injection

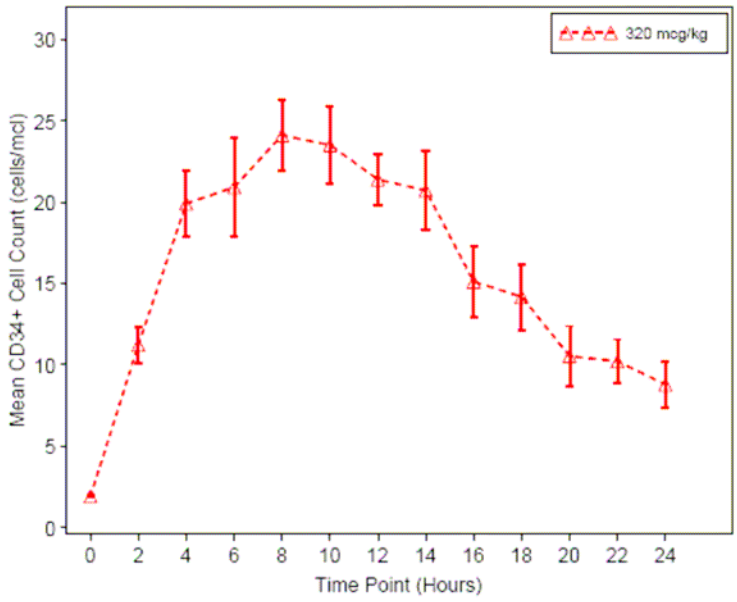


Table 7. Adverse Events Experienced by at least 2 Subjects [N, (%)] in a Treatment Group

Adverse Event by Preferred Term	240 µg/kg N=4	320 µg/kg N=6	All Subjects N=10
Paresthesia	1 (25)	6 (100)	7 (70)
Injection site erythema	1 (25)	6 (100)	7 (70)
Chest discomfort	0	4 (67)	4 (40)
Nausea	2 (50)	1 (17)	3 (30)
Diarrhea	2 (50)	0	2 (20)

Considering the efficacy results and the fact that subjects in the higher dose group experienced more AEs and AEs of greater intensity, the 320 µg/kg dose was not considered to provide an advantage over 240 µg/kg plerixafor injection.

Secondary pharmacology

No interactions other than between plerixafor and G-CSF have been studied. Drug interactions have not been observed in clinical trials with plerixafor. In clinical studies of patients with Non-Hodgkin's lymphoma, the addition of rituximab to a mobilisation regimen of Mozobil and G-CSF did not impact patient safety or CD34+ cell yield.

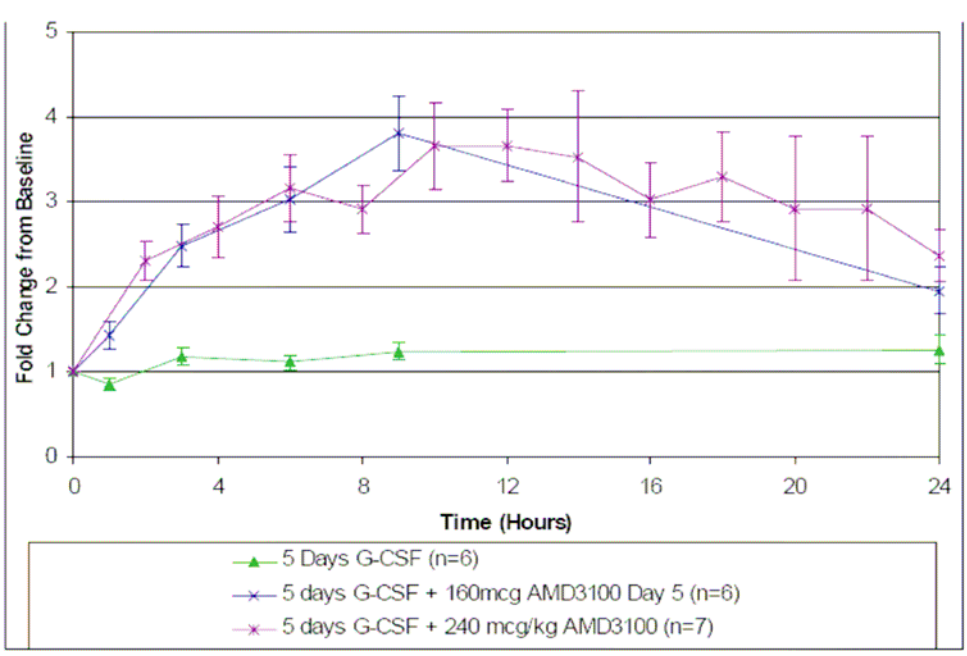
AMD3100-1003

The objective of this study was to carry out an analysis of the effect of plerixafor when given alone or with G-CSF to mobilize progenitor cells after pre-treatment with G-CSF. The table below summarizes the different patient groups and treatment schedules.

Group	# Subjects	Treatment on day 5
A	6	10 µg/kg G-CSF + 160 µg/kg plerixafor injection
AA (A apheresis)	3	10 µg/kg G-CSF + 160 µg/kg plerixafor injection
B	6	160 µg/kg plerixafor injection
C	6	10 µg/kg G-CSF
CC (C apheresis)	3	10 µg/kg G-CSF
D	4	10 µg/kg G-CSF + 240 µg/kg plerixafor injection
E	3	10 µg/kg G-CSF + 240 µg/kg plerixafor injection

In all groups G-CSF was given for 4 d before the randomized treatment on day 5 in the different groups A-E. Groups D and E were admitted to hospital for serial CD34+ measurements, group D for 12 h and group E for 24 h. Peak response concerning CFU-GM progenitors occured at 6 h in the group given plerixafor 160 µg/kg + G-CSF, thus earlier than the peak CD34+ response.

Figure 5. Mean Fold-Change in PB CD34+ Cell Counts From Baseline in Healthy Subjects After an Injection of Plerixafor (160 µg/kg or 240 µg/kg) After 5 Days of G-CSF in Study 1003



Subjects receiving G-CSF + 240 µg/kg plerixafor produced peak increases of CD34+ cells in PB at 10-14 hours post-dose. In this study of the combination of G-CSF + plerixafor peak increases of CD34+ cells were similar with plerixafor doses of 160 µg/kg and 240 µg/kg.

Progenitor cell mobilization by combined 10 µg/kg G-CSF and 160 µg/kg plerixafor injection and treatment yielded approximately 5-fold, 3-fold and 3-fold mean relative increases in peripheral blood CFU-GM, BFU-E and CFU-GEMM, respectively, all at 6 hours post-dose. The increase in peripheral blood CFU-GM levels paralleled the increase in peripheral blood CD34+ levels.

The difference between CD34+ cell numbers in patients treated with G-CSF + plerixafor 160 µg/kg (AA) and the G-CSF treated patients (CC) was larger than the respective differences concerning CFU. Similar large increases in CFUs were observed for the G-CSF + plerixafor (AA) and the G-CSF alone (CC) groups.

Study AMD3100-1004

This was a phase 1 study carried out to evaluate the safety and effect on circulating CD34+ cells of a single dose of 160 µg/kg, 240 µg/kg, or 320 µg/kg of plerixafor administered by subcutaneous injection to patients with Non-Hodgkin's Lymphoma or Multiple Myeloma

A single dose of either 160 µg/kg , 240 µg/kg or 320 µg/kg plerixafor gave clear increases in PB CD34+ cells/µl at 4 h and 6 h. There was no positive dose- response relationship for the median values in NHL patients. The patients receiving 320 µg/kg had frequent measurements of CD34+ cells in PB up to 24 h after dosing. In NHL and MM patients receiving 320 µg/kg plerixafor injection mean peak fold-increases in PB CD34+ cells of 9.3 and 12.3 respectively were observed 8-10 hours post-dose. Mean peak fold-increases at 6 hours ranged from 4.4 to 9.4 across the 6 disease/dose groups studies.

In the mobilization and apheresis part of the study where G-CSF was given for 4 d before plerixafor 320 µg/kg and apheresis, 3 MM patients collected the target of >5 x10^6 CD34+ cells /kg in 2 or fewer apheresis days. The yields for the 3 NHL patients were much lower and and 2 of 3 were poor mobilisers. Three MM patients and 2 NHL patients were transplanted with cells collected following plerixafor and showed PMN engraftment generally within 12 days and no later than 15 days from transplantation. Graft durability was shown up to the last follow-up contact.

Overall, the most common AEs reported were injection site erythema (12/21, 57.1 %), fatigue (7/21, 33.3 %), paresthesia (5/21, 23.8 %) and bone pain (5/21, 23.8 %). Injection site erythema was most commonly reported for the 240 µg/kg plerixafor injection treated NHL patients (3/3, 100 %) and the 320 µg/kg plerixafor injection treated MM patients (3/3, 100 %). The incidence of fatigue was highest for the 320 µg/kg plerixafor injection treated NHL patients (3/5, 60.0 %) and the incidence of paresthesia was highest for the160 µg/kg plerixafor injection treated MM patients (2/3, 66.7 %). Bone pain was only reported for NHL (3/5, 60.0 %) and MM patients (2/3, 66.7 %) treated with 320 µg/kg plerixafor injection who were also treated with G-CSF which is commonly associated with bone pain

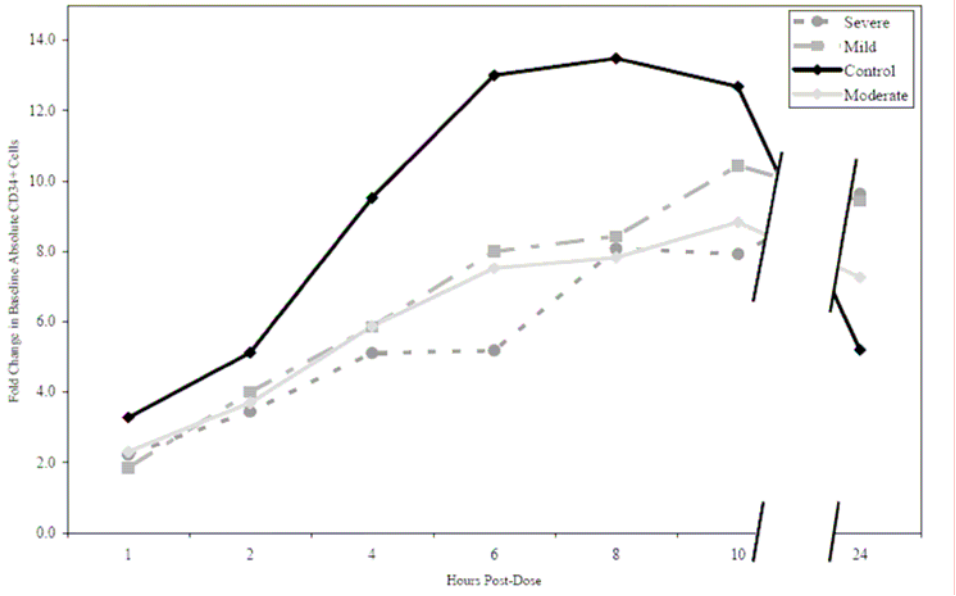
Study AMD3100-1101

This was a phase 1 study of the safety, pharmacokinetics, and haematological activity of plerixafor (240 µg/kg) in subjects with renal impairment. Twenty three subjects (17 with renal impairment and 6 healthy controls) were enrolled in this study. Subjects were stratified into 4 cohorts:

Cohort	**Number of subjects**	**Average Renal Clearance (mL/min)**
Severe impairment	6	<31, not requiring dialysis
Moderate impairment	6	31-50
Mild impairment	5	51-80
Control	6	>90

The delayed excretion and thereby increased systemic exposure of a single dose of plerixafor 240 µg/kg with higher degrees of renal impairment as described in the PK report, was associated with a delayed peak mobilization of CD34+ cells as seen below. In the patient group with severe renal impairment, the highest measurement for CD34+ cells was at 24 h, with an unknown peak before or after this timepoint.

Figure 6. Mean Fold-Change From Baseline in Absolute PB CD34+ Cell Counts Over Time by Cohort in Study 1101



Discussion on clinical pharmacology aspects

The pharmacokinetic and pharmacodynamic profile of plerixafor were consistent, whether in healthy subjects given plerixafor alone or oncology patients given plerixafor plus G-CSF. No major objections regarding the pharmacokinetics and pharmacodynamics of plerixafor were raised. However, the following issues were identified by the CHMP with regards to the clinical pharmacokinetic and pharmacodynamic studies submitted.

1. The CHMP requested that the Applicant indicate whether plerixafor had been applied at other injection sites besides the abdominal area, and what effects this had had on the pharmacokinetics of the medicinal product. Although pharmacokinetics upon subcutaneous injection at various injection sites was not compared, the Applicant provided data which indicated that PD appears similar when given via the abdomen or upper arm.

2. The Applicant was requested to discuss dose instructions for subjects with moderate and severe renal impairment with respect to dose, frequency of plerixafor administration and time to apheresis as pharmacodynamics were also affected by renal impairment. The CHMP considered that the proposed dose reduction of 1/3 in severe renal impairment needed to be further justified.

From the Applicant's responses, PK data and safety data were considered to support dose adjustment of 160 µg/kg in patients with renal impairment 20-50 ml/min. However, the PK data did not support dose adjustment of 160 µg/kg in patients with severe renal impairment <20 ml/min and there was only limited efficacy and safety data in this population. Therefore, the CHMP considered that no dose recommendations can be made for this population. In line with these conclusions, the SPC was amended as follows:

Proposal applicant 4.2

Special populations
Renal impairment
Patients with creatinine clearance 20-50 ml/min should have their dose of plerixafor reduced by one-third to 0.16 mg/kg/day (see section 5.2). Clinical data with this dose adjustment are limited. There is insufficient clinical experience to make alternative posology recommendations for patients with a

creatinine clearance <20 mL/min, as well as to make posology recommendations for patients on haemodialysis.

In addition to this, the Applicant proposed to use the Cockcroft-Gault formula to estimate the creatinine clearance in patients with severe renal dysfunction.

3. The CHMP considered that a weight cut-off should be proposed for when a flat, maximum dose should be used. A maximal absolute dose of 40 mg/day was proposed by the applicant and in patients with dose adjustment of 0.16 mg/kg a maximal dose of 27 mg/day was proposed. These dose adjustments were considered adequate by the CHMP. Section 4.2 of the SPC was amended accordingly.

Clinical efficacy

There were two phase 2 efficacy and safety studies with plerixafor in conjunction with G-CSF (granulocyte-colony stimulating factor) in patients with non Hodgkin lymphoma (NHL) and multiple myeloma (MM); study AMD3100-2101 and AMD3100-2106. In addition, there were two phase 3 placebo-controlled efficacy and safety studies with plerixafor in conjunction with G–CSF in patients with non Hodgkin lymphoma (AMD3100-3101) and in patients with multiple myeloma (AMD3100-3102). There were 8 clinical pharmacology studies and 10 supportive studies providing additional safety, efficacy and clinical pharmacology data.

- Dose response study(ies)

Six dose response studies were conducted, in healthy subjects and oncology patients with NHL and MM . Based on the safety and efficacy observed in the Phase 1 studies and one Phase 2 study, as described below, the 240-µg/kg dose was chosen as the dose to go forward in the clinical development programme for plerixafor. Because in proof-of principle study (AMD 3100-2101), the CD34+ yield per aphaeresis was higher at the 240 µg/kg dose compared to the 160 µg/kg dose, the higher of the 2 doses (240 µg/kg) was chosen.

Protocol AMD3100-1004
This was the first study conducted in cancer patients. A minimum of 12 patients and a maximum of 24 were to be entered. There were 2 groups – those with NHL and those with MM. Each group was further divided into 2 cohorts – the first was to be treated with 160 µg/kg of AMD3100 and the second with 240 µg/kg of plerixafor. If 3 patients in a cohort were safely dosed, then 3 patients were entered into the second cohort. If both groups were safely dosed in each cohort, the study of 12 patients would be complete. The purpose of the study, other than safety, was to measure the change in CD34+ cell numbers at 0, 4, and 6 hours to determine if there was an increase in cell peak at times similar to what was seen in volunteers. The study completed an enrolment of 13 patients (1 patient was replaced due to a technical error in cell processing). Seven patients received 240 µg/kg of plerixafor. There were no drug-related SAEs and no unusual or unexpected AEs. Efficacy data showed up to a 7-fold increase in circulating CD34+ cells. The study was amended to evaluate a 320 µg/kg dose .

Protocol AMD3100-2101
This was the first Phase 2 study in NHL and MM patients. Twenty-five patients were entered. In summary, patients underwent mobilization with one regimen of either (A) 5 days of 10 µg/kg of G-CSF or (B) 5 days of 10 µg/kg of G-CSF plus 160 µg/kg of AMD3100 on day 5.Patients were apheresed one hour after the G-CSF alone dose or 6 hours after the morning G-CSF plus AMD3100 dose for up to 4 days to achieve a target of 5×10^6 CD34+ cells/kg. (G-CSF ± AMD3100 were given on days after day 5 if apheresis proceeded beyond day 5.) After a rest period, patients received the opposite regimen (A after B or B after A) and were apheresed. They underwent chemotherapy and transplantation. The purpose was to determine safety, apheresis yields, and transplantation success. The protocol was amended to dose the patients at 240 µg/kg of AMD3100. Eight were dosed at 160

μg/kg and the rest at 240 μg/kg. The protocol was further amended such that the G-CSF alone regimen is always used first. There were no drug related SAEs or unexpected AEs.

Protocol AMD3100-2102
This study evaluated the benefit of plerixafor in poor mobilisers with MM and whether myeloma cells are mobilized. A dose of 240 μg/kg of plerixafor was added to their G-CSF mobilization regimen on the evening of Day 4 of G-CSF. Apheresis began on Day 5 after the morning dose of G-CSF. Eighteen patients entered this study and 7/10 mobilized enough cells for single or tandem transplants. Myeloma cells were not mobilized as assessed by a two parameter DNA procedure that determines the monoclonicity and level of elevation of immunoglobulin (Ig) chains in lymphoma cells.

Protocol AMD3100-2103
This study in NHL patients evaluated mobilization and whether NHL cells were mobilized. A dose of 240 μg/kg of plerixafor was added to their G-CSF mobilization regimen on the evening of day 4 of G-CSF. Thirteen patients entered this study. NHL cells were not mobilized.

Protocol AMD3100-2104
This study evaluated the effect of 240 μg/kg of plerixafor when added to a chemotherapy mobilizing regimen that included G-CSF. Eighteen patients entered the study.

Protocol AMD3100-2105
This study evaluated the effect of 240 μg/kg of plerixafor when used to mobilize MM and NHL patients with a 10:00 pm study drug dosing and an 8:00 am apheresis schedule. Forty-one patients entered the study.

- Main study(ies)

Study AMD 3100-3101 (referred to below as 3101) and AMD 3100-3102 (referred to below as 3102) were two phase 3 placebo-controlled efficacy and safety studies with plerixafor in conjunction with G–CSF in patients with NHL (3101) and in patients with MM (3102).

AMD3100-3101, a randomized, double-blind and placebo-controlled study conducted in patients with non-Hodgkin lymphoma (NHL). A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Comparative Trial of AMD3100 (240 μg/kg) Plus G-CSF (10 μg/kg) Versus G-CSF (10 μg/kg) Plus Placebo to Mobilize and Collect ≥ 5 x 10^6 CD34+ cells/kg in Non-Hodgkin's Lymphoma Patients for Autologous Transplantation.

AMD3100-3102, a randomized, double-blind and placebo-controlled study conducted in patients with multiple myeloma (MM).A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Comparative Trial of AMD3100 (240 μg/kg) Plus G-CSF (10 μg/kg) Versus G-CSF (10 μg/kg) Plus Placebo to Mobilize and Collect ≥ 6 x 10^6 CD34+ cells/kg in Multiple Myeloma Patients for Autologous Transplantation

METHODS

Study Participants

Inclusion

Patients had to meet the following criteria to participate in this study:

1. Age 18 to 78 years
2. Biopsy-confirmed diagnosis of NHL is to have been done prior to the first mobilization, excluding all types of chronic lymphocytic leukaemia (Study 3101) or Diagnosis of MM (Study 3102).
3. Eligible for autologous transplantation
4. In first or second CR or PR
5. > 4 weeks since last cycle of chemotherapy (Rituxan was not considered chemotherapy for the

purpose of study 3101; thalidomide, dexamethasone and Velcade were not considered prior chemotherapy for the purpose of study 3102)
6. ECOG performance status of 0 or 1
7. Patient has recovered from all acute toxic effects of prior chemotherapy
8. WBC count > 2.5 x109/l (Study 3101)
9. Absolute PMN count > 1.5 x109/l
10. PLT count > 100 x109/l
11. Serum creatinine x 2.2 mg/dl
12. SGOT, SGPT, and total bilirubin < 2.5 upper limit of normal (ULN) (Study 3101)
13. Cardiac and pulmonary status sufficient to undergo apheresis and transplantation
14. Negative for HIV
15. Signed informed consent
16. Patients of childbearing potential agree to use an approved form of contraception

Exclusion

Patients were excluded from the study for any of the following reasons:

1. A co-morbid condition which, in the view of the investigators, renders the patient at high risk from treatment complications
2. Failed previous stem cell collections or collection attempts (Study 3101)
3. Prior autologous or allogeneic transplant (Study 3102)
4. Received bone-seeking radionuclides (e.g., holmium) (Study 3102)
5. Received more than 2 cycles of alkylating agent combinations (Study 3102)
6. < 6 weeks off BCNU prior to first dose of G-CSF
7. Active CNS involvement
8. A residual acute medical condition resulting from prior chemotherapy (Study 3102)
9. Active brain metastases or carcinomatous meningitis
10. Bone marrow involvement >20% (Study 3101)
11. Received radiation therapy to the pelvis (Study 3101)
12. Post-transplant chemotherapy and/or radiation therapy below the diaphragm is anticipated (Study 3101)
13. Received GM-CSF or Neulastawithin 3 weeks prior to the first dose of G-CSF for Mobilization
14. Received G-CSF within 14 days prior to the first dose of G-CSF for mobilization (Study 3101)
15. Received prior radio-immunotherapy with Zevalin or Bexxar (Study 3101)
16. Received thalidomide, dexamethasone, and/or Velcade within 7 days prior to the first dose of G-CSF (Study 3102)
17. Active infection requiring antibiotic treatment
18. Fever (temperature > 38 °C/100.4 °F)
19. Positive pregnancy test in female patients
20. Lactating females
21. Abnormal ECG with clinically significant rhythm disturbance (ventricular arrhythmias), or other conduction abnormality in the last year that in the opinion of the investigator warrants exclusion of the subject from the trial
21. Patients who previously received experimental therapy within 4 weeks of enrolling in this protocol or who are currently enrolled in another experimental protocol during the mobilization phase.
23. Patients whose apheresis product will be further selected and purified.

Treatments
The studies were divided into the following protocol-defined phases:

1. -G-CSF mobilisation (up to 8 days in duration)
   - treatment/aphaeresis (up to 4 days in duration) up to the day prior to chemotherapy ablation.
2. - chemotherapy ablation
   - transplantation (cell transplantation within 5 weeks of last aphaeresis)

ending with engraftment (day of the latter of PMN cell and PLT engraftment following transplantation

3. - post-engraftment follow-up (100 days, 6 and 12 months)

In study 3101 and 3102 patients were treated with 10 µg/kg G-CSF for 4 days followed by 240 µg/kg plerixafor or placebo. This scheme was continued till the target number of CD34+ cells was reached (Table 8).

Table 10. Study scheme

Timepoint	**Morning Dose**	**Evening Dose**	**Apheresis Schedule**
Day 1	G-CSF 10 µg/kg		
Day 2	G-CSF 10 µg/kg		
Day 3	G-CSF 10 µg/kg		
Day 4	G-CSF 10 µg/kg*	Evening: Plerixafor 240 µg/kg Or placebo	10-11 hours after plerixafor/placebo: G-CSF and Aphaeresis*
Day 5	G-CSF 10 µg/kg*	Plerixafor 240 µg/kg Or placebo*	Aphaeresis* 10-11 hours after plerixafor/placebo
Day 6	G-CSF 10 µg/kg*	Plerixafor 240 µg/kg Or placebo*	Aphaeresis* 10-11 hours after plerixafor/placebo
Day 7	G-CSF 10 µg/kg*	Plerixafor 240 µg/kg Or placebo*	Aphaeresis* 10-11 hours after plerixafor/placebo

*aphaeresis with prior plerixafor/placebo for up to a maximum of 4 or until ≥ 5 x 10^6 CD34+ cells/kg were collected.

Rescue procedure

Patients who failed to mobilise were eligible to enter an open-label rescue procedure where, after a minimum 7-day rest period, they received another treatment course of G-CSF followed by G-CSF + plerixafor and collection of cells.

Objectives

Primary

Study 3101: The primary objective is to determine if NHL patients mobilized with G-CSF plus 240 µg/kg plerixafor are more likely to achieve a target number of ≥ 5 × 106 CD34+ cells/kg in 4 or less days of apheresis than NHL patients mobilized with G-CSF plus placebo.

Study 3102: The primary objective is to determine if MM patients mobilized with G-CSF plus 240 µg/kg plerixafor are more likely to achieve a target number of ≥ 6 × 106 CD34+ cells/kg in 2 or less apheresis days than MM patients mobilized with G-CSF plus placebo.

Secondary

1. To evaluate the safety of G-CSF plus plerixafor (240µg/kg) compared to G-CSF plus placebo in NHL patients.
2. To compare NHL patients mobilized with G-CSF plus 240 µg/kg plerixafor versus patients mobilized with G-CSF plus placebo with respect to the number of patients who achieve a minimum of 2 × 106 CD34+ cells/kg in 4 or less days of apheresis (Study 3101)
3. To compare the 2 treatment arms with respect to the number of days of apheresis required to reach the target of ≥ 5 × 106 CD34+ cells/kg (Study 3101).
4. To determine if MM patients mobilized with G-CSF plus plerixafor (240 µg/kg) are more likely to achieve a target number of ≥ 6 × 106 CD34+ cells/kg in 4 or less apheresis days than MM patients mobilized with G-CSF plus placebo (Study 3102).

5. To compare MM patients mobilized with G-CSF plus 240 μg/kg plerixafor versus patients mobilized with G-CSF plus placebo with respect to the number of patients who achieve a minimum of 2 × 106 CD34+ cells/kg in 4 apheresis days (Study 3102).
6. To compare the 2 treatment arms with respect to the number of days of apheresis required to reach the target of ≥ 6 × 106 CD34+ cells/kg (Study 3102)
7. To compare the 2 treatment arms with respect to PMN and PLT engraftment times
8. To compare the 2 treatment arms for graft durability

Outcomes/endpoints

Primary Endpoint
The primary endpoint was treatment success, i.e. the proportion of patients achieving the target number of CD34+ cells within a predefined number of aphaeresis sessions as defined for each study.

In trial 3101 the protocol-defined target was ≥ 5 × 106 CD34+ cells/kg within 4 days of apheresis. In trial 3102: The protocol-defined target was ≥ 6 × 106 CD34+ cells/kg within 2 or less days of apheresis.

Secondary Endpoints
A number of secondary endpoints were defined in the protocol:

1. Percentage of patients achieving a minimum transplantable number of CD34+ cells
(2 × 106 CD34+ cells/kg) in 4 or less days of apheresis
2. Number of days of aphereses required to reach ≥ 5 × 106 CD34+ cells/kg
3. Number of days to PMN engraftment and to PLT engraftment
4. Percentage of patients with durable engraftment at 100 days post-transplant

An efficacy objective specific to EMEA was to determine if NHL patients mobilized with G-CSF + 240 μg/kg plerixafor were more likely to achieve a target number CD34+ cells/kg in 4 or fewer days of apheresis and also have successful polymorphonuclear cell (PMN) and platelet (PLT) engraftments than NHL patients mobilized with G-CSF + placebo.Therefore, the CHMP considered that composite endpoints should be given more weight for the assessment of efficacy, in particular those taking into account both the target number of cells and successful engraftment. The following endpoints were thus defined for Study 3101 by CHMP as important secondary endpoints.

1) Target number of cells ≥ 2 x 106 CD 34 + cells/kg in ≤ 4 days of aphaeresis and successful PMN and PLT engraftment;
2) Target number of cells ≥ 5 x 106 CD 34 + cells/kg in ≤ 4 days of aphaeresis and successful PMN and PLT engraftment;
3) PMN count ≥ 0.5 x 109/L for 3 consecutive days or ≥ 1.0 x 109/L for 1 day and PLT count ≥ 20 x 109/L for 7 consecutive days without patient receiving a transfusion in the prior 7 days.

Similarly, in Study 3012, an efficacy objective specific to EMEA was to determine if MM patients mobilized with G-CSF + 240 μg/kg plerixafor were more likely to achieve a target number of CD34+ cells/kg in 2 or fewer days of apheresis and also have successful polymorphonuclear cell (PMN) and platelet (PLT) engraftments than MM patients mobilized with G-CSF + placebo. Therefore, the following endpoints were defined for Study 3102 by CHMP as important secondary endpoints.

1) Target number of cells ≥ 6 x 106 CD 34 + cells/kg in ≤ 2 days of aphaeresis and successful PMN and PLT engraftment.
2) PMN count ≥ 0.5 x 10^9/L for 3 consecutive days or ≥ 1.0 x 10^9/L for 1 day and PLT count ≥ 20 x 10^9/L for 7 consecutive days without patient receiving a transfusion in the prior 7 days.

Graft durability
Graft durability was defined as maintenance of acceptable blood counts at 100 days, 6 months and 12 months post-transplantation according to at least 2 of the 3 following criteria.

1. PLT count > 50 x 10^9/L without transfusion for a least 2 weeks prior to the follow up visit

2. Haemoglobin level ≥ 10 g /dL with no erythropoietin or transfusions for at least 1 month prior to follow up visit.
3. PMN > 1 x 10^9/L with no G-CSF for at least 1 week prior to the follow-up visit.

Graft failure was defined as 1 or both of the following**:**

1. No durable graft in 2 of the 3 cell lines of red blood cells, leucocytes and platelets (using definitions of durability above) at 100 days (and 6 and 12 months) post-transplantation
2. For the purposes of the 100-day evaluation, an additional criterion was applied. If 100-day data were not available, and the patient's absolute neutrophil count (ANC) did not rise above 0.5 x 10^9/L by 28 days post-transplantation, the patient was considered not to have maintained a durable graft at the 100-days visit.

PMN Engraftment

Defined as PMN counts ≥ 0.5 x 10^9/L for 3 consecutive days or ≥ 1.0 x 10^9/L for 1 day.

PLT Engraftment

Defined as PLT counts ≥ 20 x 10^9/L for the first of 7 consecutive days without receiving a transfusion in the prior 7 days.

Sample size

Study 3101 was powered to show that the treatment success rate among the G + AMD3100 patients is at least 20 percentage points different from the treatment success rate among the G + Placebo patients. In AMD3100-2101, 10 of 15 (or 66.7%) of the patients reached a target of 5 × 106 CD34+ cells within 4 days of apheresis after receiving G-CSF + plerixafor, while only 3 of 15 (or 20%) of the patients reached the target after receiving G-CSF alone.

Study 3102 was powered to show that the treatment success rate among the G + AMD3100 patients is at least 20 percentage points different from the treatment success rate among the G + Placebo patients. In Protocol AMD3100-2101, 7 of 10 (or 70%) of the patients reached a target of 6 × 106 CD34+ cells/kg within 2 days of apheresis after receiving G-CSF plus plerixafor, while only 2 of 10 (or 20%) of the patients reached the target after receiving G-CSF alone.

With a minimum of 93 patients per treatment group, and assuming that the treatment success rate for the G-CSF + Placebo patients is 30%, the studies will be able to declare a 20-point difference in treatment success rates using a test for comparing 2 independent population proportions with a 2-sided $\alpha = 0.05$ level of significance and a $1-\beta = 0.80$ level of statistical power. The studies were powered for the Per-Protocol analysis to show a 20-point difference between treatment groups in the percentage of patients reaching the target of 5 × 106 CD34+ cells/kg within 4 days of apheresis (Study 3101) and the target of 6 × 106 CD34+ cells/kg within 2 days of apheresis (Study 3102).

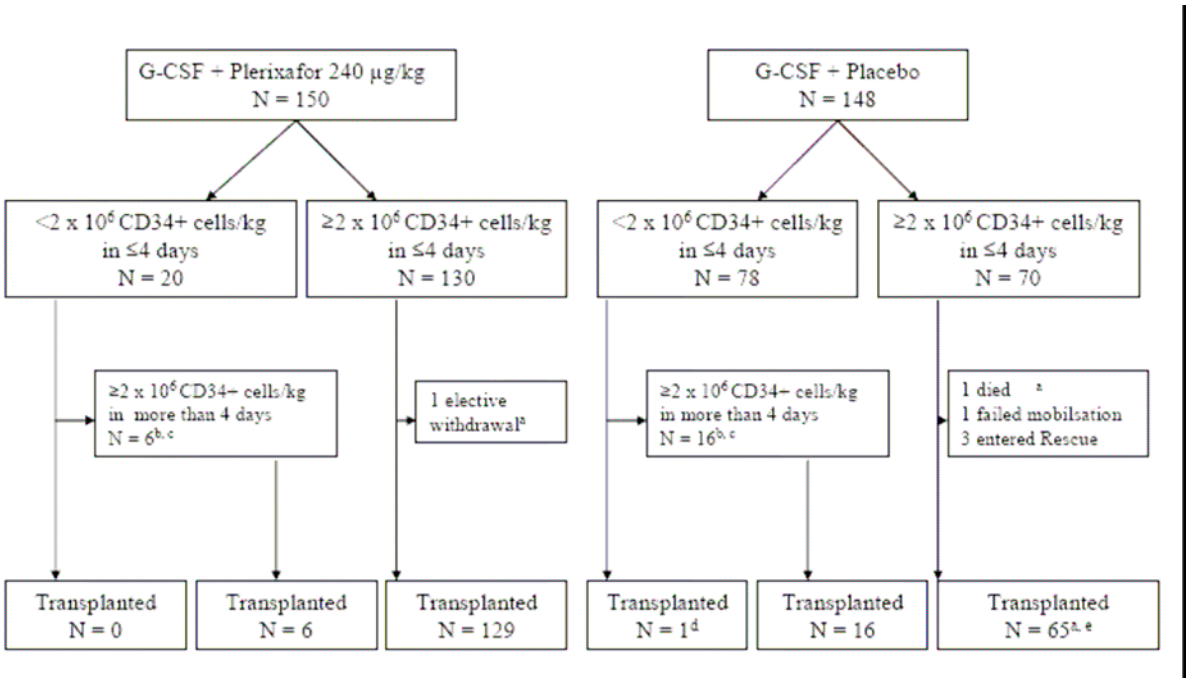
The number of subjects that participated in the pivotal studies is summarised in the table below. The majority of the patients completed the study and were transplanted.

Table 11. Patients in study 3101 and 3102 that completed the study

study	period	completed					
		yes		no		still on study	
3101		**G-CSF + plerixafor**	**G-CSF + placebo**	**G-CSF + plerixafor**	**G-CSF + placebo**	**G-CSF + plerixafor**	**G-CSF + placebo**
	G-CSF mobilisation	147	142	3	6	0	0
	Treatment /aphaeresis	135	82	12	60	0	0
	Pre-transplant chemotherapy	135	82	0	0	0	0
	Transplantation	135	82	0	0	0	0
	Post-transplantation /follow-up	66	43	20	10	49	29
3102	G-CSF mobilisation	144	150	4	4	0	0
	Treatment /aphaeresis	142	136	2	7	0	0
	Pre-transplant chemotherapy	142	136	0	0	0	0
	Transplantation	142	136	0	0	0	0
	Post-transplantation /follow-up	82	76	10	15	50	46

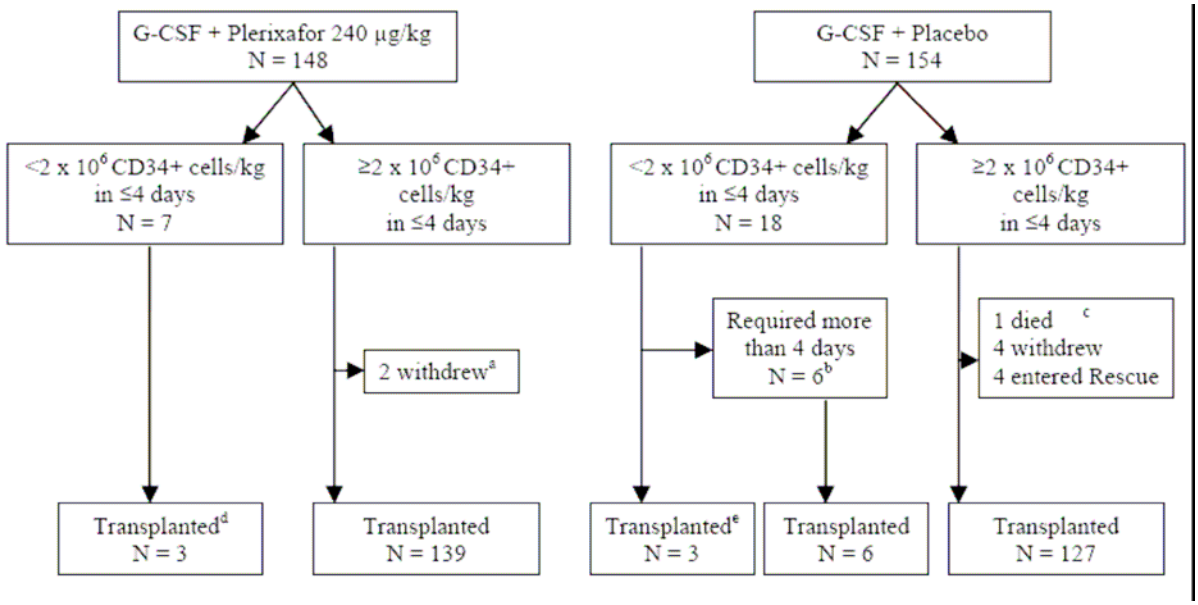
This data was provided in the Clinical Study Reports dated 01 April 2008 (study 3101) and 31 March 2008 (study 3102) at the time of MAA filing. Additional data was provided in the Clinical Study Reports dated 28 October 2008 and 16 October 2008 respectively. Data not shown.

Figure 7. Summary of patients with HSC transplants study 3101



Data from the interim study report dated 1st April 2008 was provided at the time of MAA filing. Additional data from the final study report dated 28 October 2008 were provided on 15 December 2008.

Figure 8. Summary of patients with HSC transplants study 3102



Data from the interim study report dated 31 March 2008 was provided at the time of MAA filing. Additional data from study reports dated 28 October 2008 were provided on 15 December 2008.

Randomisation

In study 3101 patients determined to be eligible for the study were randomised prior to receiving the first dose of G-CSF in a 1:1 ratio, stratified by study centre. In study 3102 eligible patients were stratified by study centre, platelet count (<0.2 x 10^6/dL versus ≥ 0.2 x 10^6/dL), and type of transplant planned (single or tandem) prior to randomisation. The study randomization schedules were generated prior to enrolling the first patient into the study. Patient enrolment and patient treatment assignment were managed through a centralized patient randomization website.

Patients randomized to receive plerixafor**:**
The patient's actual body weight was used to calculate the volume of plerixafor to be administered. The patient's weight had to be obtained within 7 days prior to dosing. The volume of study drug, plerixafor (240 mg/kg), to be given to patients was calculated as follows: 0.012 ´ patient's actual body weight (in kg) = dose to be administered (in ml)

Patients randomized to receive placebo:
The volume of placebo to be administered was determined using the same calculation as for plerixafor to ensure the same volume for injection.

Blinding (masking)

Studies 3101 and 3102 were double-blind, comparative studies. Each study center was assigned a unique number. This number was used as part of the patient number assigned to each patient as they enrolled in each of the studies. Once a patient had been screened and deemed eligible to enter the study, a patient number for that patient was assigned using centralized randomization. Patient numbers were assigned in sequential order of enrolment. A 5-digit number was assigned to the patient, with the first 2 digits representing the study center and the last 3 digits being the sequential number for the patient.

Patients who met the study inclusion and exclusion criteria were randomized prior to receiving the first dose of G-CSF for mobilization. The pharmacist responsible for dispensing study treatment logged on to the study patient randomization website to randomize the patient. The pharmacist the drew up the appropriate treatment (plerixafor or placebo) into a syringe and attached a label with the patient number, patient initials, date, volume, and "AMD3100/Placebo" (the label will not identify the treatment arm). This information was documented in the drug accountability binder. The administration of the study treatment was documented on the patient's CRF at the time that the study treatment is administered to the patient. Only the pharmacist knew the treatment assignment for the patient. The pharmacist had to ensure that the study investigator, study site personnel, sponsor, and the patient remained blinded to the actual treatment the patient received.

Statistical methods

The percentage of patients achieving treatment success were compared between the two treatment groups using Cochran/Mantel-Haenzel chi-square, stratified by investigator. Categorical data was summarized using frequency tables, presenting the patient counts and the percentage of patients in that group falling into the category. The Cochran/Mantel-Haenzel chi-square was used for analyzing contingency tables for between-group differences, stratified by investigator and PLT count at study entry. McNemar's chi-square was used to assesswithin-subject group differences in a bivariate response variable.

All demographic and analytical between-group differences for continuous variables were analyzed parametrically using the analysis-of-variance and non-parametrically using the Wilcoxon test or the analysis-of-variance of ranks. Equivalent methods were used for analyzing within-subject group differences. The paired t-test and the sign-rank test were used to test the null hypothesis that the mean within-subject measurement is equal to zero. The SAS system was used to perform all analyses.

Time-to-Event parameters were summarized using Kaplan-Meier methods, while treatment group differences in the resulting survival curves were analyzed using the Wilcoxon test and the Log-Rank test. If the event was not observed for a patient, then the patient was censored in the analysis on the last day he/she was evaluated for the event.

A p-value of 0.05 or less was considered to be statistically significant. The p-values of all tests were reported without any correction for the multiplicity of tests performed. A final data analysis plan was developed prior to locking the CRF database at the conclusion of the study. The statistical plan included mock-ups of all tables, listings, and figures to be included in a summary report, as well as documentation of all data derivations and statistical methods utilized.

Intent-to-Treat Analysis

All patients who enrol and were randomized in the study were included in the Intent-to-Treat analysis. Patients were analyzed according to the treatment group to which they were randomized. The total number of CD34+ cells collected at the time the patient completes their last apheresis, regardless of whether or not the patient completes 4 aphereses, was used to determine if the patient could be classified as a treatment success. The day of the last apheresis was used to derive each of the Days-to-Reach-Target parameters. Patients who terminated the study prior to engraftment were classified as having not reached engraftment in the analyses. No other special data handling algorithms were used for imputing missing data. The Intention-to-Treat analysis was used to establish the efficacy of plerixafor based on the primary endpoint (treatment success).

Per-Protocol Analysis

All patients completing 4 aphereses, who did not need to complete all 4 aphereses because they had reached the target of 5 x 10^6 CD34+ cells/kg, or who did not complete all 4 aphereses because they failed to collect 0.8 x 10^6 CD34+ cells/kg within 2 apheresis days were included in the Per-Protocol analysis. Patients were analyzed according to the treatment that they received. No other special data handling algorithms were used for imputing missing data. The Per-Protocol analysis was used as supportive in the assessment of efficacy of plerixafor.

Data Assumptions for Patients Not Completing Study

Patients who did not complete the study according to the protocol.were included in the Intent-to-Treat analysis but excluded from the Per-Protocol analysis. The conventions and data assumptions described below were used for imputing missing data in the Intent-to-Treat analysis.

Patients not completing G-CSF mobilization regimen

These patients were categorized as treatment failures in the Intent-to-Treat analyses. They were be excluded from the Per-Protocol analysis and from the Safety analysis. Any patient not completing their mobilization regimen of G-CSF fell into this category. Patients did not complete their mobilization regimen due to an AE or death, disease progression, G-CSF administration technical problems, or withdrawal of consent.

Patients not getting study treatment (plerixafor or placebo)

These patients were categorized as treatment failures in the Intent-to-Treat analyses. They were excluded from the Per-Protocol analysis and from the Safety analysis. Any patient who completed their mobilization regimen of G-CSF but did not receive study treatment fell into this category. Patients did not get their study treatment (plerixafor or placebo) due to an AE or death, disease progression, study treatment administration technical problems, or withdrawal of consent.

Patients not completing 4 days of apheresis

These patients were categorized as treatment failures in the Intent-to-Treat analyses. They were included in the Safety analysis, but were excluded from the Per-Protocol analysis if they receive their study treatment (pleixafor or placebo) but did not complete their apheresis due to an AE or death, disease progression, technical problems with the apheresis product or procedure, or withdrawal of consent.

Patients with incomplete apheresis yield data

These patients were categorized as treatment failures in the Intent-to-Treat analyses. They were excluded from the Per-Protocol analysis but were included in the Safety analysis. Any patient missing yield data from one or more aphereses fell into this category. Patients did not have apheresis yield data due to an AE, technical problems with the apheresis product or procedure, or withdrawal of consent.

Patients with poor yield

Patients with poor yield were defined as patients who do not collect ³ 0.8 ´ 106 CD34+ cells/kg after 2 days of apheresis or patients who fail to collect at least 2 ´ 106 CD34+ cells/kg in 4 or less days of apheresis. These patients were categorized as treatment failures in both the Intent-to-Treat analyses and the Per-Protocol analyses. They were also included in the Safety analysis.

Data Analysis

Change-from-Baseline Parameters

Within-subject changes from pre-treatment values for a parameter were evaluated using "Change-from-Baseline" and was calculated as: Change-from-Baseline = post-AMD3100 dosing value – pre-AMD3100 dosing value such that a positive value indicates an increase from the pre-AMD3100 value to the post-AMD3100 value, whereas a negative result indicated the opposite. Change-from-Baseline was calculated for each timepoint after the first dosing of plerixafor.

Time-to-Event Parameters

The following secondary efficacy parameters are time-to-event parameters: Days to Reach Target of 5 x 106 CD34+ cells/kg, Days to Reach Target of 2 x 106 CD34+ cells/kg, Days to PMN engraftment, and Days to PLT engraftment.

For the Days to Reach Target parameters, the number of days is calculated as: Number of Days = SAS Date of Event – SAS Date of First Apheresis

For the Days to Engraftment parameters, the number of days is calculated as: Number of Days = SAS Date of Event – SAS Date of Last Transplant

If the event was not observed for the patient, then the Number of Days were be calculated at the last day the patient was evaluated for the event.

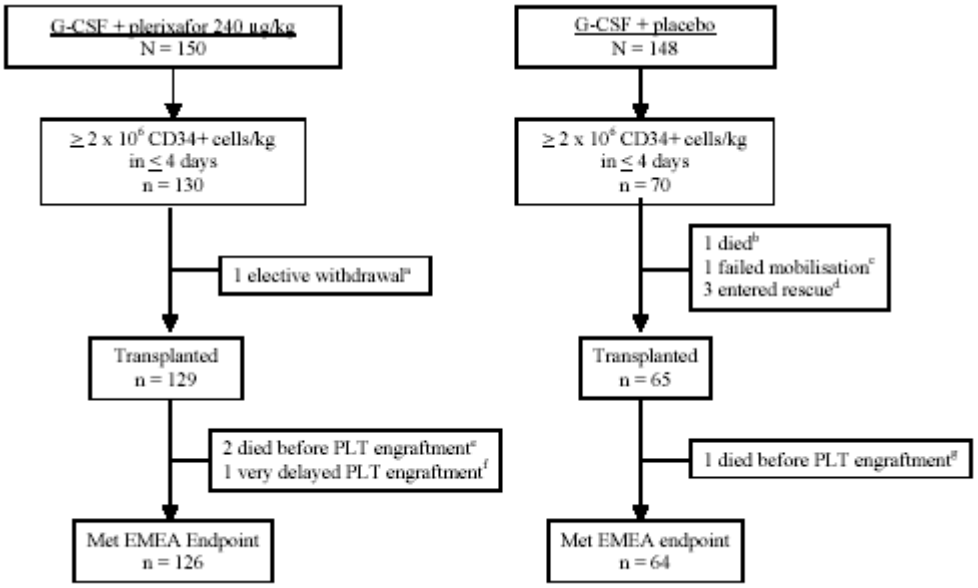
RESULTS

- **Participant flow**

The subjects entering study 3101 and 3102 and reaching the EMEA endpoint, are summarised in the Figures below.

Figure 9. Participants flow in Study 3101 (Clinical Study Report dated 01 April 2008)

Summary of patient transplants, engraftment and
EMEA composite primary endpoint
≥ 2 x 106 CD34+ cells/kg ≤ 4 aphaeresis days AND
successful PMN and PLT engraftment



Summary of patient transplants, engraftment and
EMEA composite primary endpoint
≥ 5 x 10^6 CD34+ cells/kg ≤ 4 aphaeresis days) AND
successful PMN and PLT engraftment

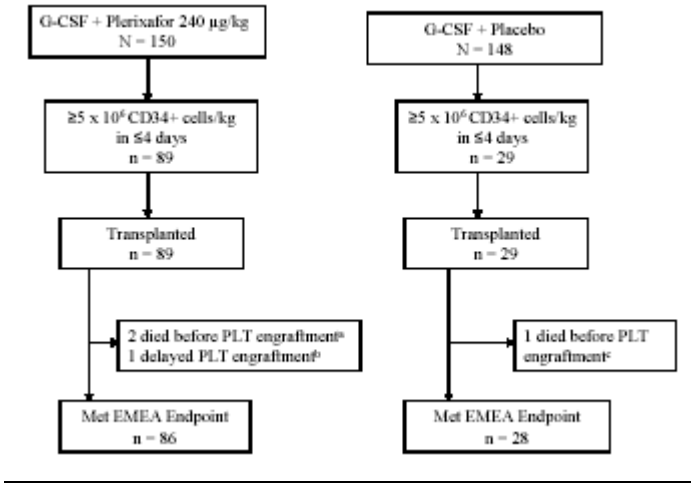
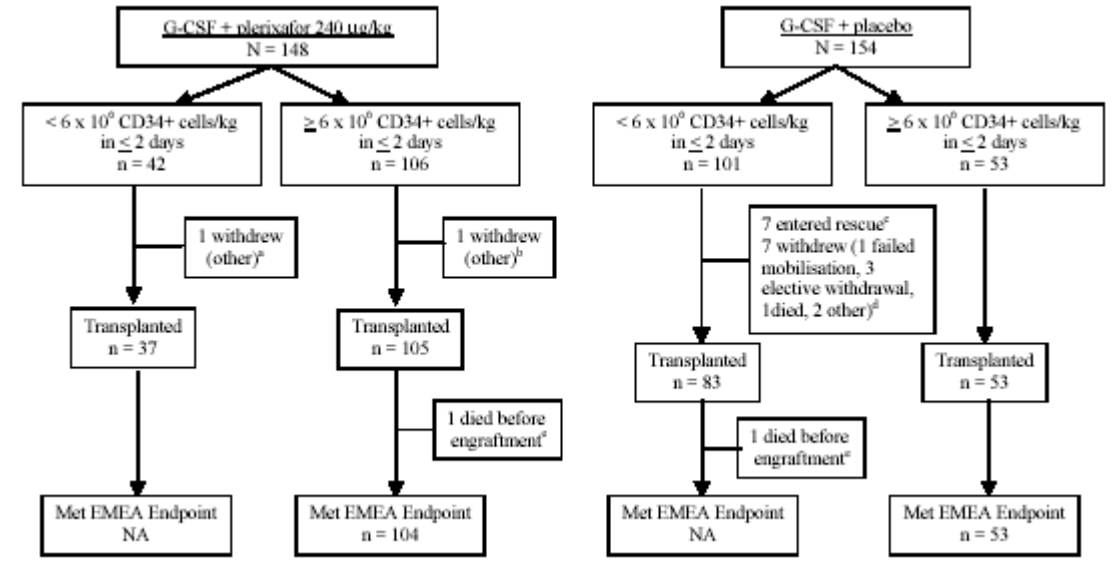


Figure 10. Participants flow in Study 3102 (Clinical Study Report dated 31 March 2008)

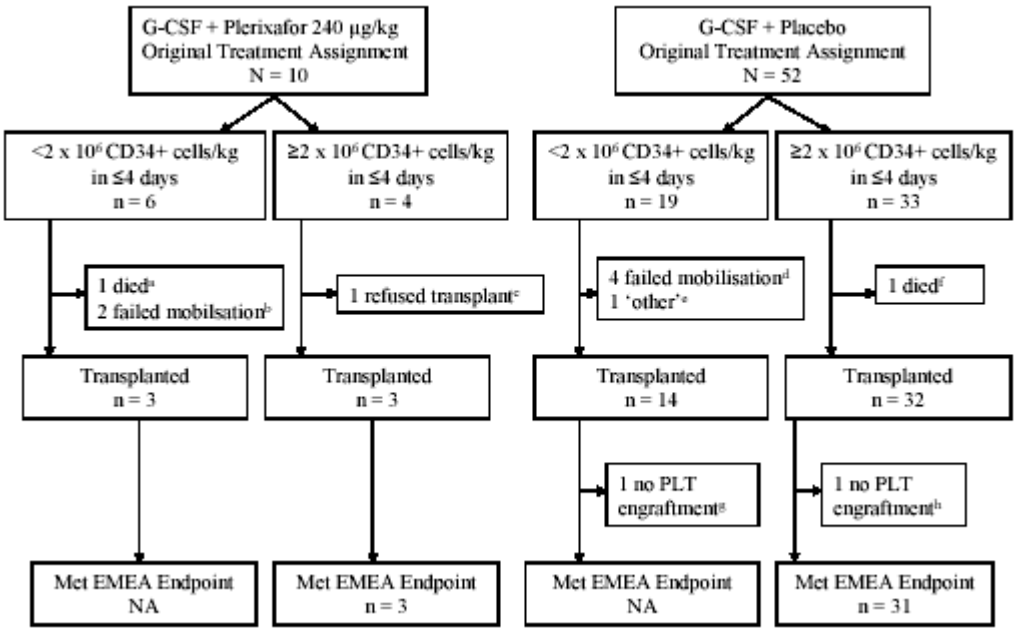


Rescue procedure

A total of 62 patients from the 3101 study(10/150 G-CSF + plerixafor and 52/148 G-CSF + placebo patients) who failed to collect 2 x 10^6 CD34+ cells/kg in the initial treatment/aphaeresis period entered the open-label rescue procedure, were they received another 4-day mobilisation regimen of G-CSF + plerixafor.

An overview of these patients is given in the flow chart below. Seven patients (all placebo patients) in the 3102 failed to collect 2 x 10^6 CD34+ cells/kg in the initial treatment/aphaeresis period ('poor mobilisers') and entered the open-label rescue procedure. They received another 4-day mobilisation regimen of G-CSF + plerixafor.

Figure 11. Rescue patients in study 3101 (Clinical Study Report dated 01 April 2008)


G-CSF + Plerixafor 240 µg/kg
Original Treatment Assignment
N = 10
G-CSF + Placebo
Original Treatment Assignment
N = 52
<2 x 10⁶ CD34+ cells/kg
in ≤4 days
n = 6
≥2 x 10⁶ CD34+ cells/kg
in ≤4 days
n = 4
<2 x 10⁶ CD34+ cells/kg
in ≤4 days
n = 19
≥2 x 10⁶ CD34+ cells/kg
in ≤4 days
n = 33
1 died[a]
2 failed mobilsation[b]
1 refused transplant[c]
4 failed mobilisation[d]
1 'other'[e]
1 died[f]
Transplanted
n = 3
Transplanted
n = 3
Transplanted
n = 14
Transplanted
n = 32
1 no PLT
engraftment[g]
1 no PLT
engraftment[h]
Met EMEA Endpoint
NA
Met EMEA Endpoint
n = 3
Met EMEA Endpoint
NA
Met EMEA Endpoint
n = 31

Recruitment
In the protocols for Studies 3101 and 3102, NHL and MM patients who were expected to be adequate to good mobilizers were entered. Given that the number of patients at risk of being poor mobilizers varies between 20% and 35%, the number of patients required to meet the target of ≥ 5 × 106 CD34+ cells/kg or achieving a minimum dose of ≥ 2 × 106 CD34+ cells/kg was difficult to estimate.

Study 3101:
All patients with NHL (except CLL) who have completed their chemotherapy regimen prior to PB stem cell mobilization and autologous transplantation could be screened for entry into this study. Prior to mobilization, they did not have fever or signs of infection and did not have worsening of their disease. Study period : 18 January 2005 – last patient's 12-month visit: 20 December 2007.

Study 3102:
Patients with MM who had completed their chemotherapy regimen prior to peripheral blood (PB) stem cell mobilization and autologous transplantation were screened for entry into this study. Prior to mobilization, they did not have fever or signs of infection and did not have worsening of their disease.
Study period : 4 February 2005 – last patient's 12-month visit: 22 January 2008.

Conduct of the study

Study 3101:
There were 7 amendments Protocol Version 1.0 and 1 protocol clarification letter. There were also 2 other versions of the protocol, Protocol Version 2.0 (incorporating Amendments 1-6) and Protocol Version 3.0 (incorporating Amendments 1-7). All of the protocol amendments were instituted after initiation of study enrollment. No protocol amendments were made to the study endpoints or efficacy analysis.

Study 3102:
There were 8 amendments to the original protocol, and 2 protocol clarification letters. There were also 2 other versions of the protocol. Since Version 2.0, containing all changes from Amendments 1 – 7 (dated 2 Sep 2005) was never sent to sites, a corrected version, Version 3.0 (dated 16 Sep 2005) contained all changes from Amendments 1-8. No protocol amendments were made to the study endpoints or efficacy analysis.

Baseline data

Baseline characteristics in the phase 3 studies are summarised in the table below.

Table 12. Baseline characteristics in phase 3 studies

Characteristic	Study Number			
	3101: NHL Patients		3102: MM Patients	
	G + plerixafor N = 150	G + placebo N = 148	G + plerixafor N = 148	G + placebo N = 154
Age (years)				
Mean (SD)	55.1 (10.9)	57.5 (10.3)	58.2 (8.4)	58.4 (8.6)
Median	56.0	59.0	58.5	59.0
Min - Max	29 - 75	22 - 75	28 - 75	28 - 75
Gender, n (%)				
Male	100 (66.7)	102 (68.9)	100 (67.6)	107 (69.5)
Female	50 (33.3)	46 (31.1)	48 (32.4)	47 (30.5)
Ethnic Origin, n (%)				
Caucasian	136 (90.7)	140 (94.6)	117 (79.1)	128 (83.1)
African-American	6 (4.0)	1 (0.7)	18 (12.2)	14 (9.1)
Asian	2 (1.3)	2 (1.4)	1 (0.7)	3 (1.9)
Hispanic/Latino	5 (3.3)	4 (2.7)	11 (7.4)	4 (2.6)
Other	1 (0.7)	1 (0.7)	1 (0.7)	5 (3.2)
Stage of Disease at Initial Diagnosis, n (%)				
I	15 (10.0)	10 (6.8)	28 (18.9)	19 (12.3)
II	15 (10.0)	32 (21.6)	29 (19.6)	44 (28.6)
III	29 (19.3)	44 (29.7)	91 (61.5)	90 (58.4)
IV	86 (57.3)	61 (41.2)	NA	NA
Missing	5 (3.3)	1 (0.7)	0 (0.0)	1 (0.6)
Stage of Disease at Study Entry, n (%)				
I	6 (4.0)	12 (8.1)	28 (18.9)	15 (9.7)
II	15 (10.0)	28 (18.9)	25 (16.9)	42 (27.3)
III	32 (21.3)	34 (23.0)	83 (56.1)	83 (53.9)
IV	72 (48.0)	52 (35.1)	NA	NA
Missing	25 (16.7)	22 (14.9)	12 (8.1)	14 (9.1)
Prior Chemotherapy, n (%)				
Yes	145 (96.7)	140 (94.6)	144 (97.3)	148 (96.1)
No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Missing	5 (3.3)	8 (5.4)	4 (2.7)	6 (3.9)

Note: Updated information was provided in Clinical Study Reports dated 28 October 2008 (Study 3101) and 16 October 2008 (Study 3102). Data not shown.

Numbers analysed

The patients used for the primary analysis were those who received G-CSF mobilisation,subsequent treatment (plerixafor or placebo) and apheresis to determine the CD34+ collection yield.

Table 13. Patients in study 3101 and 3102 that completed the study

study	**period**	**completed**					
		yes		**no**		**still on study**	
3101		**G-CSF + plerixafor**	**G-CSF + placebo**	**G-CSF + plerixafor**	**G-CSF + placebo**	**G-CSF + plerixafor**	**G-CSF + placebo**
	G-CSF mobilisation	147	142	3	6	0	0
	Treatment /aphaeresis	135	82	12	60	0	0
	Pre-transplant	135	82	0	0	0	0

	chemotherapy						
	Transplantation	135	82	0	0	0	0
	Post-transplantation /follow-up	66	43	20	10	49	29
3102	G-CSF mobilisation	144	150	4	4	0	0
	Treatment /aphaeresis	142	136	2	7	0	0
	Pre-transplant chemotherapy	142	136	0	0	0	0
	Transplantation	142	136	0	0	0	0
	Post-transplantation /follow-up	82	76	10	15	50	46

Note: Updated information was provided in Clinical Study Reports dated 28 October 2008 (Study 3101) and 16 October 2008 (Study 3102). Data not shown.

Table 14.Summary of patients with HSC transplants study 3101

	G + Plerixafor 240 µg/kg N = 150	**G + Placebo N = 148**
Total Patients Transplanted	135	82
Reached ≥ 2 x 10^6 CD34+ cells/kg in 4 or Fewer Apheresis Days	129[a]	65[a,b]
Did Not Reach 2 x 10^6 CD34+ cells/kg in Fewer than 4 Apheresis Days		
Required More than 4 Apheresis Days	6[c,d]	16[c,d]
Did Not Reach 2 x 10^6 CD34+ cells/kg	0	1[e]
Total Patients Not Transplanted	15	66
Withdrawn from Study During G-CSF Mobilisation	3[f]	6[f]
Withdrawn from Study During Treatment/Apheresis	2[g]	8[g]
Entered Rescue procedure	10	52

Note: Updated information was provided in Clinical Study Reports dated 28 October 2008. Data not shown.

Table 15. Summary of patients with HSC transplants study 3102

	G + Plerixafor 240 µg/kg N = 148	G + Placebo N = 154
Total Patients Transplanted	142	136
Reached ≥ 2 x 10^6 CD34+ cells/kg in Fewer than 4 Apheresis Days	139[a]	127[b,c]
Did Not Reach 2 x 10^6 in Fewer than 4 Apheresis Days	3[e]	9
Received Additional Mobilisation and Reached ≥ 2 x 10^6 CD34+ cells/kg According to Central Lab	0	5[d]
Did Not Reach 2 x 10^6 cells/kg According to Central Lab	2[e]	3[f]
Received Additional Mobilisation and Did Not Reach 2 x 10^6 CD34+ cells/kg According to Central Lab	1[g]	1[h]
Total Patients Not Transplanted	6	18
Withdrawn from Study During G-CSF Mobilisation	4[i]	4[i]
Withdrawn from Study During Treatment/Apheresis	2[j]	7[j]
Entered Rescue Procedure	0	7

Note: Updated information was provided in Clinical Study Reports dated 16 October 2008. Data not shown.

Outcomes and estimation

The tables below summarize the results of the two phase III studies 3101 and 3102.

Table 16. Summary of study 3101

		G-CSF + Plerixafor	G-CSF + Placebo	95% CI	p-value
Primary endpoint Patients with ≥ 5 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis		89/150 (59.3%)	29/148 (19.6%)	29.6, 49.9	<0.001
Composite endpoint (EMEA) Patients with ≥ **2** x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis AND successful PMN and PLT engraftment		126/150 (84.0%)	64/148 (43.2%)	30.9, 50.7	<0.001
Composite Secondary endpoint (EMEA) Patients with ≥ **5** x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis AND successful PMN and PLT engraftment		86/150 (57.3%)	28/148 (18.9%)	28.3, 48.5	< 0.001
Secondary endpoint Patients with ≥ 2 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis		130/150 (86.7%)	70/148 (47.3%)	29.7, 49.1	< 0.001
Engraftment success and time to engraftment (n)					
	PMN engraftment	135/135 (100%)	82/82 (100%)		
	Median time to PMN engraftment	10 days	10 days	0.8, 1.5	0.33
	PLT engraftment	132/135 (97.8%)	81/82 (98.8%)		
	Median time to PLT engraftment	20 days	20 days	0.8, 1.4	0.63
Patients reaching target					
	Aphaeresis day 1	41 (27.9%)	6 (4.2%)		
	Aphaeresis day 2	71	20		

		(49.1%)	(14.2%)		
	Aphaeresis day 3	81 (57.7%)	27 (21.6%)		
	Aphaeresis day 4	89 (65.6%)	29 (24.2%)		
	Median time to target	3.0 days	NE= not estimable	2.4, 5.6	< 0.001
Patients not reaching target by aphaeresis day 4		58 (39.5%)	113 (79.6%)		
patients maintaining a durable graft					
	100 days	128/135 (94.8%)	78/82 (95.1%)		0.67
	6 months	120/123 (97.6 %)	77/78 (98.7%)		0.7
	12 months	110/112 (98.2.0%)	65/65 (100%)		0.56
PB CD34+ cell count x 10^6, mean (SD)					
	Day 4 cells count (cells/µL)	12.0 (12.0) n=131	12.5 (18.5) n=124		0.79
	Day 5 cells count (cells/µL)	53.2 (47.4) n=129	19.2 (23.7) n=122		< 0.001
	Mean fold increase (day 4 to day 5)	6.1 (5.4) n=125	1.9 (1.5) n=118		< 0.001
Overall survival (Cox proportional hazards regression)		132/150 (88.0%)	129/148 (87.2%)		

Plerixafor dose 240 µg/kg

Note: The primary endpoint data was provided in the Clinical Study Report dated 01 April 2008, at the time of MAA filing. The secondary endpoint data was based on the Clinical Study Report dated 28 October 2008.

Table 17. Summary of study 3102

		G-CSF + Plerixafor	G-CSF + Placebo	95% CI	p-value
Primary endpoint Patients with ≥ 6 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis		106/148 (71.6%)	53/154 (34.4%)	26.8, 47.6	<0.001
Composite Secondary endpoint (EMEA) Patients with ≥ **6** x 10^6 CD34+ cells/kg ≤ 2 days of aphaeresis AND successful PMN and PLT engraftment		104/148 (70.3%)	53/154 (34.4%)	25.3, 46.4	<0.001
Secondary endpoint Patients with ≥ 2 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis		141/148 (95.3%)	136/154 (88.3%)	0.8, 13.1	0.031
Secondary endpoint Patients with ≥ 6 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis		112/148 (95.3%)	79/154 (51.3%)	13.9, 34.9	< 0.001
Engraftment success and time to engraftment (n)					
	PMN engraftment	141/142 (99.3%)	136/136 (100%)		
	Median time to PMN engraftment	11 days	11 days	0.8, 1,3	0.69
	PLT engraftment	141/142 (99.3%)	135/136 (99.3%)		
	Median time to PLT engraftment	18 days	18 days	0.7, 1.1	0.18
Patients reaching target					
	Aphaeresis day 1	78	26		

		(54.2%)	(17.3%)		
	Aphaeresis day 2	106 (77.9%)	53 (35.3%)		
	Aphaeresis day 3	112 (86.8%)	71 (48.9%)		
	Aphaeresis day 4	112 (86.8%)	79 (55.9%)		
	Median time to target	1 day	4 days	1.9, 3.4	< 0.001
Patients not reaching target by aphaeresis day 4		32 (22.0%)	71 (47.3%)		
patients maintaining a durable graft					
	100 days	140/142 (98.6%)	133/136 (97.8%)	-2.3, 3.9	0.70
	6 months	133/135 (98.5%)	125/127 (98.4%)	-2.9, 3.1	0.92
	12 months	127/128 (95.3%)	148/154 (96.1%)	-2.2,2.3	0.98
PB CD34+ cell count X 10^6, mean (SD)					
n		122	123		
	Day 4 cells count (cells/µL)	32.5 (43.2)	34.3 (43.6)		0.75
	Day 5 cells count (cells/µL)	143.3 (151.6)	67.3 (185.9)		< 0.001
	Mean fold increase (day 4 to day 5)	6.4 (6.8)	2.4 (7.3)		< 0.001
Overall survival (Cox proportional hazards regression)		141./148 (95.3%)	148/154 (96.1%)	0.4, 3.6	

Note: The primary endpoint data was provided in the Clinical Study Report dated 31 March 2008 at the time of MAA filing. The secondary endpoint data was based on the Clinical Study Report dated 16 October 2008.

Study 3101

The proportion of patients maintaining a durable graft using modified platelet criteria for graft durability (PLT ≥ 20 x 10^9/L without transfusions for at least 2 weeks prior to the visit) were similar in both groups at day 100, 6 months and 12 months.

The measurement time period for fold increase was: from the morning of day 4 (just prior to G-CSF dose) to the morning of day 5 (prior to first aphaeresis). On Day 4 prior to the first dose of plerixafor or placebo the PB CD34+ cell count was similar in both arms. On day 5, 10-11 hours after the first dose, the mean PB CD34+ cell count was significantly higher ($p<0.001$) in the G-CSF + plerixafor group (53.5 cells/µL) compared to G-CSF + placebo (19.2 cells/µL). The mean fold increase was also significantly higher ($p<0.001$) in the plerixafor group.
In addition, the proportion of patients with <10, <15 and <20 PB CD34+ cells were higher in the placebo group.

Overall survival was an exploratory efficacy endpoint as well, no difference between the treatment arms were seen. Overall survival (without rescue patients): 18 patients died and 132 alive in the plerixafor group versus 19 patients died and 148 alive in the placebo group.

Study 3102

The proportion of patients maintaining a durable graft using modified platelet criteria for graft durability (PLT ≥ 20 x 10^9/L without transfusions for at least 2 weeks prior to the visit) were similar in both groups at day 100, 6 months and 12 months.

The measurement time period for fold increase was: from the morning of day 4 (just prior to G-CSF dose) to the morning of day 5 (prior to first aphaeresis). On Day 4 prior to the first dose of plerixafor or placebo the PB CD34+ cell count was similar in both arms. On day 5, 10-11 hours after the first dose, the mean PB CD34+ cell count was significantly higher ($p<0.001$) in the G-CSF + plerixafor

group (143.3 cells/μL) compared to G-CSF + placebo (67.3 cells/μL). The mean fold increase was also significantly higher ($p<0.001$) in the plerixafor group.
In addition, the proportion of patients with <10, <15 and <20 PB CD34+ cells were higher in the placebo group.

Overall survival was an exploratory efficacy endpoint as well, no difference between the treatment arms were seen. Overall survival: 7 patients died and 141 alive in the plerixafor group versus 6 patients died and 148 alive in the placebo group.

Rescue procedure: Study 3101

A total of 62 patients entered the rescue procedure: 10/150 (6.7%) of the patients in the G + plerixafor group and 52/148 (35.1%) of the patients in the G + placebo group. In the initial treatment/apheresis period, these patients failed to collect 2 x 10^6 CD34+ cells/kg (59 according to both central and local laboratory data; 3 patients [in the G + placebo group] collected ≥ 2 x 10^6 cells/kg according to central laboratory data but not according to local laboratory data). After entering the rescue procedure, 37 (59.7%) of the patients collected the minimum number of CD34+ cells required for transplantation (≥ 2 x 10^6 CD34+ cells/kg) in 4 or fewer days of apheresis: 4/10 (40.0%) of the Rescue patients from the G + plerixafor group and 33/52 (63.5%) of the Rescue patients from the G + placebo group. In addition, 7 of the patients who collected ≥ 2 x 10^6 CD34+ cells/kg also collected ≥ 5 x 10^6 CD34+ cells/kg in 4 or fewer days of apheresis (7/62, 11.3%; all from the G + placebo group). These results were based on the data provided in the Clinical Study Report dated 01 April 2008 at the time of MAA filing.

Rescue Procedure: Study 3102
The Rescue patients were patients who failed mobilization (i.e., who did not collect ≥ 0.8 x 10^6 CD34+ cells/kg after 2 days of apheresis, who did not collect at least 2 x 10^6 CD34+ cells/kg in 4 or fewer days of apheresis, or patients who were planned for tandem transplant and did not collect at least 4 x 10^6 CD34+ cells/kg in 4 or fewer apheresis days) and chose to enter the open label rescue procedure. Seven patients entered the rescue procedure, 4 of whom did not collect at least 2 x 106 CD34+ cells/kg (3 of these had been planned for tandem transplant), and 2 of whom were planned for tandem transplant and did not collect at least 4 x 10^6 CD34+ cells/kg according to the local laboratory (central laboratory values, used for efficacy parameters, may have varied from these results). The seventh rescue patient was planned for a tandem transplant, and collected exactly 4 x 106 CD34+ cells/kg according to the local laboratory, but collected fewer cells according to the central laboratory. All of these patients were initially treated with placebo in the randomized trial.
During the rescue procedure, 7/7 (100%) of Rescue patients achieved a minimum target dose of ≥ 2 x 10^6 cells/kg in 4 or fewer apheresis days, while 2/7 (28.6%) of the patients achieved the primary endpoint of ≥ 6 x 10^6 CD34+ cells/kg in 2 or fewer apheresis days. Three of seven patients (42.9%) met the ≥ 6 x 10^6 CD34+ cells/kg in 4 or fewer apheresis days endpoint. Of the Rescue patients, 6/7 were initially planned to receive tandem transplant, and in the rescue procedure 4/6 of these (5/7 overall). These results were based on the data provided in the Clinical Study Report dated 31 March 2008 at the time of MAA filing.

Rituximab patients
A small number of patients could be enrolled in the study if they received rituximab (currently used in combination chemotherapy for NHL). There were 6 patients in the G-CSF + plerixafor and 7 patients in the G-CSF + placebo group. Nine of these 13 patients collected the minimum of CD34+ cells (≥ 2 x 10^6 cells/kg) and 11 underwent transplantation. Durable grafts at 12 months were as follows: 4/4 in the G-CSF + plerixafor and 4/5 patients in the G-CSF + placebo group. These results were based on the data provided in the Clinical Study Report dated 28 October 2008.

Ancillary analyses

- Analysis performed across trials (pooled analyses and meta-analysis)

No analysis were performed across trials.

- Clinical studies in special populations

No clinical studies in special populations were performed.

- Supportive study(ies)

The main supportive studies conducted were as follows:
- 2 open-label phase 2 studies (2101 and 2106).

- 4 Phase 2 studies of plerixafor in conjunction with G-CSF in oncology patients
- 1 Phase 2 study of plerixafor alone in oncology patients.
- 1 Phase 2 study of plerixafor in conjunction with G-CSF and chemo-mobilization in oncology patients.
- 1 Phase 2 study of plerixafor in conjunction with G-CSF and rituximab in oncology patients.
- 2 Phase 2 studies of plerixafor in conjunction with G-CSF in poor mobilisers
- 1 Phase 2 study of plerixafor alone in patients with HIV
- Compassionate Use Programme (**CUP**) of plerixafor in conjunction with G-CSF in oncology patients.
- 6 ongoing clinical studies

The following is a short summary of the results from these supportive studies.

Study 2101
This was a multicentre, crossover study in 25 patients with NHL (n=15) and MM (n=10). Patients were randomly assigned to receive G-CSF + plerixafor or G-CSF alone as initial mobilising regimen, followed by a 2-week washout and remobilisation with the alternated regimen. After 8 patients the dose from 160 µg/kg was raised to 240 µg/kg because equal or better CD34+ cell mobilisation was found with this dose in the healthy volunteer study and in 1 oncology study at that time. Additionally the start of plerixafor was changed from day 5 to day 4 and G-CSF run-in was changed from 3 to 4 days. The initial study design was a randomised crossover study with patients randomised to either G-CSF + plerixafor first followed by G-CSF alone or the reverse. After 12 patients the randomisation was removed and the remaining 13 patients received G-CSF alone in the mobilisation period.

Primary objective
To evaluate the difference in number of CD34+ cells/kg collected after mobilisation with G-CSF and plerixafor compared with that collected after mobilisation.

Table 18. Summary of study 2101

	NHL patients		MM patients	
	G-CSF + Plerixafor	G-CSF	G-CSF + Plerixafor	G-CSF
Primary endpoint Patients with ≥ 2 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis	15 (100%)	7 (46.7%)	10 (100%)	9 (90%)
Primary endpoint Patients with ≥ 5 x 10^6 CD34+ cells/kg ≤ 4 days of aphaeresis	10 (66.7%)	3 (20.0%)	10 (100%)	5 (50%)

Study 2106

This was a single centre study to assess the efficacy and safety of G-CSF and plerixafor 240 µg/kg in patients with Hodgkin's disease (n=22). The number of cells collected and the rate of failure to collect a minimum of $\geq 2 \times 10^6$ CD34+ cells/kg were compared to historical controls that had mobilised with G-CSF alone.
In- and exclusion criteria were similar to phase 3 studies (see above), with the exception of the waiting periods for chemotherapeutics (at least 1 week from last dose of Velcade® and dexamethasone in phase 3 studies) and the exclusion of the use of bone-seeking therapeutic radionuclides in the phase 3 studies (not excluded in phase 2).

Primary objective
To determine the proportion of HD patients who had $\geq 5 \times 10^6$ CD 34+ cells/kg after HSC mobilisation with G-CSF and plerixafor. The number of PB HSCs collected and the rate of failure to collect $\geq 2 \times 10^6$ CD34+ cells/kg were compared to 2 sets of data generated from historical controls that had mobilised with G-CSF alone.

Table 19. Summary of study 2106

	Open label historical controls with HD (n=22)
	G-CSF + Plerixafor
Primary endpoint Patients with $\geq 2 \times 10^6$ CD34+ cells/kg ≤ 4 days of aphaeresis	20 (91%)
Primary endpoint Patients with $\geq 5 \times 10^6$ CD34+ cells/kg ≤ 4 days of aphaeresis	15 (68%)

Study 2108
In this study 9 patients with MM were mobilized with plerixafor 240 µg/kg alone for up to 4 consecutive days. All patients achieved at least 2×10^6 CD34+ cells/kg and all were transplanted but only 6 were followed up 12 months after transplantation, all having durable grafts. Less than half of the patients achieved more than 4×10^6 CD34+ cells/kg. Median times to PMN engraftment was 10.5 days and the median time to PLT engraftment was 21 days in the 7 patients (6 for PLTs) with engraftment data. The study was terminated early (originally 20 patients were planned for enrolment.

Study 2105
This open-label, single-arm, multi-center study evaluated the safety and efficacy of plerixafor injection 240 µg/kg, used in addition to a standard 10 µg/kg G-CSF mobilization regimen, for the collection of peripheral blood stem cells for autologous transplantation. Enrolment ended when Phase 3 plerixafor trials were initiated. A total of 49 patients (23 NHL, 26MM) were enrolled and analyzed. The results are supportive of those for the studies 3101 and 3102 with identical treatment design.

Study 2104
This study assessed the safety and efficacy of plerixafor 240 µg/kg for HSC mobilization in patients with MM and NHL when administered at least 6 days following a regimen of mobilizing chemotherapy and G-CSF 10 µg/kg daily. 40 (26 MM and 14 NHL) patients received a regimen of mobilizing chemotherapy and G-CSF, following which 1 apheresis collection was performed when CD34+ counts of ≥ 20 cells/µL were measured in PB. The second and subsequent apheresis collections were performed following plerixafor treatment. Mean apheresis yields were increased in the second apheresis (Day 2, post-plerixafor), when compared with the first (Day 1, pre-plerixafor) apheresis, for both MM and NHL patient groups. Overall, 19 of 21 (90.5 %) patients with MM and 9 of 13 (69.2 %) patients with NHL achieved a greater apheresis yield on Day 2. In a subgroup of patients the mean rate of increase of PB CD34+ cells was 4.75-fold greater following the first dose of plerixafor than the pre-plerixafor rate.

Study 2109
Five patients with MM or NHL having started standard G-CSF mobilization were enrolled. A PB CD34+ cell count of 5 to 19 CD34+ cells/μL at Day 5 was required for study entry. If the patient's PB CD34+ cell count on Day 5 was 5 to 7 cells/μL, then he/she was predicted to be a poor mobiliser. The patient did not undergo apheresis that day and received a dose of plerixafor 240 μg/kg in the evening. This process of an evening dosing with plerixafor followed by G-CSF and apheresis the next morning was repeated for up to a total of 3 days of apheresis or until $\geq 5 \times 10^6$ cells/kg were collected. The study was terminated early due to competing resources with the Phase 3 studies (planned enrolment was 15 patients) and no analyses of efficacy were made

Study 2112
In this ongoing study, 40 patients with diagnoses of NHL (27 patients), MM (5 patients), HD (6 patients), AML (1 patient, before amendment), and desmoplastic small round cell tumor (DMRCT: 1 patient) were enrolled after having previously failed mobilization (planned enrolment 100 patients). Patients received G-CSF and plerixafor in the same posology as for the pivotal studies.

Study 2102
In this study, 10 patients with MM in Group A were required to have had a previous mobilization from which they were unsuccessful in collecting an adequate number of CD34+ cells for transplantation (usually $\geq 2 \times 10^6$ cells/kg). 10 patients enrolled in Group B were required to have received previous chemotherapy and G-CSF treatment as a mobilization regimen who, following recovery of their WBC count (> 2,000 cells/μL for at least 2 days), had a PB CD34+ count of 5 to 12 cells/μL. Or, patients in Group B were required to have received extensive prior chemotherapy and a pre-mobilization PLT count of 100,000 to 150,000/μL (2 measurements obtained within 1 week). Patients in Group B were expected to yield total apheresis collections of $< 1 \times 10^6$ CD34+ cells/kg, which would be insufficient for transplant. All 20 patients in group A and B received mobilizing treatment with daily G-CSF 10 μg/kg and plerixafor 240 μg/kg. Overall, 17 patients received either 1 or 2 transplants. PMN engraftment was observed for all patients and PLT engraftment was observed for 16/17 patients (94.1 %). No PLT engraftment data were recorded for 1 patient. All 12 patients assessed at 12 months post-transplant were determined to have a durable graft.

Study 2103
In this study of safety and preliminary efficacy, 13 patients with NHL received plerixafor 240 μg/kg after 4 days of daily G-CSF 10-16 μg/kg, and were transplanted after apheresis with collection of 2-5 x 10^6 CD34+ cells/kg.

Study C201
22 patients (8 NHL, 14 MM) in Canada were mobilized with G-CSF and plerixafor (same regimen as study 3101 and 3102), transplanted and followed.

Study EU21
35 patients (4 NHL, 31 MM) in Germany received 48 transplants after mobilization with G-CSF + plerixafor given as in the randomized pivotal studies 3101 and 3102. Platement engraftment was achieved after a median of 11 days and PMN engraftment was achieved after a median of 14 days. In a laboratory study, G-CSF + plerixafor mobilized CD34+ PBPCs from 3 patients expressed significantly higher amounts of genes that potentially promote superior engraftment after myeloablative therapy than G-CSF mobilized CD34+ PBPCs from another 3 patients. The median number of PB CD34+ cells achieved as determined by the central lab was 7.4 x 10^6 cells/kg overall, 8.3 x 10^6 cells/kg in patients with NHL, and 7.1 x 10^6 cells/kg in patients with MM. As determined by the central laboratory, 94% of patients reached the minimum target PB CD34+ cell collection of ≥ 2 x 10^6 cells/kg. Two patients with MM did not meet the minimum target PB CD34+ cell collection; both patients were transplanted and successfully engrafted

Study 2113
In this study with planned enrolment of 30 patients, hitherto 20 patients with HD or NHL have been enrolled, where CD20+ lymphomas have received rituximab 1 x / week in 3 weeks from the week

before starting G-CSF + plerixafor whereas CD20- lymphomas have received only G-CSF + plerixafor.(data not shown).

Study 2001
This was a dose-escalating study in HIV patients investigating safety, pharmacokinetics and antiviral activity of intravenous plerixafor treatment up to doses 160 µg/kg/h for 10 consecutive days (data not shown).

AMD3100-CUP (enrolment ongoing)
The compassionate use program (CUP) was an option for patients who had failed previous conventional therapies for stem cell collection or based upon a low PB CD34+ cell count following conventional therapy were not considered by the physician to have a reasonable chance of collecting enough cells for autologous transplantation (data not shown).

- Discussion on clinical efficacy

Efficacy was shown by 2 randomized placebo-controlled studies of the combination of G-CSF + plerixafor compared to G-CSF + placebo in NHL and MM respectively concerning stem cell mobilization and successful autologous transplantation. Consistent results were shown also in a study of Hodgkin´s disease. In addition, the combination of G-CSF + plerixafor was shown to be more effective than G-CSF alone for harvest of CD34+ cells in all 25 patients in a cross-over study in NHL and MM. Several other studies support these findings of efficacy in combination with G-CSF.

In NHL, 70 (47.3%) of the patients being treated with G-CSF + placebo , collected the minimum number of CD34+ cells required for transplantation (≥ 2 x 106 CD34+ cells/kg) in 4 or fewer days of apheresis during the first treatment phase. In contrast, for those poor mobilisers who did not reach the target number of CD34+ cells during the first treatment phase, 37 (59.7%) of them collected the minimum number of CD34+ cells required for transplantation in 4 or fewer days of apheresis, after entering the rescue procedure.

In MM, 136 (88.3%) of the patients being treated with G-CSF + placebo , collected the minimum number of CD34+ cells required for transplantation (≥ 2 x 106 CD34+ cells/kg) in 4 or fewer days of apheresis during the first treatment phase. In contrast, for those poor mobilisers who did not reach the target number of CD34+ cells during the first treatment phase, 7 (100%) of them collected the minimum number of CD34+ cells required for transplantation in 4 or fewer days of apheresis, after entering the rescue procedure.

In contrast to the quantitative results of CD34+ cell numbers, engraftment data have not been shown advantageous for the combination of G-CSF + plerixafor compared to G-CSF alone. No dose-response relationship between plerixafor mobilized CD34+ cell numbers infused at transplantation and time to engraftment has been shown or evaluated although such a relationship was shown for both treatment arms when pooled together in studies 3101 and 3102.

The following major objections were raised with regards to the clinical efficacy data submitted.

1. The CHMP considered that a broad indication (patients with lymphoma and multiple myeloma) was not approvable, mainly because the possibility of tumour cell mobilization has not been sufficiently addressed to either confirm or exclude it. Following the Applicant's responses, tumour cell mobilisation by plerixafor was only sought for in a limited number of patients. However, from these studies little or no mobilisation of tumour cells was detected, using the recommended dose.

The mode of action of plerixafor, which is different from growth factors, is such that binding of the CXCR4 receptor, which is present also on tumour cells, may enhance mobilisation of tumour cell. This effect may be dose dependent. If this effect is quantitative also the quantitative expression on the tumour cell may play a role. It was also argued that growth factors like G-CSF may also mobilise

tumour cells, but it can equally not be excluded that growth factors and plerixafor may mobilise stem cells with a different potential, the stem cells mobilised by plerixafor being in part the cells with malignant stem cell potential

For both these reasons the CHMP warranted caution in the use of plerixafor in patients with lymphoma and multiple myeloma, who are considered good mobilisers, even if these limited studies did not show a significant difference in the number of mobilised stem cells in both arms, determined with markers characteristic for mature B cells or plasma cells. An overview of progression free survival in the pivotal studies presented was considered necessary. The Applicant committed to extend the long-term follow (LTF) up for our two controlled Phase 3 studies AMD3100-3101 and AMD3100-3102 to 5 years, including evaluation of relapse, progression-free survival (PFS), and overall survival. However, it should be noted that the original Phase 3 studies and the protocol for the long-term follow-up studies were not designed to capture PFS (overall survival was captured). In addition to this, special warnings for the potential for tumor cell mobilization in patients with lymphoma and multiple myeloma, as well as tumor cell mobilization in leukaemia patients were adequately reflected in section 4.4 of the SPC.

For patients with MM and lymphoma, whose cells mobilise poorly,, treated with G-CSF alone, the CHMP considered that the risk benefit ratio was different to that of the overall population. Given that in myeloma and in relapsed large B cell lymphoma the chance of cure is greater after high dose treatment, it was considered of importance to make an effort to harvest a sufficient transplant. However, it is in this group where the chance of mobilisation of tumour cells may also be greater, because poor mobilisation may be caused by infiltration of malignant cells in the bone marrow. Therefore, the CHMP considered it important for the Applicant to obtain further data on tumour cell mobilisation in the poor mobilisor group (compared with the previous mobilisation attempt with G-CSF). Hence, tumour cell mobilisation specifically in poor mobilisers will be addressed by the Applicant by evaluation of PFS (as well as other long-term outcomes) by use of a registry, in collaboration with EBMT. To this effect, the Applicant submitted an outline of the design of the registry (Proposed Plan: To Demonstrate The Safety Of Mozobil To Mobilize Stem Cells In Poor Mobilisers With Lymphoma Or Multiple Myeloma).

2. The CHMP considered that it remained unclear whether Mozobil could induce tumour cell mobilization. Therefore, the Applicant was asked to present a review of the topics CXCR4 expression in myeloma and lymphoma (including Hodgkin´s disease) and to discuss whether CXCR4 should be considered a marker for tumour stem cells in these malignant disorders, regarding all relevant and modern knowledge from the literature and other sources. An extensive overview was given on the expression of the CXCR4 receptor expression on the different cells. From the data presented, the CHMP noted that this receptor is also expressed on lymphoma and myeloma cells. The function of this receptor and the effect of inhibition remains ill-understood in the clinical setting. Therefore, a mobilising effect on lymphoma and myeloma cells was neither shown, nor excluded in the data presented. Also a protective (e.g anti-apoptotic) effect on tumour cell elimination by chemotherapeutic agents or corticosteroids was suggested in cell lines and not excluded in the therapeutic setting. In conclusion, the CHMP was of the opinion that these uncertainties underlined the precautions raised in the first major objection and that monitoring not only of cell contamination of the graft but also of clinical parameters was necessary. To this effect, the Applicant committed to extend the long-term follow (LTF) up for our two controlled Phase 3 studies AMD3100-3101 and AMD3100-3102 to 5 years, including evaluation of relapse, progression-free survival (PFS), and overall survival. The Applicant also committed to assess available tumour cell mobilization lymphoma and/or multiple myeloma animal models that could provide meaningful data comparing different mobilization regimens (See non-clinical discussion).

3. The CHMP requested that the Applicant justify the additional benefit of Mozobil over G-CSF alone in the broad population. The Applicant was requested to discuss the apheresis technique and its clinical relevance for demonstrating which type of patients may benefit from plerixafor therapy, considering the currently available therapeutic alternative of G-CSF. In addition to this, the CHMP

requested the indication to include only poor mobilisers, as the clinical usefulness of plerixafor as add-on therapy had been clearly shown for this subset of patients.

In its responses, the Applicant clearly discussed the apheresis technique, which was deemed to be fully in accordance with the current international guidelines. However, with regards to the benefit/risk of plerixafor for a broad population , the CHMP considered that no new information had been provided to modify their opinion to restrict the indication to poor mobilisers. Overall, addition of plerixafor may improve the success rate of mobilisation of enough haematological stem cells in some instances, but in all other aspects (safety, predictability in peak CD34 content or number of aphereses) G-CSF was considered by CHMP to be reliable. In conclusion, the CHMP considered that an additional benefit of Mozobil in a broad indication was not substantiated.

To address the risk of potential tumour cell mobilisation with plerixafor, the Applicant proposed the following measures to provide reassurance on the long-term safety of the drug:

- to extend the long- term follow-up (LTF) for the two controlled Phase 3 studies from 3 to 5 years, including evaluation of relapse, progression-free survival, and overall survival.
- to develop with CHMP a long-term, post-approval transplant registry

When consulted in this matter, the SAG-Oncology agreed that in an indication including patients with lymphoma and multiple myeloma without restrictions, more precise data about the risks of tumour cell mobilisation would be necessary in order to establish that the benefits outweigh the risks. However, in patients in whom mobilisation is, or can be expected to be, poor the risk was considered acceptable if there are no alternative treatment options for mobilisation. In this population careful long term follow-up was recommended in order to rule out a detriment in terms of PFS. Any available safety and efficacy data in this subgroup alone should be provided. What constitutes "patients in whom mobilisation is, or can be expected to be, poor" was difficult to define although, generally, treatment with G-CSF alone should be given for at least 4 days before concluding that mobilisation is poor. In some situations, the previous treatment history may clearly indicate a high risk of poor mobilisation (e.g., multiple treatments with fludarabine). In other situations, it may depend on the planned treatment and how many cells one is aiming to mobilise.

Based on this evidence, the CHMP agreed to restrict the indication to poor mobilisers, considered as patients who failed to reach enough CD34+ cells in the peripheral blood after stimulation with G-CSF + chemotherapy or with G-CSF alone. Given that the number of CD34+ cells needed to consider a patients a poor mobiliser may vary in different transplant centres, the CHMP preferred not to define details on this subject and to leave the decision to the individual centres.

4. The CHMP requested that the Applicant clarify the apparently low efficacy in the G-CSF alone group. In its responses, the Applicant argued that differences in the definition of mobilisation failure, different G-CSF schedules or apheresis techniques, made comparison with literature data difficult. However, these definitions were the same for the control arm and the study arm in the studies under evaluation. The doses of G-CSF used in the studies (10mugr/kg once daily) were the same as recommended by the marketing authorisation holder for mobilisation after chemotherapy and mostly used in clinical practice.

The CHMP noted that in general, one or two aphereses are sufficient to obtain the required amount of stem cells with this dose. A study, cited by the Applicant, of Stiff et al. (2000) to confirm their data on the success rate in the G-CSF alone group, was performed in heavily pre-treated patients, not in the general population identified for autologous stem cell transplantation. In most protocols, autologous stem cell transplantation is part of the first treatment (multiple myeloma) or is used in patients after induction of their first relapse (chemosensitive large B cell lymphoma patients). In the survey by Bensinger et al (2008) a failure rate was found between 5 and 30 %. Both studies underlined that in most cases mobilisation with G-CSF is successful, which makes a broad indication for addition of plerixafor not reasonable, as the use of this drug leads also to additional toxicity.

Several characteristics can predict failure, such as pretreatment, mobilising chemotherapy and (previous) localisation of malignant cells in the bone marrow. The best indicator for mobilisation outcome is the estimation of CD34+ cells in the blood in advance of mobilisation. The CHMP noted that this was not scheduled in the studies under discussion: all patients proceeded to apheresis. This could also in part explain the relatively high failure rate. Overall, from the data given by the Applicant and from the cited literature, a sufficient explanation for the relative low mobilisation rate in the G-CSF alone arm was not identified.

When consulted on this matter, the SAG-Oncology stated it is difficult to speculate about the reasons for the observed results in the G-CSF alone arm. A number of important prognostic factors could have contributed to this observation, including definition of failure in published series, previous treatments, different dose and G-CSF regimens with or without chemotherapy, indications and patient populations. Overall, the SAG acknowledged that the effect observed for GSF alone was low but it was difficult to conclude on the significance of this result. In addition to this, the effect of Mozobil added to G-CSF plus chemotherapy could not be estimated or extrapolated based on the data submitted from the pivotal studies, which only concerned the addition of Mozobil to G-CSF alone.

The following additional concerns were raised with regards to the clinical efficacy data submitted for this application.

2. The CHMP requested that the Applicant demonstrate that plerixafor does not inhibit CYP2A6, CYP2B6, CYP2C8 and CYP2E1. The Applicant submitted results of such study and adapted the SPC accordingly: *Plerixafor is not metabolized in vitro using human liver microsomes or human primary hepatocytes and does not exhibit inhibitory activity in vitro towards the major drug-metabolising CYP450 enzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5)*

3. The CHMP considered that the Applicant should attempt new ways of analyzing and presenting data concerning the dose-response relationship between CD34+ cell numbers and time to engraftment, especially for the patients treated in the G-CSF + plerixafor arm of studies 3101 and 3102.

According to the data submitted, the median transplanted cell doses were thus 40% and 36% higher in the plerixafor treated than in the placebo treated groups in studies 3101 and 3102 respectively. In addition to these analyses, some further descriptive analyses could further elucidate the actual relationships between cell dose and haematodynamic response. Therefore, the Applicant performed simple correlation plots for the plerixafor and placebo treatment arms separately showing relationships between transplanted cell doses and platelet (and other blood cell) counts as continuous variables for appropriately chosen time points.

4. The Applicant was requested to discuss the impact of previous treatments (especially chemotherapies used before the mobilisation) on the effectiveness of plerixafor. In the responses, the Applicant concluded that the use of prior fludarabine and Revlimid did not affect CD34+ stem cell mobilisation. Therefore, the CHMP considered that although the number of patients with prior chemotherapywere very small, the data provided indicated a comparable CD34+ stem cell mobilisation in patients who had previously been treated with chemotherapy.

Clinical safety

Studies contributing to the analysis of safety are summarized in the table below. Safety parameters included physical examination, vital signs, the incidence of AEs, serious AEs, graft failures, medical history, injection site assessment, monitoring of concomitant medications and laboratory evaluations.

Table 20. Overview of all studies with plerixafor used for safety analysis

study		Type and number of subjects	Aim
98-01 1002		Healthy subjects	

1003 1005		N=76	
1004	Phase I	Oncology patients N=21	To show that single dose of plerixafor could mobilise CD34+ cells
1101	Phase I	Renal impairment non-oncology patients N=17	To study the safety, pharmacokinetics and haematological activity of plerixafor in subjects with renal impairment.
2001	Phase I/II	HIV-patients N=40	Dose escalation study of the safety and antiviral activity of plerixafor administered by continuous i.v. route.
2101	Phase II	NHL, MM N=25	To assess the potential advantage of G-CSF+ plerixafor over G-CSF alone
2102 2103		NHL N=20 MM N=13	To assess efficacy of plerixafor In predicted and poor mobilisers
2104	Phase II	N=44	To evaluated the use of G-CSF + plerixafor with chemotherapy mobilisation
2105		NHL and MM N=49	
2106		HD N=22	
2108	Phase II	Plerixafor monotherapy N=9	To show that plerixafor mobilised CD34+ cells were capable of prompt and durable engraftment
2109		NHL and MM N=5	
2112	Phase II	N=40	
2113	Phase II	NHL, NH disease N=15	To evaluate the concomitant use of G-CSF + plerixafor and rituximab
EU21		NHL and MM N=35	To obtain evidence of efficacy and safety .
C201	Follow up ongoing		
CUP		Subset: Paediatric patients N=8	
Compassionate use program	Enrolment ongoing	N=368	
3101 and 3102	Phase 3	NHL and MM N=614	To obtain definitive evidence of efficacy and to define safety profile of plerixafor compared to placebo

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- Patient exposure

Data of 21 clinical trials and 1 compassionate use program are presented. Doses ranged from 160-320 µg/kg, with >95% treated with plerixafor 240 µg/kg.

For the evaluation of safety the data were divided in 5 periods:.

Period 1 mobilisation and apheresis
Period 2 myeloablative chemotherapy, transplantation and post-transplantation through engraftment
Period 3 post-engraftment
Period 4 equivalent to period 2 pertain exclusively to the 2nd transplant for patients undergoing tandem transplant
Period 5 equivalent to period 3 pertain exclusively to the 2nd transplant for patients undergoing tandem transplant

An overview is presented in the tables below.

Table 21. Patients enrolled and treated in the 21 studies and CUP

Treatment	N
Ever received plerixafor[a, b]	**1161**
Received placebo then plerixafor	59
Received plerixafor alone[a]	139
Received G + plerixafor[b]	963
Received G-CSF + placebo	**239**
Received G-CSF but never received plerixafor or placebo[c]	**26**
Total patients enrolled and treated	**1426**

The table below summarizes drug exposures in all oncology studies in patients with NHL, MM and HD.

Table 22. Drug exposure in all oncology studies in patients with NHL, MM and HD

	G-CSF + plerixafor				G-CSF + placebo		
	NHL	MM	HD	total	NHL	MM	total
Cumulative dose of G-CSF/body weight (µg/kg)							
N	242	255	38	537	144	150	294
Mean (SD)	66.5 (19.7)	63.4 (31.3)	70.1 (25.0)	65.3 (26.2)	66.3 (14.4)	69.9 (13.7)	68.1 (14.1)
Median	63.5	59.6	60.5	60.1	69.3	72.0	70.0
Range	10-176	14-402	48-159	10-402	20-90	22-107	20-107
missing	2	0	1	3	1	0	1
Cumulative dose of plerixafor or placebo (mg)							
N	241	251	38	532	142	148	290
Mean (SD)	56.3 (30.6)	44.6 (30.0)	56.2 (45.2)	50.8 (32.0)	65.2 (26.1)	64.1 (24.2)	64.6 (25.1)
Median	52.0	38.4	40.7	44.0	64.0	61.6	64.0
Range	10-196	11-165	15-323	10-232	13-146	18-132	13-146
missing	3	4	1	8	3	2	5
Cumulative dose of plerixafor or placebo /body weight (µg/kg)							
N	239	251	37	529	142	148	290
Mean (SD)	655.4 (333.3)	551.0 (385.7)	619.3 (402.9)	603.9 (366.5)	738.7 (231.7)	748.1 (234.7)	743.5 (232.9)
Median	507.1	478.5	484.9	483.9	744.9	740.5	742.7
Range	156-	225-	210-	156-	236-	236-	236-

	2057	2345	1746	2345	1014	1116	1116
missing	5	4	2	11	3	2	5
Average daily dose of plerixafor or placebo /body weight (µg/kg) N (%)							
160	5 (2.0%)	4 (1.6%)	0	9 (1.7%)	0	0	0
240	234 (95.9%)	245 (96.1%)	37 (94.9%)	518 (95.9%)	142 (97.9%)	148 (98.7%)	290 (98.3%)
320	0	1 (0.4%)	0	1 (0.2%)	0	0	0
>400	0	1 (0.4%)	0	1 (0.2%)	0	0	0
missing	5 (2.0%)	4 (1.6%)	2 (5.1%)	11 (2.0%)	3 (2.1%)	2 (1.3%)	5 (1.7%)
Number of G-CSF administrations							
N	244	255	39	540	144	150	294
Mean (SD)	6.7 (1.8)	6.4 (2.8)	6.6 (2.6)	6.5 (2.4)	6.9 (1.2)	7.1 (1.0)	7.0 (1.1)
Median	6.0	6.0	6.0	6.0	7.0	7.0	7.0
Range	1-19	3-35	4-16	1-35	2-8	4-8	2-8
Missing	0	0	0	0	1	0	1
Number of plerixafor or placebo administrations							
n	241	251	38	532	142	148	290
Mean (SD)	2.8 (1.5)	2.3 (1.6)	2.6 (1.7)	2.5 (1.6)	3.1 (1.0)	3.1 (1.0)	3.1 (1.0)
Median	2.0	2.0	2.0	2.0	3.0	3.0	3.0
range	1-11	1-10	1-8	1-11	1-4	1-4	1-4
missing	3	4	1	8	3	2	5
Number of days exposed							
n	244	255	39	540	144	150	294
Mean (SD)	6.7 (1.8)	6.5 (3.1)	6.7 (2.5)	6.6 (2.5)	7.0 (1.1)	7.1 (1.0)	7.0 (1.1)
Median	6.0	6.0	6.0	6.0	7.0	7.0	7.0
range	1-19	3-39	4-16	1-39	2-8	4-8	2-8
missing	0	0	0	0	1	0	1

For poor mobilisers the mean (SD) cumulative dose of G-CSF / body weight was 82.6 µg/kg (± 41.7) and the mean number of administrations was 8.3. The mean cumulative dose of plerixafor was 906.0 µg/kg / body weight (± 441.7) with an average of 3.8 administrations. 68.7 % of the poor mobilisers had NHL, 25.2% MM, 4.6% HD and 1.5% other. The majority had stage III disease. The poor mobilisers population consisted of 72 patients from phase 2 studies and 59 patients from phase 3 studies. 16/131 (11.9%) of the patients died. Twelve point two percent (12.2 %) of the poor mobilisers died. All patients had prior chemotherapy and 35/131 (26.7%) had received prior radiotherapy

Of the patients in all oncology studies, all patients except 1 patient in the G-CSF + placebo had received prior chemotherapy, 27% of the G-CSF + plerixafor and 26% of the G-CSF + placebo patients had received prior radiotherapy. Stage I and III disease were similar in both groups, however stage IV disease was greater in the G-CSF + plerixafor group (23%) compared to 17% of the G-CSF + placebo group.

The table below present the disposition of the patients in all oncology studies at the time of MAA filing, 50% plerixafor and 40% placebo patients completed the pivotal studies and 33 and 25% resp. In the pivotal studies 3 patients in the plerixafor and 1 patient in the placebo arm discontinued due to unacceptable AEs.

Table 23. Disposition of patients in all oncology studies

Parameter	G + Plerixafor (N = 540)				G + Placebo (N = 295)		
	NHL (N = 244)	MM (N = 255)	HD (N = 39)	Total (N = 540)	NHL (N = 145)	MM (N = 150)	Total (N = 295)
Disposition for patients in Protocols: 2101, 2102, 2103, 2105, 2106, 2109, 2112 EU21 and C201	93	107	29	231	0	0	0
Completed treatment period, n (%)							
Yes	86 (92.5)	102 (95.3)	28 (96.6)	218 (94.4)	N/A	N/A	N/A
No	7 (7.5)	5 (4.7)	1 (3.4)	13 (5.6)	N/A	N/A	N/A
If no, reason period not completed, n (%)							
Unacceptable adverse events	1 (1.1)	1 (0.9)	0 (0.0)	2 (0.9)	N/A	N/A	N/A
Intercurrent illness	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	N/A	N/A	N/A
Elective withdrawal	0 (0.0)	3 (2.8)	0 (0.0)	3 (1.3)	N/A	N/A	N/A
Noncompliance	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	N/A	N/A	N/A
Subject died	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	N/A	N/A	N/A
Lost to follow up	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	N/A	N/A	N/A
Failed mobilization	1 (1.1)	0 (0.0)	1 (3.4)	2 (0.9)	N/A	N/A	N/A
Other	5 (5.4)	1 (0.9)	0 (0.0)	6 (2.6)	N/A	N/A	N/A
On-study patient deaths[a], n (%)	11 (11.8)	6 (5.6)	0 (0.0)	17 (7.4)	N/A	N/A	N/A

Parameter	G + Plerixafor (N = 540)				G + Placebo (N = 295)		
	NHL (N = 244)	MM (N = 255)	HD (N = 39)	Total (N = 540)	NHL (N = 145)	MM (N = 150)	Total (N = 295)
Disposition for patients in Protocols: 2113, 3101 and 3102[b]	151	148	10	309	145	150	295
Completed study, n (%)							
Yes	67 (44.4)	83 (56.1)	0 (0.0)	150 (48.5)	42 (29.0)	75 (50.0)	117 (39.7)
No	34 (22.5)	15 (10.1)	9 (90.0)	58 (18.8)	74 (51.0)	29 (19.3)	103 (34.9)
Ongoing	50 (33.1)	50 (33.8)	1 (10.0)	101 (32.7)	29 (20.0)	46 (30.7)	75 (25.4)
If no, reason not completed, n (%)							
Unacceptable adverse events	2 (1.3)	1 (0.7)	0 (0.0)	3 (1.0)	1 (0.7)	0 (0.0)	1 (0.3)
Intercurrent illness	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Elective withdrawal	3 (2.0)	4 (2.7)	0 (0.0)	7 (2.3)	1 (0.7)	5 (3.3)	6 (2.0)
Noncompliance	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.3)
Subject died	14 (9.3)	4 (2.7)	0 (0.0)	18 (5.8)	10 (6.9)	5 (3.3)	15 (5.1)
Lost to follow up	2 (1.3)	0 (0.0)	1 (10.0)	3 (1.0)	0 (0.0)	1 (0.7)	1 (0.3)
Failed mobilization	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.3)	5 (3.4)	4 (2.7)	9 (3.1)
Entered rescue	10 (6.6)	0 (0.0)	0 (0.0)	10 (3.2)	52 (35.9)	7 (4.7)	59 (20.0)
Other	2 (1.3)	6 (4.1)	0 (0.0)	8 (2.6)	4 (2.8)	7 (4.7)	11 (3.7)
On-study patient deaths[a,c], n (%)	14 (9.3)	4 (2.7)	0 (0.0)	18 (5.8)	13 (9.0)	6 (4.0)	19 (6.4)

Parameter	NHL (N = 244)	MM (N = 255)	HD (N = 39)	Total (N = 540)	NHL (N = 145)	MM (N = 150)	Total (N = 295)
All Oncology patient deaths[a, c, d], n (%)	25 (10.2)	10 (3.9)	0 (0.0)	35 (6.5)	13 (9.0)	6 (4.0)	19 (6.4)
Patients with AEs leading to study discontinuation, treatment discontinuation, interruption, or modification, n (%)	6 (2.5)	4 (1.6)	1 (2.6)	11 (2.0)	4 (2.8)	2 (1.3)	6 (2.0)

In the all oncology group, the most common reason for treatment period not completed was "other" which included: entry criteria not met, patient refusal to undergo additional aphaeresis, patient stopped aphaeresis due to poor collections and site error.

- Adverse events

During period 1 in the pivotal phase III studies, 96.3% of G-CSF + plerixafor and 65.1% G-CSF + placebo experienced at least 1 AE. 65.1% of G-CSF + plerixafor and 42.7% G-CSF + placebo experienced an AE related to study treatment. An overview of AEs in the phase III studies is summarised in the table below.

Table 23. Overview of AEs in phase 3 placebo-controlled studies

	G + Plerixafor						G + Placebo					
	(1)	(2)	(3)	(4)	(5)	Overall	(1)	(2)	(3)	(4)	(5)	Overall
	(N = 298)	(N = 278)	(N = 277)	(N = 32)	(N = 32)	(N = 298)	(N = 295)	(N = 217)	(N = 217)	(N = 24)	(N = 24)	(N = 295)
AEs	**287 (96.3)**	129 (46.4)	52 (18.8)	13 (40.6)	4 (12.5)	291 (97.7)	**277 (93.9)**	95 (43.8)	36 (16.6)	4 (16.7)	1 (4.2)	285 (96.6)
Deaths[a]	**0 (0.0)**	1 (0.4)	17 (6.1)	0 (0.0)	0 (0.0)	18 (6.0)	**4 (1.4)**	0 (0.0)	15 (6.9)	0 (0.0)	0 (0.0)	19 (6.4)
SAEs	**12 (4.0)**	62 (22.3)	45 (16.2)	7 (21.9)	4 (12.5)	111 (37.2)	**17 (5.8)**	44 (20.3)	34 (15.7)	2 (8.3)	1 (4.2)	85 (28.8)
Related AEs	**194 (65.1)**	2 (0.7)	1 (0.4)	0 (0.0)	0 (0.0)	195 (65.4)	**126 (42.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	126 (42.7)
Related SAEs	**2 (0.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)	**2 (0.7)**[b]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
AEs Leading to Study or Treatment Discontinuation	**5 (1.7)**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	6 (2.0)	**6 (2.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.0)

The table below is an overview of the incidence of adverse events in all oncology studies submitted.

Table 24. Overview of AEs in all oncology studies during mobilization and treatment/aheresis (period 1)

	G + Plerixafor (N = 540)				G + Placebo (N = 295)		
	NHL (N = 244)	MM (N = 255)	HD (N = 39)	Total (N = 540)	NHL (N = 145)	MM (N = 150)	Total (N = 295)
AEs	238 (97.5)	235 (92.2)	29 (74.4)	504 (93.3)	138 (95.2)	139 (92.7)	277 (93.9)
Deaths	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.1)	1 (0.7)	4 (1.4)
SAEs	12 (4.9)	7 (2.7)	1 (2.6)	21 (3.9)	11 (7.6)	6 (4.0)	17 (5.8)
Related AEs	163 (66.8)	152 (59.6)	21 (53.8)	338 (62.6)	60 (41.4)	66 (44.0)	126 (42.7)
Related SAEs	2 (0.8)	0 (0.0)	0 (0.0)	2 (0.4)	1 (0.7)	1 (0.7)	2 (0.7)
AEs Leading to Study Discontinuation, Treatment Discontinuation or Modification	6 (2.5)	2 (0.8)	1 (2.6)	9 (1.7)	4 (2.8)	2 (1.3)	6 (2.0)

In the oncology studies the most frequently occurring AEs in period 1 were; diarrhoea, nausea, injection site reaction, back and bone pain, headache, arthralgia, hypomagnesaemia, fatigue and paraesthesia (mostly in > 10%), with diarrhoea, nausea and injection site reaction the most frequent in the plerixafor patients. Most AEs were mild-moderate. The incidence of AEs was generally lowest in HD patients; however this group was also the smallest.

Thrombocytopenia was found more frequent in G-CSF + plerixafor in the oncology patients' analysis compared to the phase 3 studies (2.2 versus 1.0%). Related AEs more frequently recorded in plerixafor during period 1 were: thrombocytopenia, injection site reaction, pyrexia, flatulence, vomiting, fatigue, peripheral oedema, catheter site pain, hypokalaemia, hypomagnesaemia, insomnia, diarrhoea, nausea and anxiety.

For poor mobilisers the table below presents the incidence of AEs and SAEs. During period 1: 126/131 (96.2%) of the poor mobiliser patients experienced at least 1 AE and 87/131 (66.4%) were considered to be related to study treatment.

Table 26. Overview of AEs in poor mobilisers

	Study Period					
	(1)	(2)	(3)	(4)	(5)	Overall
	(N = 131)	(N = 116)	(N = 112)	(N = 17)	(N = 17)	(N = 131)
AEs	**126 (96.2)**	46 (39.7)	22 (19.6)	4 (23.5)	2 (11.8)	129 (98.5)
Death	**1 (0.8)**	1 (0.9)	13 (11.6)	0 (0.0)	1 (5.9)	16 (12.2)
SAEs	**7 (5.3)**	25 (21.6)	21 (18.8)	4 (23.5)	2 (11.8)	47 (35.9)
Related AEs[a]	**87 (66.4)**	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	89 (67.9)
Related SAEs[a]	**0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AEs leading to study discontinuation, treatment discontinuation or modification	**3 (2.3)**	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	4 (3.1)

The most frequently occurring side effects occurring in the Phase III studies are summarised in the table below, and were nausea, diarrhoea, fatigue, injection site reaction (>5%) and were mostly reported in period 1 when plerixafor/placebo and G-CSF was administrated. Diarrhoea, nausea and injection site reaction typically occurred more frequently in the plerixafor arm. In addition, vomiting, oral paraesthesia, abdominal pain flatulence, catheter site pain, pyrexia, peripheral oedema, and hypokalaemia occurred (5-10%). Mucosal inflammation occurred especially in period 2 and can be related to chemotherapy. The most common AEs in the poor mobilisers were comparable to the phase 3 studies. In the oncology studies the most frequently occurring AEs in period 1 were also comparable to the phase 3 studies.

Table 27. Common AEs (≥ 5%) in phase 3 studies

	G + Plerixafor						G + Placebo					
AE Period	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**Overall**	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**Overall**
	N = 298	**N =278**	**N =277**	**N = 32**	**N = 32**	**N = 298**	**N =295**	**N =217**	**N = 217**	**N = 24**	**N = 24**	**N = 295**
System Organ Class Preferred Term	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Any AE	**287 (96.3)**	129 (46.4)	52 (18.8)	13 (40.6)	4 (12.5)	291 (97.7)	**277 (93.9)**	95 (43.8)	36 (16.6)	4 (16.7)	1 (4.2)	285 (96.6)
Blood and lymphatic system disorders												
Febrile neutropenia	**1 (0.3)**	24 (8.6)	2 (0.7)	4 (12.5)	0 (0.0)	30 (10.1)	**0 (0.0)**	18 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	18 (6.1)
Gastrointestinal disorders												
Diarrhea	**112 (37.6)**	12 (4.3)	2 (0.7)	0 (0.0)	0 (0.0)	119 (39.9)	**49 (16.6)**	11 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)	57 (19.3)
Nausea	**102 (34.2)**	22 (7.9)	1 (0.4)	3 (9.4)	0 (0.0)	116 (38.9)	**64 (21.7)**	19 (8.8)	0 (0.0)	0 (0.0)	0 (0.0)	77 (26.1)
Vomiting	**29 (9.7)**	14 (5.0)	0 (0.0)	3 (9.4)	0 (0.0)	43 (14.4)	**18 (6.1)**	10 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)	26 (8.8)
Paresthesia oral	**22 (7.4)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22 (7.4)	**25 (8.5)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	26 (8.8)
Flatulence	**20 (6.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (6.7)	**11 (3.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (3.7)
Abdominal pain	**13 (4.4)**	1 (0.4)	2 (0.7)	0 (0.0)	0 (0.0)	16 (5.4)	**7 (2.4)**	3 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	10 (3.4)

	G + Plerixafor						G + Placebo					
AE Period	(1) N = 298	(2) N =278	(3) N =277	(4) N = 32	(5) N = 32	Overall N = 298	(1) N =295	(2) N =217	(3) N = 217	(4) N = 24	(5) N = 24	Overall N = 295
System Organ Class Preferred Term	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
General disorders and administration site conditions												
Fatigue	**80 (26.8)**	6 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	86 (28.9)	**74 (25.1)**	3 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	77 (26.1)
Injection site erythema	**78 (26.2)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	78 (26.2)	**14 (4.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (4.7)
Mucosal inflammation	**1 (0.3)**	41 (14.7)	1 (0.4)	5 (15.6)	0 (0.0)	45 (15.1)	**1 (0.3)**	28 (12.9)	0 (0.0)	1 (4.2)	0 (0.0)	30 (10.2)
Catheter site pain	**32 (10.7)**	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	34 (11.4)	**40 (13.6)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	40 (13.6)
Pyrexia	**18 (6.0)**	5 (1.8)	6 (2.2)	0 (0.0)	2 (6.3)	31 (10.4)	**19 (6.4)**	8 (3.7)	4 (1.8)	2 (8.3)	0 (0.0)	33 (11.2)
Edema peripheral	**27 (9.1)**	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	27 (9.1)	**28 (9.5)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	28 (9.5)
Pain	**24 (8.1)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (8.1)	**26 (8.8)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	26 (8.8)
Injection site pruritus	**17 (5.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	17 (5.7)	**2 (0.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Metabolism and nutrition disorders												
Hypokalemia	**45 (15.1)**	4 (1.4)	0 (0.0)	1 (3.1)	0 (0.0)	49 (16.4)	**49 (16.6)**	2 (0.9)	1 (0.5)	0 (0.0)	0 (0.0)	51 (17.3)

	G + Plerixafor						G + Placebo					
AE Period	(1) N = 298	(2) N =278	(3) N =277	(4) N = 32	(5) N = 32	Overall N = 298	(1) N =295	(2) N =217	(3) N = 217	(4) N = 24	(5) N = 24	Overall N = 295
System Organ Class Preferred Term	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Hypomagnesemia	**26 (8.7)**	2 (0.7)	0 (0.0)	1 (3.1)	0 (0.0)	28 (9.4)	**28 (9.5)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	29 (9.8)
Musculoskeletal and connective tissue disorders												
Bone pain	**95 (31.9)**	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	96 (32.2)	**105 (35.6)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	105 (35.6)
Back pain	**54 (18.1)**	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	56 (18.8)	**64 (21.7)**	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	66 (22.4)
Arthralgia	**39 (13.1)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	39 (13.1)	**36 (12.2)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	37 (12.5)
Pain in extremity	**15 (5.0)**	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	16 (5.4)	**21 (7.1)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (7.1)
Nervous system disorders												
Headache	**67 (22.5)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	67 (22.5)	**62 (21.0)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	63 (21.4)
Paresthesia	**60 (20.1)**	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	61 (20.5)	**64 (21.7)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	64 (21.7)
Dizziness	**31 (10.4)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	31 (10.4)	**18 (6.1)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	19 (6.4)

	G + Plerixafor						G + Placebo					
AE Period	(1) N = 298	(2) N =278	(3) N =277	(4) N = 32	(5) N = 32	Overall N = 298	(1) N =295	(2) N =217	(3) N = 217	(4) N = 24	(5) N = 24	Overall N = 295
System Organ Class Preferred Term	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Psychiatric disorders												
Insomnia	**21 (7.0)**	3 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	24 (8.1)	**15 (5.1)**	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	16 (5.4)
Anxiety	**16 (5.4)**	4 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	20 (6.7)	**13 (4.4)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (4.7)
Skin and subcutaneous tissue disorders												
Rash	**9 (3.0)**	7 (2.5)	1 (0.4)	0 (0.0)	0 (0.0)	17 (5.7)	**10 (3.4)**	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	12 (4.1)

AEs occurring in >10% of patients in period 1 in either treatment group were: diarrhoea, nausea, bone pain, fatigue, injection site erythema, headache, paraesthesia, back pain, hypokalaemia, arthralgia, catheter site reaction and dizziness. Bone pain is a common adverse event of G-CSF. Diarrhoea, nausea, vomiting, flatulence, injection site erythema/pruritis and dizziness occurred more frequently in the G-CSF + plerixafor patients. The frequencies are presented in the table below.

Table 28. Common AEs (> 10%) in period 1 in the Phase 3 studies

	G-CSF + plerixafor N = 298 Period 1	G-CSF + placebo N = 295 Period 1
Any AE	287 (96.3%)	277 (93.9%)
Gastrointestinal		
Diarrhoea	112 (37.6%)	49 (16.6%)
Nausea	102 (34.2%)	64 (21.7%)
Vomiting	29 (9.7)	18 (6.1%)
Oral paraesthesia	22 (7.4%)	25 (8.5%)
Flatulence	20 (6.7%)	11 (3.7%)
General disorders and administration site reaction		
Fatigue	80 (26.8%)	74 (25.1%)
Injection site erythema	78 (26.2%)	14 (4.7%)
Catheter site pain	32 (10.7%)	40 (13.6%)
Oedema peripheral	27 (9.1%)	28 (9.5%)
Pain	24 (8.1%)	26 (8.8%)
Pyrexia	18 (6.0%)	19 (6.4%)
Injection site pruritus	17 (5.7%)	2 (0.7%)
Metabolism and nutrition disorders		
Hypokalaemia	45 (15.1%)	49 (16.6%)
Hypomagnesaemia	26 (8.7%)	28 (9.5%)
Musculoskeletal and connective tissue disorders		
Bone pain	95 (31.9%)	105 (35.6%)
Back pain	54 (18.1%)	64 (21.7%)
Arthralgia	39 (13.1%)	36 (12.2%)
Pain in extremity	15 (5.0%)	21 (7.1%)
Nervous system disorders		
Headache	67 (22.5%)	62 (21.0%)
Paraesthesia	60 (20.1%)	64 (21.7%)
Dizziness	31 (10.4%)	18 (6.1%)
Psychiatric disorders		
Insomnia	21 (7.0%)	15 (5.1%)
Anxiety	16 (5.4%)	13 (4.4%)

- Serious adverse event/deaths/other significant events

Phase 3 studies

The proportion of patients with at least 1 SAE in Period 1 was low and was similar for the 2 treatment groups (4.0% for G-CSF + plerixafor compared with 5.8% for G-CSF + placebo). The majority of the SAEs occurred in Periods 2 and 3, during which patients received ablative chemotherapy and were no longer receiving study treatment (plerixafor or placebo). The incidence of SAEs was (in the G + plerixafor versus G + placebo groups, respectively): 22.3% versus 20.3% in Period 2 and 16.2% versus 15.7% in Period 3. Treatment discontinuation due to AEs was similar in G-CSF + plerixafor and G-CSF + placebo (both in 6 patients).

Oncology studies

In Period 1, SAEs were seen in 3.9% of G-CSF + plerixafor and in 5.8% of G-CSF + placebo patients. The majority of SAEs occurred in Period 2 and 3: 18.4% and 15.8 resp in G-CSF + plerixafor versus 20.3 % and 15.7% resp. in G-CSF + placebo patients. Drug hypersensitivity occurred in 2 plerixafor patients versus none of the G-CSF placebo patients. Renal and urinary disorders occurred in 7 plerixafor patients and 1 of the placebo patients.. A total of 11 (2.0%) of the patients in the G + plerixafor group and 6 (2.0%) in the G + placebo group experienced AEs that led to study discontinuation, study treatment discontinuation, or treatment modification. 54 Deaths occurred of whom 50 after transplantation (exception were 4 placebo patients which died before transplantation).
Serious adverse events that were seen more frequently in plerixafor compared to placebo are presented in the table below.

Table 29. Cardiac, Renal, Urinary, and Vascular SAEs more frequently seen in plerixafor compared to placebo in all oncology studies

	G-CSF + plerixafor						G-CSF + placebo					
Period	1	2	3	4	5	total n=540	1	2	3	4	5	total n=295
Any AEs	3.9%	18.4%	15.8%	23.8%	15.9%	33.3%	5.8%	20.3%	15.7%	8.3%	4.2%	28.8%
Cardiac disorders												
Atrial fibrillation	1 0.2%	1 0.2%	1 0.2%	1 1.6%	1 1.6%	5 0.9%	2 0.7%	3 1.4%	0	0	0	5 1.7%
Myocardial infarction	0	0	3 0.6%	0	0	3 0.6%	0	0	0	0	0	0
Atrial flutter	0	0	2 0.4%	0	0	2 0.4%	0	0	0	0	0	0
Cardiac arrest	0	0	1 0.2%	0	1 1.6%	2 0.4%	0	0	0	0	0	0
Cardiac failure congestive	0	0	2 0.4%	0	0	2 0.4%	0	0	0	0	0	0
Drug hyper-sensitivity	0	0	1 0.2%	1 1.6%	0	2 0.4%	0	0	0	0	0	0
Graft versus host failure	0	1 0.2%	1 0.2%	0	0	2 0.4%	0	0	0	0	0	0
Renal and urinary disorders												
Acute renal failure	1 0.2%	1 0.2%	1 0.2%	0	1 1.6%	4 0.7%	1 0.3%	0	0	0	0	1 0.3%
Renal failure	0	1 0.2%	0	0	0	3 0.6%	0	0	0	0	0	0
Vascular disorders												
hypotension	1 0.2%	1 0.2%	2 0.4%	1 1.6%	1 1.6%	6 1.1%	0	1 0.5%	0	0	0	1 0.3%
Deep vein thrombosis	1 0.2%	0	3 0.6%	0	0	4 0.7%	0	0	1 0.5%	0	0	1 0.3%
Orthostatic hypotension	0	3 0.6%	0	0	0	3 0.6%	0	0	1 0.5%	0	0	1 0.3%

There were 3 patients with SAEs in period 1 that were ascribed to study treatment; hypotension and dizziness (1 G-CSF + plerixafor patient), thrombocytopenia (1 G-CSF + plerixafor patient) and non-cardiac chest pain (1 G-CSF + placebo patient).

Overall the incidence of patients with at least 1 SAEs was higher in plerixafor compared to placebo patients; 33.3% versus 28.8%. When analysed by cancer type; the MM patients had the highest incidences of vomiting, nausea and mucosal inflammation. There were 9 SAEs which were related to the study drug. Six patients experienced systemic reactions related to plerixafor with urticaria, anxiety, periorbital swelling, dyspnoea, hypoxia and hypotension and chest pain or tachycardia.

Poor mobilisers

Overall, the proportion of poor mobilisers with at least 1 serious adverse event was 35.9%, which was similar to that for the G+ plerixafor group in the Phase 3 studies (37.2%).

Cardiovascular Disorders

SAEs as cardiovascular disorders occurred in 4/298 and 5/295 (resp plerixafor and placebo) of the phase 3 studies, deep venous thrombosis 4/298 and 1/295 and hypotension in 2/298 and 1/295 of plerixafor and placebo patients resp. In period 3 myocardial infarction (MI) was seen in 3 plerixafor (1 death) and 1 placebo patient. Cardiac disorders were seen in 14/540 plerixafor patients in all oncology studies versus 5/295 in placebo patients. This concerned atrial fibrillation with 5 patients in both treatment groups and the rest concerned myocardial infarction, atrial flutter, cardiac arrest, cardiac congestive failure (in 9 plerixafor patients, mostly in period 3). In G-CSF + plerixafor in all periods cardiac disorders were seen in 10/131 patients and hypotension in 4/131 (poor mobilisers). A total of 7 plerixafor-treated patients had myocardial infarctions (one fatal), occurring 14 days-10 months after

the last plerixafor administration. Two additional patients had MI in the CUP. Patients with cardiac arrhythmias were excluded from the phase 3 studies.

Death or Treatment Discontinuation
A total of 108 patients died in the 21 studies and compassionate use program; 88/1161 (7.6%) who received plerixafor and 20/239 (8.4%) who received G-CSF + placebo. A total of 7/88 patients who died had received placebo and then entered the rescue procedure and received plerixafor.

The incidence and causes of death were similar in G-CSF + placebo and G-CSF + plerixafor treated patients, with disease progression as the most common cause after transplantation (phase 3 studies, all oncology studies and poor mobilisers). It was stated that there were no noticeable differences between deaths in plerixafor or placebo patients.

47 patients experienced AEs that led to discontinuation, or study treatment discontinuation, interruption or modification; 38/1161 (3.3%) who ever received plerixafor and 9/239 (3.8%) who received placebo.

Graft Failures
There were two graft failures (plerixafor patients) in phase 3 studies; which were stated not to be related to the study treatment. One patient had MDS and the other had values defined as graft failure at day 100 but at 12 months there was no evaluable disease.

Infections
Infections: were seen in 24.8% in the plerixafor and 21.1% in the placebo treated patients. Staphylococcal infections were seen in 7 plerixafor patients and none of the placebo treated patients (4 coagulase negative staphylococci). Other bacterial infections and lung infections were similar in both groups.

Systemic Reactions
In total, there were 6 plerixafor-treated patients with systemic (hypersensitivity) reactions including: urticaria (2), periorbital swelling (2), dyspnoea (1) hypoxia (1) and hypotension (1). In the phase III studies 7 placebo and 7 plerixafor treated patients experienced hypotension within 24 hours after drug administration.

- Laboratory findings

Hypomagnesaemia and hypocalcaemia, which had been observed in animal studies with supratherapeutic dose and also in some phase 2 studies, could not be detected in the pivotal clinical studies. Hypokalaemia was seen more frequently in plerixafor treated patients. 31.2% versus 23.0%. In addition, thrombocytopenia was more frequently seen in plerixafor 80.7% versus 71.9%.

- Safety in special populations

No studies were conducted on plerixafor's effect on the ability to drive. No specific studies evaluating the safety of plerixafor in elderly patients were conducted. However, a substantial number of patients aged 65 years or more were evaluated in the pivotal trials. The safety of plerixafor was evaluated in 8 patients in CUP program, who were < 18 years, with NHL, medulloblastoma, Ewing sarcoma, brain tumour and oestrogenic sarcoma. The median age was 14 years. AEs reported were: nausea, vomiting, headache, respiratory failure, progressive encephalopathy, citrate toxicity, coagulopathy, catheter related complication, thrombocytopenia and injection site reaction.

The safety of plerixafor in patients with renal failure was measured in an open label phase I study. Patients were divided into 3 cohorts: creatinine clearance 51-80 ml/min, 31-50 ml/min and < 31 ml/min not requiring dialysis. Patients were followed for 48 hours after plerixafor administration. The primary objective was to assess effects of impaired renal function on the pharmacokinetics of single dose of 240 µg plerixafor sc. 10/17 patients with renal impairment experienced AEs (58.5%); none were severe. Most frequently reported AEs were: gastrointestinal (41.2%), nervous system disorders

(29.4%) and general disorders and administration site reactions (29.4%). AEs in > 10% of patients: diarrhoea (23.5%), injection site erythema (17.6%), paraesthesia (17.6) and injection site reaction (11.8%). A reduction in renal clearance of plerixafor was observed in patients with creatinine clearance < 30 ml/min.

- Safety related to drug-drug interactions and other interactions

No drug interaction studies were submitted, given that there are no critical drug interactions to be expected from the in vitro studies conducted.

Tumour cell mobilisation

Myeloma cell mobilisation was assessed in 10 patients with MM in study 2101. Determination of aneuploid myeloma cells was made by flow cytometry which measured light chain cytoplasmic immunoglobulin content versus DNA content; the presence of 1% abnormal plasma cells in the bone marrow or peripheral blood. 50 peripheral blood samples were analysed in 10 patients. One patient had 2% kappa cells with DNA index of 1 and also contained 1% lambda cells with a DNA index of 1. The rest contained light chain restricted cells below 1%. No plasma cells with aneuploid DNA content were seen.

Aphaeresis samples of 11 patients from study 3101 and 2101 were analysed with qBCL-2 assay, this can detect BCL-2 translocations down to 0.002% or 1/50.000 cells. No translocations were found in the samples of plerixafor treated patients. In addition, no plerixafor treated patient had NHL cells (assay sensitivity: 1 lymphoma cell in 10.000 cells).

- Discontinuation due to adverse events

Overall 47 patients experienced AEs that led to discontinuation, or study treatment discontinuation, interruption or modification; 38/1161 (3.3%) who ever received plerixafor and 9/239 (3.8%) who received placebo. AEs that led to study discontinuation were similar in both treatment arms (2.0%) in the oncology studies (data not shown).

- Post marketing experience

There is no postmarketing experience with plerixafor in the EU.

- Discussion on clinical safety

Overall, the safety profile of plerixafor was considered established from the clinical studies submitted.
A total of 108 patients died in the 21 studies and compassionate use program; 88/1161 (7.6%) who ever received plerixafor and 20/239 (8.4%) who received G-CSF + placebo. A total of 7/88 patients who died had received placebo and then entered the rescue procedure and received plerixafor. Overall 47 patients experienced AEs that led to discontinuation, or study treatment discontinuation, interruption or modification; 38/1161 (3.3%) who ever received plerixafor and 9/239 (3.8%) who received placebo. The sample size of patients was considered limited for the detection of uncommon side effects.

Most common AEs (> 10%) in the treatment period were: diarrhoea, nausea and injection site reaction; 37.6%, 34.2% and 26.2% in the plerixafor + G-CSF versus 16.6%, 21.7% and 4.7% in the placebo + G-CSF group. Common AEs (≥1% to <10%) were headache, dizziness, flatulence, abdominal pain, vomiting, abdominal distension, dry mouth, stomach discomfort, constipation, dyspepsia, hypaesthesia oral, arthralgia, hyperhidrosis, erythema, fatigue, insomnia, injection site reactions, malaise and injection site rash.

AEs that were related to study medication that were recorded more frequently in plerixafor during period 1 were: thrombocytopenia, injection site reaction, pyrexia, flatulence, vomiting, fatigue, peripheral oedema, catheter site pain, hypokalaemia, hypomagnesaemia, insomnia and anxiety. Of the SAEs hypotension, cardiac disorders, deep venous thrombosis and the systemic reactions seem to occur more in plerixafor treated patients.

Importantly, the CHMP consulted the SAG-Oncology with regards to the theoretical risk of tumour cell mobilisation in myeloma and lymphoma patients. Based on pharmacological grounds, SAG indicated that there is a risk of tumour cell mobilisation, and this is of additional concern as it may involve tumor cells that have become more resistant. Although the risk was considered a hypothesis at this stage (and equally, an opposite antitumour effect might also be hypothesised as claimed by the applicant As there are no relevant clinical or nonclinical data that provide reassurance about this risk of tumour cell mobilisation, he SAG recommended that data from adequate non-clinical models together with reliable PFS data with adequate follow-up (minimum follow-up in the order of 1-2 years for all patients) needed to be presented before this risk could be considered acceptable in the broad population of patients with lymphoma and multiple myeloma without further restrictions.

In its responses, the Applicant considered that the best approach to assess the clinical relevance of tumour cell contamination would be to assess the long-term clinical outcomes of patients receiving transplanted haematopoietic stem cell products. Therefore, the Applicant has extended the long-term follow (LTF) up for our two controlled Phase 3 studies (AMD3100-3101 and AMD3100-3102) to 5 years, including evaluation of relapse, progression-free survival (PFS), and overall survival. Tumour cell mobilisation specifically in poor mobilisers will be addressed by evaluation of PFS (as well as other long-term outcomes) by use of a registry, in collaboration with EBMT. The CHMP considered that this registry should include patients that are mobilised with 1. G-CSF alone, 2. G-CSF plus chemotherapy, 3. G-CSF plus chemotherapy and Mozobil. Also patients with off label use should be examined. In addition, the CHMP requested that the Applicant present a detailed proposeal for the registry.

Based on the clinical safety studies submitted, the following concerns were also identified:

1. The CHMP requested that the Applicant provide more detailed information of all patients experiencing systemic drug reactions in phase I, II, and III studies to assess the importance of systemic anaphylactic reactions, given that this is a major health concern, and underestimation of the problem could happen on the basis of the limited data provided thus far. Following the Applicant's responses the CHMP concluded that the systemic reactions reported were generally mild to moderate and their incidence was very low (0.7%), making this adverse drug reaction acceptable.

2. The CHMP requested that the Applicant justify not restricting the indication to patients with proven CXCR4 negative tumours given that the disruption of the binding between CXCR4 and SDF-1 by plerixafor must be assumed to result in the mobilisation of tumour cells. The CHMP, taking into consideration the Applicant's responses, concluded that the Applicant should provide additional data on the extent to which plerixafor could induce tumour cell mobilisation. Given that this is a considerable safety concern in the pharmacotherapeutic class of growth factors, a new medicinal product such as plerixafor, with similarities in the mechanism of action, should require clinical data to exclude the possibility of tumour cell mobilisation. Therefore, the Applicant conducted a review of CXCR4 expression in myeloma and lymphoma patients, and discussed whether CXCR4 should be considered a marker for tumour stem cells in these malignant disorders.

3. The CHMP requested that the Applicant further discussed the incidence of cardiovascular disorders amongst the plerixafor + G-CSF treated patients and the placebo + G-CSF treated patients. Based on the Applicant's responses, which included a QTc study, the CHMP concluded that the incidence of several cardiac disorders was not higher in patients treated with plerixafor compared to placebo except for myocardial infarction. Therefore, in Section 4.8 of the SPC, this adverse event was sufficiently reflected.

Given that the company submitted data from the QT-study, in which signs and symptoms of hypotension were observed, a warning on manifestations of vasovagal reactions was included in sections 4.8 and 4.4 of the SPC.

4. The CHMP requested that the Applicant discuss two graft failures in the phase III studies, which were only seen in patients receiving plerixafor. From the total safety database submitted by the

Applicant, 4 out of 1400 patients developed graft failure. The CHMP considered that an incidence of < 0.3% graft failure is low, acceptable, and in line with the published literature.

5. The Applicant was asked to discuss central nervous system adverse events observed in the clinical setting, given that plerixafor could potentially directly induce such effects. Based on the Applicant's responses, the CHMP noted that headache, dizziness, paraesthesias, diarrhoea, nausea and vomiting were duly included in Section 4.8 of the SPC.

6. Hyperleukocytosis (WBC count above 100 x109/l) occurred in at least one patient given G-CSF + plerixafor (study 3101). The Applicant was therefore asked to provide a review of cases with hyperleukocytosis with or without symptoms from all clinical studies / CUP and to make suggestions on how to reduce the risks. From the review provided by the Applicant, the CHMP concluded that hyperleukocytosis was indeed more frequent in Mozobil-treated patients. However, the occurrence of to only one associated manifestations, i.e. dyspnoea, was uncommon and temporary. No relevant thrombotic events related to hyperleukocytosis were observed. In summary, the data did not suggest that plerixafor conferred an increased risk of adverse events associated with hyperleucocytosis syndrome. To this effect, the following text special precaution was included in section 4.4 of the SPC: Administration of Mozobil in conjunction with G-CSF increases circulating leukocytes as well as haematopoietic stem cell populations. White blood cell counts should be monitored during Mozobil therapy. Clinical judgment should be exercised when administering Mozobil to patients with peripheral blood neutrophil counts above 50,000 cells/µl.

7. The CHMP noted a tendency towards more infections after tandem transplantation in G-CSF + plerixafor mobilized patients than for the G-CSF + placebo tandem transplant group, implying vague questions regarding the quality of the stem cells after storage for the patients treated with plerixafor mobilization. Therefore, the Applicant was asked to investigate the quality of stored harvested stem cells after varying durations by methods including CFU enumerations, comparing this between the treatment groups and generally discussing the engraftment quality of the stem cell products after storage.

From the Applicant's responses, the data indicate that the engraftment quality of the harvested stem cells was not adversely impacted by the storage conditions utilized in the plerixafor clinical trials. Identical storage conditions would be expected in the clinical use of the marketed product. Therefore, the Applicant did not consider an additional study assessing engraftment quality after varying durations of storage to be necessary, and committed to monitor adverse events in tandem transplanted patients. The CHMP was in agreement with the Applicant with regards to this point.

8. Section 4.4 of the SPC was amended to include the amount of sodium in the formulation of plerixafor:

Sodium
Mozobil contains less than 1 mmol sodium (23 mg) per dose, i.e. essentially 'sodium- free'.

3.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan.

Table Summary of the risk management plan

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine)
Identified risks		
Urticaria, eye swelling, hypoxia, and dyspnoea (allergic reactions)	Routine: Postmarketing surveillance and reported as separate analysis in PSURs	**Warning included in Section 4.4 of the SPC** Mozobil has been uncommonly associated with potential systemic reactions related to subcutaneous injection such as urticaria, periorbital swelling, dyspnoea, or hypoxia (see section 4.8). Symptoms responded to treatments (e.g., antihistamines, corticosteroids, hydration or supplemental oxygen) or resolved spontaneously. Appropriate precautions should be taken because of the potential for these reactions.
Orthostatic hypotension, syncope, postural syncope, and syncope vasovagal (vasovagal reactions)	Routine: Postmarketing surveillance and reported as separate analysis in PSURs	**Warning included in Section 4.4 of the SPC** Vasovagal reactions, orthostatic hypotension, and/or syncope can occur following subcutaneous injections (see section 4.8). Appropriate precautions should be taken because of the potential for these reactions. Also labelled in Section 4.8 of the SPC
Leukocytosis not symptomatic	Routine: Postmarketing surveillance and reported as separate analysis in PSURs	**Warning included in Section 4.4 of the SPC** Administration of Mozobil in conjunction with G-CSF increases circulating leukocytes as well as haematopoietic stem cell populations. White blood cell counts should be monitored during Mozobil therapy. Clinical judgment should be exercised when administering Mozobil to patients with peripheral blood neutrophil counts above 50,000 cells/μl.
Important Potential Risks		
Thrombocytopenia	Routine: Postmarketing surveillance	**Warning included in Section 4.4 of the SPC** *Thrombocytopenia* Thrombocytopenia is a known complication of apheresis and has been observed in patients receiving Mozobil. Platelet counts should be monitored in all patients receiving Mozobil and undergoing apheresis.
Interstitial lung disease	Routine: Postmarketing surveillance	None
Myocardial Infarction	Routine: Postmarketing surveillance	Labelled in Section 4.8 of SPC.
Paraesthesia	Routine: Postmarketing surveillance	Labelled in Section 4.8 of SPC.
Engraftment failure	Routine: postmarketing surveillance	None

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine)
Graft failure	Routine: postmarketing surveillance	None
Tumour cell mobilisation	Routine: Postmarketing surveillance, completion of Phase 3 long-term follow-up including annual reports of interim analyses, and post-approval transplant registry.	**Warning included in Section 4.4 of the SPC** Potential for tumour cell mobilisation in patients with lymphoma and multiple myeloma The effect of potential re-infusion of tumour cells has not been adequately studied. When Mozobil is used in conjunction with G-CSF for haematopoietic stem cell mobilisation in patients with lymphoma or multiple myeloma, tumour cells may be released from the marrow and subsequently collected in the leukapheresis product. The clinical relevance of the theoretical risk of tumour cell mobilisation is not fully elucidated. In clinical studies of patients with non-Hodgkin's lymphoma and multiple myeloma, mobilisation of tumour cells has not been observed with plerixafor. Tumour cell mobilisation in leukaemia patients In a compassionate use programme, Mozobil and G-CSF have been administered to patients with acute myelogenous leukaemia and plasma cell leukaemia. In some instances, these patients experienced an increase in the number of circulating leukaemia cells. For the purpose of haematopoietic stem cell mobilisation, plerixafor may cause mobilisation of leukaemic cells and subsequent contamination of the apheresis product. Therefore, plerixafor is not recommended for haematopoietic stem cell mobilisation and harvest in patients with leukaemia.

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine)
Splenomegaly	Routine: Postmarketing surveillance	**Warning included in Section 4.4 of the SPC** *Splenomegaly* In preclinical studies, higher absolute and relative spleen weights associated with extramedullary haematopoiesis were observed following prolonged (2 to 4 weeks) daily plerixafor subcutaneous administration in rats at doses approximately 4 fold higher than the recommended human dose. The effect of plerixafor on spleen size in patients has not been specifically evaluated in clinical studies. The possibility that plerixafor in conjunction with G-CSF can cause splenic enlargement cannot be excluded. Due to the very rare occurrence of splenic rupture following G-CSF administration, individuals receiving Mozobil in conjunction with G-CSF who report left upper abdominal pain and/or scapular or shoulder pain should be evaluated for splenic integrity.
Leukostasis	Routine: Postmarketing surveillance	**None specific for leukostasis** Warning in Section 4.4 for Hyperleukocytosis: Administration of Mozobil in conjunction with G-CSF increases circulating leukocytes as well as haematopoietic stem cell populations. White blood cell counts should be monitored during Mozobil therapy. Clinical judgment should be exercised when administering Mozobil to patients with peripheral blood neutrophil counts above 50,000 cells/µl.
Drug level NOS increased	Routine: Postmarketing surveillance and reported as separate analysis in PSURs	**Posology and method of administration Section 4.2** Renal impairment Patients with creatinine clearance 20-50 ml/min should have their dose of plerixafor reduced by one-third to 0.16 mg/kg/day (see section 5.2). Clinical data with this dose adjustment are limited. There is insufficient clinical experience to make alternative posology recommendations for patients with a creatinine clearance <20 ml/min, as well as to make posology recommendations for patients on haemodialysis. Based on increasing exposure with increasing body weight the dose should not exceed 27 mg/day if the creatinine clearance is lower than 50 ml/min.

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine)
Off-label use in adult and paediatric patients	Routine: Postmarketing surveillance and transplant registry.	Clear indication in the SPC.
Effect on embryo-foetal development	Routine: Postmarketing surveillance	Labelled in Section 4.6 of SPC. Mozobil should not be used during pregnancy unless the clinical condition of the woman requires treatment with plerixafor. Women of childbearing potential have to use effective contraception during treatment.
Drug exposure during pregnancy	Routine: Postmarketing surveillance	Labelled in Section 4.6 of SPC. Mozobil should not be used during pregnancy unless the clinical condition of the woman requires treatment with plerixafor. Women of childbearing potential have to use effective contraception during treatment.
Important Missing Information		
Use of plerixafor with chemomobilisation	Routine: Postmarketing surveillance Genzyme has received summaries of 3 IST protocols which are to be initiated in late 2009/early 2010 to study the use of plerixafor with chemomobilisation in patients with lymphoma and MM.	None
Safety profile in paediatric patients	A Phase 1/2 clinical trial will be conducted to study the use of plerixafor as a mobilising agent in patients from 2 years to 18 years old who require an autologous transplant following high dose chemotherapy (HDC). Any underlying pathology that requires treatment with HSCT will be considered suitable with the exception of patients with leukaemia.	None
Races other than Caucasian	Routine: Postmarketing surveillance	None
Effect on male/female fertility	Routine: Postmarketing surveillance	None
Adverse events in tandem transplant	Routine: Postmarketing surveillance Under consideration if the registry in collaboration with EBMT can be used.	None

Safety Concern	Proposed Pharmacovigilance Activities	Proposed Risk Minimisation Activities (routine)
Long-term (>1 year) safety	Routine: Completion of long-term follow-up to the Phase 3 studies and post-approval transplant registry.	None

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

3.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues, which have a negative impact on the Benefit Risk balance of the product.

Non-clinical pharmacology and toxicology

The results from single dose subcutaneous studies in rats and mice showed plerixafor can induce transient but severe neuromuscular (uncoordinated movement), sedative-like effects (hypoactivity), dyspnoea, ventral or lateral recumbency, and/or muscle spasms. Additional effects of plerixafor consistently noted in repeated dose animal studies included increased levels of circulating white blood cells and increased urinary excretion of calcium and magnesium in rats and dogs, slightly higher spleen weights in rats, and diarrhoea and tachycardia in dogs. Histopathology findings of extramedullary haematopoiesis were observed in the liver and spleen of rats and/or dogs. One or more of these findings were usually observed at systemic exposures in the same order of magnitude or slightly higher as the human clinical exposure.

Safety pharmacology studies with IV administered plerixafor in rats showed respiratory and cardiac depressant effects at systemic exposure slightly above the human clinical exposure, whilst SC administration elicited respiratory and cardiovascular effects only at higher systemic levels.

SDF-1α and CXCR4 play major roles in embryo-foetal development. Plerixafor has been shown to cause increased resorptions, decreased foetal weights, retarded skeletal development and increased foetal abnormalities in rats and rabbits. Data from animal models also suggest modulation of foetal haematopoiesis, vascularisation, and cerebellar development by SDF-1α and CXCR4. Systemic exposure at No Observed Adverse Effect Level for teratogenic effects in rats and rabbits was of the same magnitude or lower as found at therapeutic doses in patients. This teratogenic potential is likely due to its pharmacodynamic mechanism of action.

In rat distribution studies concentrations of radiolabelled plerixafor was detected in reproductive organs (testes, ovaries, uterus) two weeks after single or 7 daily repeated doses in males and after 7 daily repeated doses in females. The elimination rate from tissues was slow.

The potential effects of plerixafor on male and female fertility and post-natal development have not been evaluated in non-clinical studies.

Carcinogenicity studies with plerixafor have not been conducted. Plerixafor was not genotoxic in an adequate battery of genotoxicity tests.

Plerixafor inhibited tumour growth in *in vivo* models of non-Hodgkin's lymphoma, glioblastoma, medulloblastoma, and acute lymphoblastic leukaemia when dosed intermittently. An increase of non-

Hodgkin's lymphoma growth was noted after a continuous administration of plerixafor for 28 days. The potential risk associated with this effect is expected to be low for the intended short term duration of dosing plerixafor in humans.

Efficacy

Plerixafor is a bicyclam derivative, a selective reversible antagonist of the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal cell-derived factor-1α (SDF-1α), also known as CXCL12. Plerixafor-induced leukocytosis and elevations in circulating haematopoietic progenitor cell levels are thought to result from a disruption of CXCR4 binding to its cognate ligand, resulting in the appearance of both mature and pluripotent cells in the systemic circulation. CD34+ cells mobilised by plerixafor are functional and capable of engraftment with long-term repopulating capacity.

In two Phase III randomised-controlled studies patients with non-Hodgkin's lymphoma or multiple myeloma received Mozobil 0.24 mg/kg or placebo on each evening prior to apheresis. Patients received daily morning doses of G-CSF 10 μg/kg for 4 days prior to the first dose of plerixafor or placebo and on each morning prior to apheresis. Optimal (5 or 6 x 10^6 cells/kg) and minimal (2 x 10^6 cells/kg) numbers of CD34+ cells/kg within a given number of days, as well as the primary composite endpoints which incorporated successful engraftment are presented in the SPC; the proportion of patients reaching optimal numbers of CD34+ cells/kg by apheresis day are also presented.

Rescue patients
In study AMD3100-3101, 62 patients (10 in the Mozobil + G-CSF group and 52 in the placebo + G-CSF group), who could not mobilise sufficient numbers of CD34+ cells and thus not could not proceed to transplantation, entered into an open-label Rescue procedure with Mozobil and G-CSF. Of these patients, 55 % (34 out of 62) mobilised ≥ 2 x106/kg CD34+ cells and had successful engraftment. In study AMD3100-3102, 7 patients (all from the placebo + G-CSF group) entered the Rescue procedure. Of these patients, 100% (7 out of 7) mobilised ≥ 2 x106/kg CD34+ cells and had successful engraftment.

The dose of haematopoietic stem cells used for each transplant was determined by the investigator and all haematopoietic stem cells that were collected were not necessarily transplanted. For transplanted patients in the Phase III studies, median time to neutrophil engraftment (10-11 days), median time to platelet engraftment (18-20 days) and graft durability up to 12 months post-transplantation were similar across the Mozobil and placebo groups.

Mobilisation and engraftment data from supportive Phase II studies (plerixafor 0.24 mg/kg dosed on the evening or morning prior to apheresis) in patients with non-Hodgkin's lymphoma, Hodgkin's disease, or multiple myeloma were similar to those data for the Phase III studies.

In the placebo-controlled studies, fold increase in peripheral blood CD34+ cell count (cells/μl) over the 24-hour period from the day prior to the first apheresis to just before the first apheresis was evaluated. During that 24-hour period, the first dose of plerixafor 0.24 mg/kg or placebo was administered 10-11 hours prior to apheresis.

In pharmacodynamic studies in healthy volunteers of plerixafor alone, peak mobilisation of CD34+ cells was observed from 6 to 9 hours after administration. In pharmacodynamic studies in healthy volunteers of plerixafor in conjunction with G-CSF administered at identical dose regimen to that in studies in patients, a sustained elevation in the peripheral blood CD34+ count was observed from 4 to 18 hours after plerixafor administration with peak response between 10 and 14 hours.

Safety

Safety data for Mozobil in conjunction with G-CSF in patients with lymphoma and multiple myeloma were obtained from 2 placebo-controlled Phase III studies and 10 uncontrolled Phase II studies in 543 patients. Patients were primarily treated with daily doses of 0.24 mg/kg plerixafor by

subcutaneous injection. The exposure to Mozobil in these studies ranged from 1 to 7 consecutive days (median = 2 days).

In the two Phase III studies in non-Hodgkin's lymphoma and multiple myeloma patients (AMD3100--3101 and AMD3100-3102, respectively), a total of 301 patients were treated in the Mozobil and G-CSF group and 292 patients were treated in the placebo and G-CSF group. Patients received daily morning doses of G-CSF 10 μg/kg for 4 days prior to the first dose of plerixafor or placebo and on each morning prior to apheresis. Adverse reactions that occurred more frequently with Mozobil and G-CSF than placebo and G-CSF and were reported as related in ≥1% of the patients who received Mozobil, during haematopoietic stem cell mobilisation and apheresis and prior to chemotherapy/ablative treatment in preparation for transplantation are listed in section 4.8 of the SPC. From chemotherapy/ablative treatment in preparation of transplantation through 12 months post-transplantation, no significant differences in the incidence of adverse reactions were observed across treatment groups.

The adverse reactions reported in patients with lymphoma and multiple myeloma who received Mozobil in the controlled Phase III studies and uncontrolled studies, including a Phase II study of Mozobil as monotherapy for haematopoietic stem cell mobilisation, are similar. No significant differences in the incidence of adverse reactions were observed for oncology patients by disease, age, or gender.

Myocardial infarction
In clinical studies, 7 of 676 oncology patients experienced myocardial infarctions after haematopoietic stem cell mobilisation with Mozobil and G-CSF. All events occurred at least 14 days after last Mozobil administration. Additionally, two female oncology patients in the compassionate use programme experienced myocardial infarction following haematopoietic stem cell mobilisation with Mozobil and G-CSF. One of these events occurred 4 days after last Mozobil administration. Lack of temporal relationship in 8 of 9 patients coupled with the risk profile of patients with myocardial infarction does not suggest Mozobil confers an independent risk for myocardial infarction in patients who also receive G-CSF.

Hyperleukocytosis
White blood cell counts of 100 x 10^9/l or greater were observed, on the day prior to or any day of apheresis, in 7% patients receiving plerixafor and in 1% patients receiving placebo in the Phase III studies. No complications or clinical symptoms of hyperleukocytosis were observed.

Vasovagal reactions
In Mozobil oncology and healthy volunteer clinical studies, less than 1% of subjects experienced vasovagal reactions (orthostatic hypotension and/or syncope) following subcutaneous administration of plerixafor doses ≤ 0.24 mg/kg. The majority of these events occurred within 1 hour of Mozobil administration.

Gastrointestinal disorders
In Mozobil clinical studies of oncology patients, there have been rare reports of severe gastrointestinal events, including diarrhoea, nausea, vomiting, and abdominal pain.

Paraesthesiae
Paraesthesiae are commonly observed in oncology patients undergoing autologous transplantation following multiple disease interventions. In the placebo-controlled Phase III studies, the incidence of paresthesiae was 20.6% and 21.2% in the Mozobil and placebo groups, respectively.

Elderly patients
In the two placebo-controlled clinical studies of Mozobil, 24% of patients were ≥ 65 years old. No notable differences in the incidence of adverse reactions were observed in these elderly patients when compared with younger ones.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The Applicant performed a readability testing ("user consultation") and a satisfactory report has been provided.

Risk-benefit assessment

The composite secondary endpoint as requested by the scientific advice by the EMEA, consisted of a target number of CD34+ cells/kg and successful PMN and PLT engraftment. The results of both phase 3 studies showed that more patients in the plerixafor + G-CSF group reached the target number of CD34+ cells (a minimum of 2 x 10^6 CD34+ cells/kg and preferable 5 x 10^6 CD34+ cells/kg) and successful engraftment compared to the patients treated with G-CSF alone. Additionally, G-CSF + plerixafor mobilised the target CD34+ cells in fewer days of apheresis. These results were supported by the (limited) data of the phase 2 supportive studies. Importantly, clinical relevance of plerixafor has been shown in both phase 3 trials for a specific subgroup population (poor mobilisers).

In study AMD3100-3101 I70 (47.3%) of the patients being treated with G-CSF + placebo , collected the minimum number of CD34+ cells required for transplantation (≥ 2 x 106 CD34+ cells/kg) in 4 or fewer days of apheresis during the first treatment phase. In contrast, 62 patients (10 in the Mozobil + G-CSF group and 52 in the placebo + G-CSF group), who could not mobilise sufficient numbers of CD34+ cells and thus not could not proceed to transplantation, entered into an open-label Rescue procedure with Mozobil and G-CSF. Of these patients, 37 (59.7%) collected the minimum number of CD34+ cells required for transplantation after treatment with G-CSF + plerixafor in 4 or fewer days of apheresis. 34 (55%) patients mobilised ≥ 2 x10^6/kg CD34+ cells and had successful engraftment

In study AMD3100-3102, 136 (88.3%) of the patients being treated with G-CSF + placebo , collected the minimum number of CD34+ cells required for transplantation (≥ 2 x 106 CD34+ cells/kg) in 4 or fewer days of apheresis during the first treatment phase. In contrast, 7 patients (all from the placebo + G-CSF group) entered the Rescue procedure. All of these patients (100%) mobilised ≥ 2 x10^6/kg CD34+ cells and had successful engraftment.

However, the CHMP noted certain risks and uncertainties with regard to this medicinal product. Frequently observed adverse events related to plerixafor during its administration and apheresis were: thrombocytopenia, injection site reaction, pyrexia, flatulence, vomiting, fatigue, peripheral edema, catheter site pain, hypokalaemia, hypomagnesaemia, insomnia and anxiety. With regard to hypotension, cardiac disorders, deep venous thrombosis, systemic reactions, hypokalaemia, thrombocytopenia, hypomagnesaemia, and paraesthesia, the Applicant provided sufficient additional information, and these AE issues have been adequately addressed either in the RMP or follow-up procedures. In particular, the SPC contains a warning concerning myocardial infarction and manifestations of vasovagal reactions (including orthostatic hypotension and/or syncope). From the safety database including a recently performed phase 1 QT study, hypotension and manifestations of hypotension (vasovagal syncope/postural syncope) were observed. The QT-study showed no influence of Mozobil on the QT variables on the ECG.

Clinical and preclinical concerns were raised regarding tumour mobilisation. As a result, the Applicant provided data addressing to what extent Mozobil could induce tumor cell mobilisation or not depending on CXCR4 expression, and the consequences of any such effects for the long term outcome. Additionally, the Applicant agreed to extend the long-term follow-up of for two controlled phase III studies to 5 years, including evaluation of relapse, progression-free survival and overall survival. Tumour cell mobilization specifically in poor mobilisers will be addressed as a follow-up measure by evaluation of long-term outcomes by use of a registry in collaboration with EBMT. In

addition, the Applicant also committed to assess available tumor cell mobilization lymphoma and/or multiple myeloma animal models, comparing different mobilization regimens for tumor cell mobilization.

Patients with renal clearance <30 ml/min showed a reduction in renal clearance of plerixafor. For the safe use of plerixafor in this patient population it was deemed necessary to know whether reduction of plerixafor dose was enough to reduce accumulation of plerixafor in this patient group or that reduction in frequency of administration was also required. To this effect, the following text was included in the SPC: Patients with creatinine clearance 20-50 ml/min should have their dose of plerixafor reduced by one-third to 0.16 mg/kg/day (see section 5.2). Clinical data with this dose adjustment are limited. There is insufficient clinical experience to make alternative posology recommendations for patients with a creatinine clearance <20 mL/min, as well as to make posology recommendations for patients on haemodialysis.

Furthermore, increased exposure in obese patients may result in increased AE in the very heavy, and probably a maximum dose needed to be indicated, to avoid high exposure in the very heavy. To this effect, the following text was included in the SPC: Based on increasing exposure with increasing body weight the dose should not exceed 27 mg/day if the creatinine clearance is lower than 50 ml/min.

Therefore, based on the data submitted, the CHMP was of the opinion that the balance between efficacy and safety was not in favour of approving plerixafor for a broad indication. Although Mozobil demonstrated efficacy concerning haematopoietic stem cell mobilisation and no negative effect on durable engraftment, there was no additional efficacy of plerixafor on the engraftment variables compared to G-CSF alone. The CHMP concluded that the efficacy on the population of MM and NHL patients as a whole had not been proven for plerixafor.

In contrast, the clinical efficacy of plerixafor + G-CSF has been observed in poor mobilisers. Plerixafor shows clinical efficacy in NHL and MM patients that undergo mobilisation for autologous HSC transplantation and who have shown to be poor mobilisers after initial mobilisation with G-CSF alone (first line treatment). For this specific population, plerixafor was considered approvable as second line treatment.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- routine pharmacovigilance was adequate to monitor the safety of the product.
- no additional risk minimisation activities were required beyond those included in the product information.

Therefore, the overall Benefit/Risk ratio of Mozobil is positive as second line treatment for the specific population of patients whose cells mobilize poorly.

There is no universally accepted definition of “poor mobilisers” but some centres would regard as poor mobilisers those patients that after adequate dosage of G-CSF do not reach CD34+ cells > 20×10^6/l in the peripheral blood or do not reach within 4 days of apheresis a total of 5×10^6/kg CD34+ cells in the harvest.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Mozobil in combination with G-CSF to enhance mobilisation of haematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with lymphoma and multiple myeloma whose cells mobilise poorly as favourable and therefore recommended the granting of the marketing authorisation.