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Plerixafor Hydrochloride

Chemokine CXCR4 (SDF-1) Antagonist
Stem Cell-Mobilizing Agent

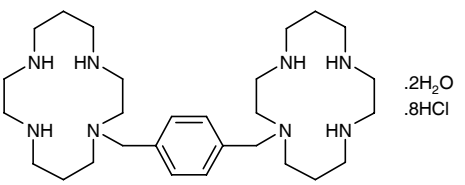
Rec INNM; USAN

AMD-3100
SDZ-SID-791
SID-791
JM-3100 (former code name)
Mozobil™

1,4-Bis(1,4,8,11-tetraazacyclotetradec-1-ylmethyl)benzene octahydrochloride dihydrate

1,1'-[1,4-Phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride dihydrate

InChI=1/C28H54N8.8ClH.2H2O/c1-9-29-15-17-31-13-3-21-35(23-19-33-11-1)25-27-5-7-28(8-6-27)26-36-22-4-14-32-18-16-30-10-2-12-34-20-24-36;;;;;;;;;;/h5-8,29-34H,1-4,9-26H2;8*1H;2*1H2



$C_{28}H_{66}Cl_8N_8O_2$
Mol wt: 830.4980

CAS: 110078-46-1 (anhydrous, free base)

CAS: 155148-31-5 (anhydrous)

EN: 196024

Abstract

The bicyclam derivative plerixafor hydrochloride (AMD-3100, Mozobil™) was originally discovered as a potent and selective anti-HIV agent; however, problems with unexpected cardiac disturbances led AnorMED (now part of Genzyme) to discontinue its development. Subsequent studies identified plerixafor as the first potent and selective nonpeptide chemokine CXCR4 (SDF-1) receptor antagonist and as a result development has focused on its use for mobilizing hematopoietic stem cells (HSCs) for transplantation as a rescue therapy after high-dose myeloablative therapy. *In vitro* and *in vivo* studies have demonstrated that plerixafor effectively mobilizes HSCs. Clinical trials have confirmed safety and tolerability and its ability to improve cell mobilization. Plerixafor has reached phase III clinical development for improving the outcome of stem cell transplantation in non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) patients.

Synthesis

Plerixafor can be prepared by several related procedures.

1) The macrocyclic tetraamine cyclam (I) is protected with *p*-toluenesulfonyl chloride at a 1:2 molar ratio, yielding the target tritosyl derivative (II) along with di- and monotosylated derivatives, which can be separated by fractional crystallization from MeOH (1). Subsequent condensation of (II) with α,α'-*p*-dibromoxylene (IIIa) gives the hexatosyl bis-cyclam (IV), which is finally deprotected by treatment with hot concentrated H_2SO_4 or with HBr in AcOH (1, 2). Alternatively, protection of (I) with di-*tert*-butyl dicarbonate provides the tri-Boc derivative (V), which is dimerized to (VI) by treatment with *p*-dibromoxylene (IIIa) and Na_2CO_3. Deprotection of (VI) is then effected by heating with aqueous HCl (3, 4). Similarly, treatment of the macrocyclic amine (I) with an excess of ethyl trifluoroacetate in the presence of Et_3N affords the tris-trifluoroacetyl cyclam (VII) as the major product. Subsequent condensation of (VII) with either *p*-dibromoxylene (IIIa) or *p*-dichloroxylene (IIIb) in the presence of KI gives the trifluoroacetyl-protected compound (VIII), which is finally deprotected by treatment with NaOH or K_2CO_3 in MeOH (5, 6). Scheme 1.

2) In a different procedure, the acyclic tetraamine (IX) is protected with *p*-toluenesulfonyl chloride to afford a separable mixture of ditosyl (X) and tritosyl compounds (XI). Condensation of (X) with *p*-dibromoxylene (IIIa) provides the tetratosyl dimer (XII), which is further tosylated to (XIII) by means of *p*-toluenesulfonyl chloride and

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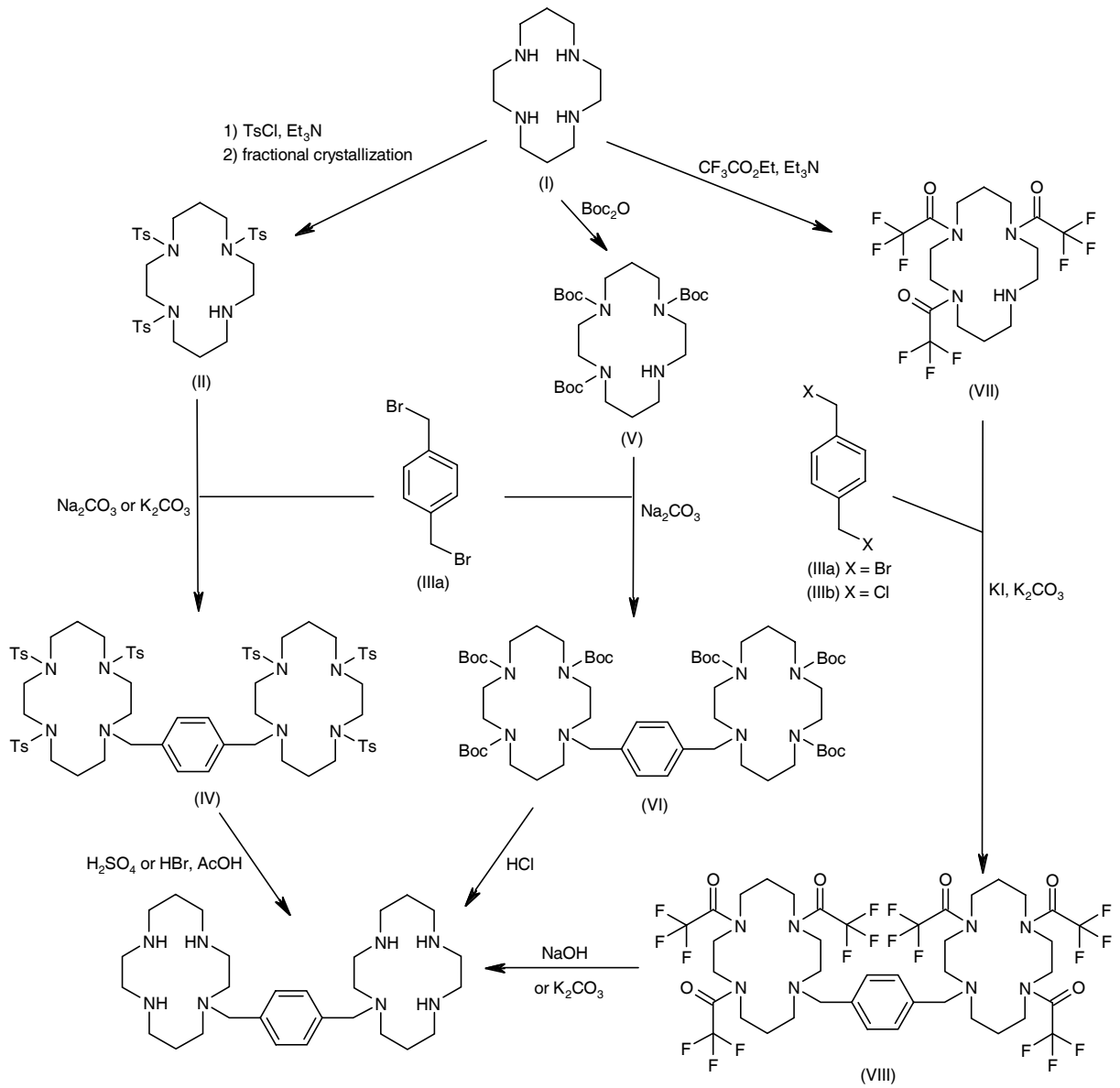
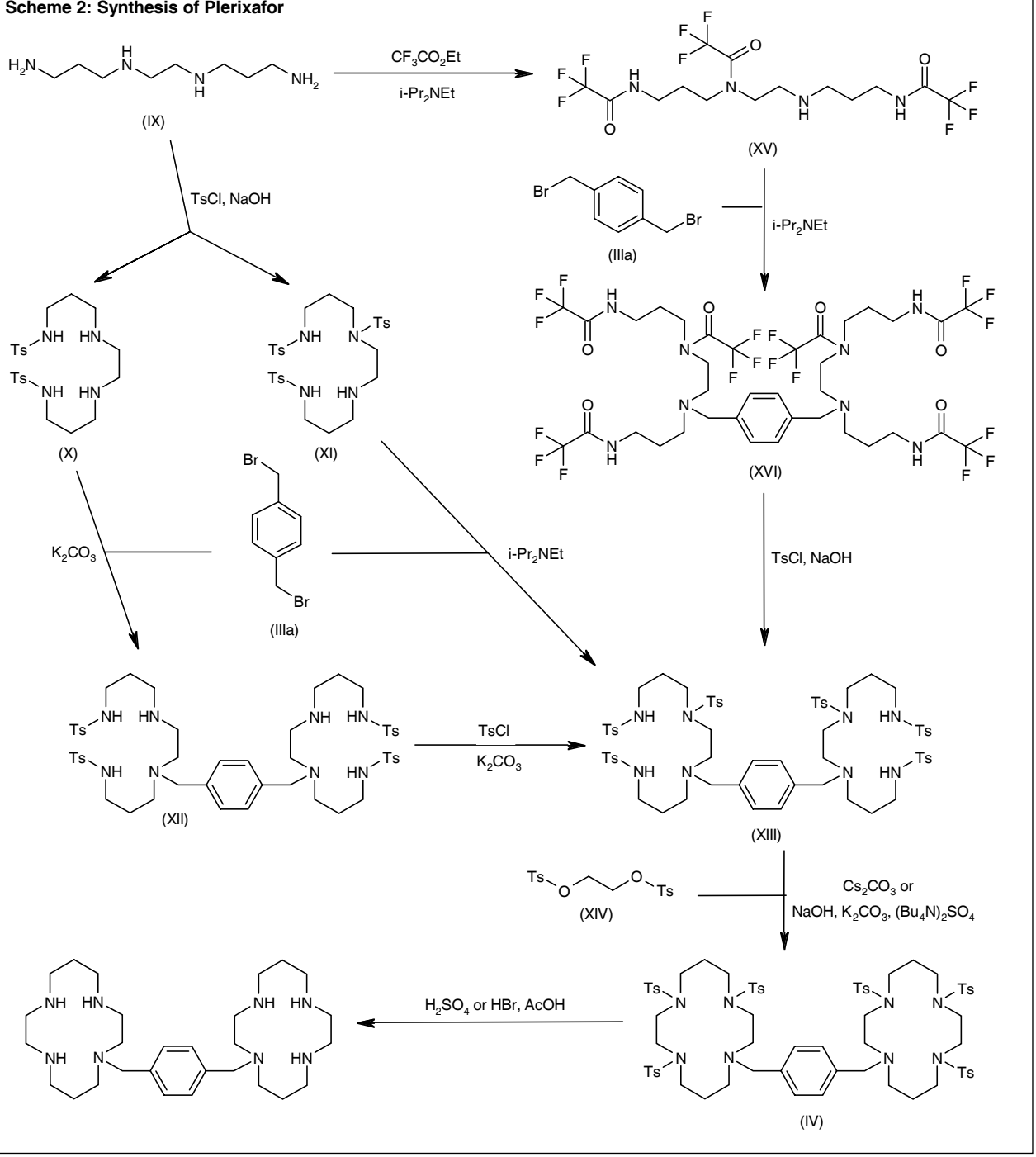
Scheme 1: Synthesis of Plerixafor



K_2CO_3. Alternatively, the hexatosyl dimer (XIII) can be obtained by condensation of the trisulfonylated tetraamine (XI) with *p*-dibromoxylene (IIIa) in the presence of *N,N*-diisopropylethylamine. Ring closure of (XIII) to furnish the bis-macrocycle (IV) is then accomplished by reaction with ethylene glycol ditosylate (XIV) in the presence of Cs_2CO_3 or under phase-transfer conditions. Subsequent acidic deprotection of (IV) gives the target compound (7). In a related approach, the open-chain tetraamine (IX) is protected with ethyl trifluoroacetate and *N,N*-diisopropylethylamine to afford the triacyl derivative (XV) as the main product, which is further condensed with dibromide (IIIa), yielding the trifluoroacetyl-protected dimer (XVI). The hexatrifluoroacetyl compound (XVI) is then converted to the hexatosyl analogue (XIII) by alkaline amide hydrolysis, followed by treatment with *p*-toluenesulfonyl chloride. Ring closure of (XIII) with ethylene glycol ditosylate (XIV) and subsequent acidic deprotection as above gives plerixafor (8, 9). Scheme 2.

3) A different protection strategy involves masking the ring nitrogens as amide groups. Methyl acrylate (XVII) is reacted with neat ethylenediamine (XVIII) to yield the aminopropionamide derivative (XIX), which is then cyclized with dimethyl malonate (XX), producing the trioxocyclam (XXI). After condensation of (XXI) with *p*-dibromoxylene (IIIa), the resulting hexaoxo bis-cyclam (XXII) is reduced to the title compound employing borane-dimethyl sulfide complex in refluxing THF (10).

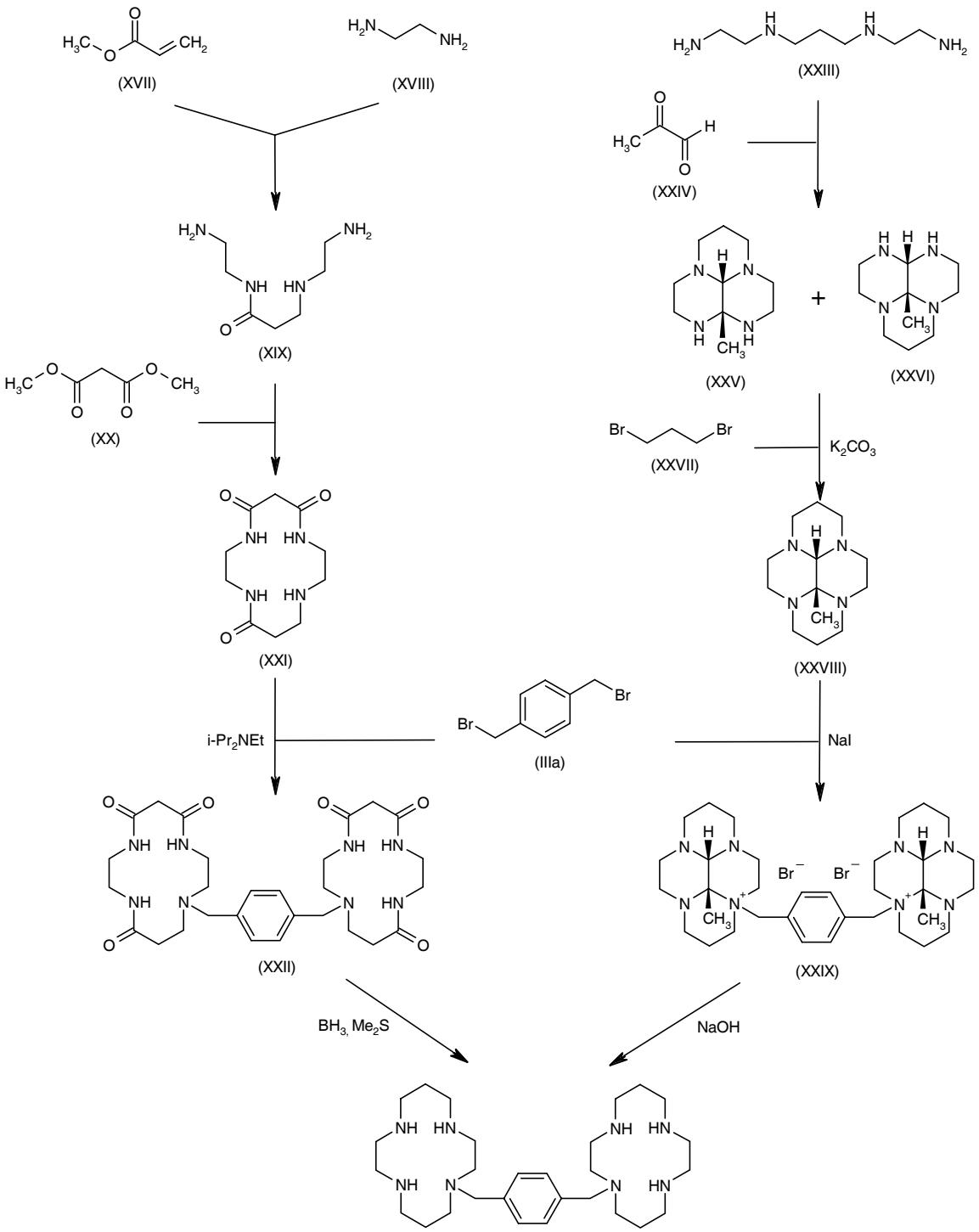
Scheme 2: Synthesis of Plerixafor



Alternatively, protection of the linear tetraamine (XXIII) with pyruvic aldehyde (XXIV) generates the tricyclic bis-aminal (XXV) along with its minor isomer (XXVI). The crude mixture of bis-aminals (XXV) and (XXVI) is then cyclized to (XXVIII) with 1,3-dibromopropane (XXVII) and K_2CO_3. After condensation of (XXVIII) with dibromide (IIIa), the resulting bis-ammonium dimer (XXIX) is hydrolyzed to the title compound upon heating with 3M NaOH (11). Scheme 3.

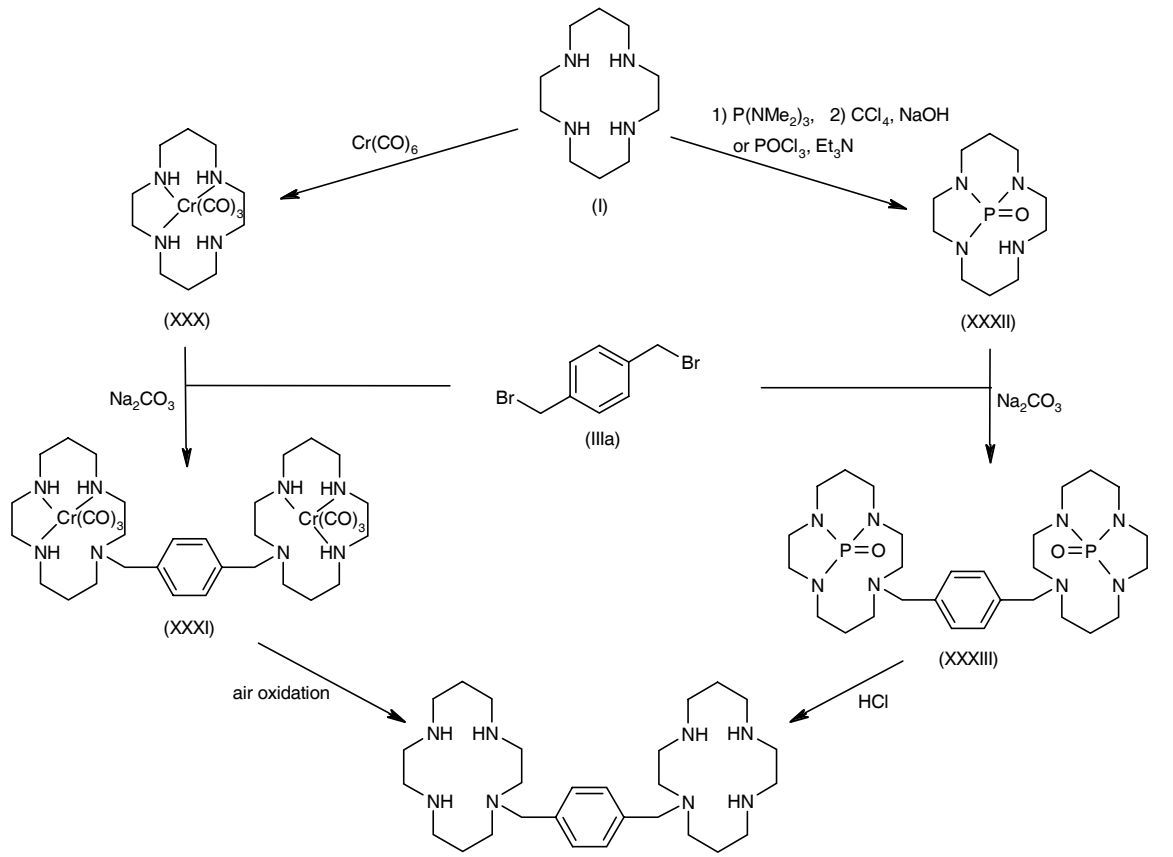
4) The tetraazacyclotetradecane (I) is protected as the chromium tridentate complex (XXX) upon heating with chromium hexacarbonyl in deaerated dibutyl ether. Subsequent condensation of the cyclam-$Cr(CO)_3$ complex (XXX) with *p*-dibromoxylene (IIIa) produces the chromium-protected bis-cyclam (XXXI), which is deprotected to plerixafor by oxidation with air in aqueous HCl (12, 13). Alternatively, protection of (I) as the phosphorotriamide (XXXII) can be accomplished by reaction with

Scheme 3: Synthesis of Plerixafor



tris(dimethylamino)phosphine, followed by oxidation with CCl_4 and NaOH (12) or by treatment with $POCl_3$ and Et_3N (14). After condensation of (XXXII) with dibromide (IIIa), the dimeric bis-phosphoramide obtained (XXXIII) is hydrolyzed to the title compound by treatment with diluted HCl (12, 14). Scheme 4.

Scheme 4: Synthesis of Plerixafor



Background

CXCR4, also known as fusin, is an α-chemokine receptor specific for stromal cell-derived factor-1 (SDF-1, more recently designated CXCL12). As a 7-transmembrane receptor coupled to G-protein-mediated signaling pathways, it transduces a signal by increasing intracellular calcium ion levels and displays potent lymphocytic chemotactic activity. It plays a role in hematopoiesis, neuronal and cardiovascular development, the spread and progression of tumors and organization of the immune system (15, 16). SDF-1α and the CXCR4 receptor have also been implicated in stem cell mobilization (17-19).

Granulocyte colony-stimulating factor (G-CSF) is the gold standard for hematopoietic stem cell (HSC)/hematopoietic progenitor cell (HPC) mobilization for transplantation. However, due to large differences in the response to G-CSF (20), other agents for use alone or in combination with G-CSF are being sought to enhance HSC mobilization. The bicyclam plerixafor hydrochloride (AMD-3100, Mozobil™) was originally discovered as a potent and selective anti-HIV agent (EC_{50} = 1-5 nM) with a novel mechanism of antiviral activity via an interaction with viral uncoating, and displaying good *in vivo* antiviral activity (2, 21-27). Early studies postulated that its molecular mechanism of action was related to the viral gp120 protein (21, 22), and subsequent investigations demonstrated that plerixafor inhibited HIV replication by binding to the CXCR4 receptor, the main co-receptor for gp120 used by X4 T-tropic strains of HIV for membrane fusion and cell entry (28-30). However, AnorMED (now Genzyme) discontinued development of plerixafor as an i.v. infusion therapy for HIV, as continuous exposure to the drug at higher doses was associated with unexpected cardiac disturbances (31). On the other hand, this same study revealed an increase in peripheral leukocytes on plerixafor due to mobilization of HSCs from bone marrow, leading to its investigation for mobilizing stem cells (21, 31).

Preclinical Pharmacology

In vitro studies confirmed that plerixafor selectively interacts with the CXCR4 receptor. Calcium flux assays with selected chemokine/cell combinations also confirmed that it does not interact with CXCR1-CXCR3 or CCR1-CCR9. In contrast, plerixafor potently and concentration-dependently inhibited CXCR4-mediated calcium signaling (IC_{50} = 0.01-0.13 μg/ml) and chemotaxis (IC_{50} = 0.13 μg/ml) in several different cell types. Furthermore, it

inhibited CXCL12-induced endocytosis of CXCR4, but did not affect phorbol ester-induced receptor internalization. Plerixafor-mediated CXCR4 agonism was ruled out due to its inability to elicit intracellular calcium flux, induce chemotaxis or trigger CXCR4 internalization (28).

Preincubation of human megakaryocytic leukemia-derived MO7e cells with 1 μM plerixafor completely blocked activation of ERK1/2 by CXCL12 and > 95% inhibition of ERK1/2 was evident upon co-incubation with plerixafor and CXCL12 (32).

Molecular modeling of the CXCR4 receptor and receptor mutagenesis identified Asp171 and Asp262, located in transmembrane domains (TM) IV and TM-VI, respectively, at each end of the main ligand-binding crevice of the CXCR4 receptor, as well as Glu288 in TMVII, as being essential for plerixafor to block the binding of CXCL12 (29, 33).

Administration of plerixafor to mice (5 mg/kg s.c.) resulted in significant increases in pluripotent hematopoietic progenitors: granulocyte-macrophage colony-forming units (CFU-GM, +8.2-fold), erythroid burst-forming units (BFU-E, +6.8-fold) and granulocyte-erythrocyte-macrophage-megakaryocyte CFUs (CFU-GEMM, +8.3-fold). Further enhanced progenitor cell levels were observed upon the administration of plerixafor 16 h after G-CSF (2.5 μg s.c. b.i.d. over 2 h), with respective mobilizations reaching 43.7-, 19.3- and 44.5-fold. Moreover, subsequent administration of the chemokine macrophage inflammatory protein-1α (MIP-1α; 5 μg i.v.) further enhanced HPC mobilization (32).

Further studies in mouse strains that exhibit differing responses to G-CSF treatment indicated greater plerixafor/G-CSF synergistic mobilization in G-CSF high responders (DBA/2 mice) *versus* poor responders (C57Bl/6 mice), with BFU-E and CFU-GEMM mobilization enhanced by 40.9- and 42-fold, respectively, in DBA/2 mice *versus* 10.7- and 11.9-fold, respectively, in the other mouse strain. This may be relevant clinically, as intraindividual variability in responses to G-CSF has been reported (34, 35).

The ability of plerixafor to mobilize self-renewing stem cells was also evaluated in mice. Plerixafor (5 mg/kg s.c.) significantly enhanced the numbers of competitive repopulating long-term marrow self-renewing stem cells (CRLTMSCs) in lethally irradiated mice receiving nonirradiated marrow cells at 3:1, 2:1 and 1:1 donor:recipient ratios (by > 80%, > 80% and approximately 20%, respectively, compared to > 5% in those treated with saline) (34, 36). Successful stem cell transplantation requires homing of transplanted cells to the bone marrow microenvironment. Studies in lethally irradiated recipients indicated no difference in syngeneic stem cell homing and engraftment between plerixafor-treated and control animals (37).

Other *in vivo* studies compared the repopulating function of $CD34^+$ cells mobilized rapidly by plerixafor (4 h) and those mobilized by G-CSF over a treatment period of 5 days. In nonobese diabetic/severely compromised immunodeficient (NOD/SCID) mice, transplantation of plerixafor-mobilized $CD34^+$ cells consistently facilitated higher engraftment levels compared to G-CSF-mobilized $CD34^+$ cells (3.4% *versus* 0.8% human cell, respectively). Plerixafor treatment may therefore offer a more rapidly obtainable source of HSCs for clinical transplantation (38). Further studies have presented evidence for identical T-cell function between groups mobilized by G-CSF and G-CSF + plerixafor, as measured by graft-*versus*-host disease (GVHD) and donor chimerism studies, supporting the use of title compound for the mobilization of allogeneic stem cells (39-41).

The kinetics of HPC mobilization with plerixafor in combination with the alkylating agent cyclophosphamide have also been examined. A single dose of plerixafor (5 mg/kg s.c.) in mice rapidly mobilized white blood cells (WBCs) and increased the mobilization of HPCs into peripheral blood, peaking at 2-3 h postadministration, with a 3-5-fold increase in WBCs and a > 20-fold increase in peripheral blood neutrophils. Plerixafor administration following an initial i.p. dose of cyclophosphamide (200 mg/kg) enhanced the increase in CFU/ml (mean 43-fold compared to 7.1- and 22.6-fold, respectively, for plerixafor and cyclophosphamide alone), which occurred 1-2 days earlier. This study provided evidence for the synergistic potential of cyclophosphamide and plerixafor for peripheral blood stem cell (PBSC) mobilization (42).

The short- and long-term engraftment potentials of both autologous and allogeneic plerixafor-mobilized peripheral blood mononuclear cells (PBMCs) were investigated in a canine model. Following total-body irradiation, infusion of autologous plerixafor-mobilized PBMCs facilitated the recovery of neutrophil and platelet counts at a median of 9 and 25 days, respectively, with all dogs displaying normal marrow function within 1 year following transplantation. Infusions of allogeneic plerixafor-mobilized PBMCs taken from dog leukocyte antigen (DLA)-identical littermates facilitated neutrophil and platelet recoveries within a median of 8 and 26 days, respectively, after total-body irradiation. In those animals receiving allogeneic transplantation, marrow function was normal and blood-donor chimerism levels were 97-100% at a median follow-up of 53 weeks (43).

Transplantation of plerixafor-mobilized $CD34^+$ cells labeled with Neo®-containing retroviral vectors into myeloablated rhesus macaques allowed for engraftment assessment. $CD34^+$ cells harvested following mobilization with a single dose of plerixafor (1 mg/kg s.c.) displayed a long-term repopulating capacity, with Neo®-marked myeloid and lymphoid cells evident for up to 32 months after transplantation. Furthermore, more plerixafor-mobilized $CD34^+$ cells were in the G1 phase of the cell cycle compared to those mobilized using a 4-day G-CSF regimen, and they exhibited more phenotypic and functional characteristics (CXCR4 and VLA-4) hypothesized to be important in the mobilization and homing of HSCs (44, 45).

Other indications

Recent studies in mice, rats and dogs have also demonstrated plerixafor's potential to preserve myocar-

dial function via the mobilization of endothelial and hematopoietic progenitor cells in models of myocardial infarction (46-49).

Additional preclinical studies have indicated the anticancer potential of plerixafor. *In vitro*, the CXCR4 antagonist was shown to inhibit the CXCL12-stimulated migration of multiple myeloma (MM) and leukemia cells, along with CXCL12-stimulated MM cell adhesion and non-Hodgkin's lymphoma (NHL) cell migration and proliferation, while promoting NHL cell apoptosis (50-54). Studies in NOD/SCID mice bearing NHL tumors demonstrated significant antitumor activity for plerixafor when given as 3 weekly s.c. injections, but enhancement of tumor growth was seen when it was administered by continuous s.c. infusion for 28 days (51). Plerixafor also inhibited the proliferative, antiapoptotic and chemotactic effects of CXCL12 in human glioblastoma multiforme and medulloblastoma cell lines. *In vivo* in mice, systemic delivery of plerixafor blocked the growth of intracranial brain tumor xenografts (55-57).

Other experiments demonstrated its ability to improve renal function and survival in mice with ischemic acute renal failure (58), and to mobilize acute promyelocytic leukemia (APL) cells from the bone marrow into the peripheral blood compartment and sensitize these cells to chemotherapy (59, 60). Evidence for plerixafor's potential in rheumatoid arthritis and asthma has also been reported (61-64).

Pharmacokinetics and Metabolism

Early preclinical pharmacokinetic studies in rats demonstrated that single oral doses of plerixafor (20 mg/kg) were poorly absorbed and highly variable, with a bioavailability of only 3%. The half-life was reported to be 0.9 h after single doses and 0.7-0.8 h upon repeated dosing. The drug was mainly eliminated renally. Subcutaneous dosing over a period of 4 weeks in rats and dogs (0.25, 1 and 4 mg/kg/day) provided peak plasma levels within 1-2 h with good dose proportionality (65).

Further studies in rabbits demonstrated that following s.c. bolus injection (25 mg/kg), peak serum levels were 33.3-36.8 μg/ml, reached at 30-60 min, decreasing to 1 μg/ml within 8 h, although plerixafor was still detectable up to 24 h postadministration. Concentration-*versus*-time data confirmed a biexponential elimination phase, with a β1 elimination half-life of approximately 1 h and a β2 half-life of about 4-5 h (66).

In dogs administered a single s.c. dose of plerixafor (4 mg/kg), peak plasma levels of 7.8-10.3 μg/ml were achieved within 1 h and drug was cleared within 24 h after administration (43).

An initial open-label pharmacokinetic and safety study was performed in 6 healthy volunteers administered escalating doses. Single 15-min i.v. infusions at doses of 10 or 20 μg/kg were associated with C_{max} values of 48 and 118 ng/ml, respectively, while median terminal half-life was reported to be 2.77 h, with a clearance of 8.19 l/h and a volume of distribution of 0.29 l/kg. Treatment was not associated with serious adverse events in either the lower or higher dose cohorts (65).

Pharmacokinetic data from 12 healthy volunteers following i.v. infusion (10, 20, 40 or 80 μg/kg) demonstrated that C_{max} and $AUC_{0-\infty}$ values were dose-dependent. Median systemic absorption following s.c. dosing (40 or 80 μg/kg) was 87%, but no drug was detectable in the blood following oral dosing (80 and 160 μg/kg). Median volume of distribution, clearance and elimination half-life values were 0.34 l/kg, 1.30 l/h and 3.6 h, respectively. Good tolerability was reported, without evidence of grade 2 toxicity or dose adjustment (67).

The exposure-response relationship of plerixafor was analyzed in 29 healthy subjects receiving a single s.c. dose of 40, 80, 160, 240 or 320 μg/kg. The pharmacokinetics of plerixafor were described by a two-compartment model with first-order absorption. Clearance and volume of distribution were estimated to be 5.17 l/h and 16.9 l, respectively. $CD34^+$ cell mobilization was best described by an indirect effect model that stimulates the entry process of $CD34^+$ from the bone marrow to peripheral blood in the form of a sigmoid maximum effect model, with estimated E_{max}, EC_{50} and equilibration times of 12.6 μg/l, 53.6 μg/l and 5.37 h, respectively (68-71).

Dose-response studies of $CD34^+$ cell mobilization and pharmacokinetic analyses were performed in healthy volunteers administered plerixafor alone (single doses of 40-320 μg/kg s.c.) or combined with G-CSF (10 μg/day over 5 days plus plerixafor 160-240 μg/kg s.c. on day 5). For plerixafor alone, $CD34^+$ cell responses peaked at a dose of 240 μg/kg at 9 h. Combination therapy (plerixafor 240 μg/kg) provided a peak effect at 14 h. Pharmacokinetic studies demonstrated a linear increase in peak drug levels with increasing dose and a half life of 3-6 h. Few adverse events were reported at the doses administered and included facial tingling, mild nausea, bloating and diarrhea. A dose of plerixafor of 240 μg/kg appeared to be optimal for $CD34^+$ mobilization with or without G-CSF (72).

Clinical Studies

Initial phase I studies recognized the potential for plerixafor when single i.v. doses of the compound augmented peripheral leukocyte counts, principally due to mobilization of $CD34^+$ HSCs from the bone marrow into the blood circulation. Mobilization of WBCs was investigated in 10 healthy volunteers who received a single s.c. injection of 80 μg/kg. A 4-fold increase in circulating $CD34^+$ cells was associated with a rapid induction of leukocytosis, with peak increases in WBCs and absolute neutrophil count observed at 6 h following injection of plerixafor, returning to baseline within 24 h. The drug was well tolerated, with 1 case each of nausea/vomiting and perioral paresthesias (73, 74). In 13 further subjects, single s.c. doses of 40-240 μg/kg plerixafor increased the number of circulating neutrophils, lymphocytes, monocytes, eosinophils and basophils at 6-9 h after administration. These effects were associated with a dose-dependent increase in the number of circulating $CD34^+$ progenitor

cells, which reached peak values of up to 10-fold higher than baseline at 9 h. Plerixafor (80 μg/kg s.c.) administered as a single dose or once daily on 3 consecutive days induced similar increases in the levels of circulating progenitor cells. All adverse events were mild and transient and the most common were erythema or stinging at the injection site, headache, perioral paresthesias, nausea and sensation of abdominal distension without diarrhea (74). The ability of plerixafor to form CFUs was examined prior to and at 1, 3, 6, 9 and 24 h after administration to these volunteers. Assays of venous blood samples demonstrated that plerixafor increased circulating levels of myeloid and erythroid progenitor cells. Peak increases in circulating CFU-GMs (+20-fold), BFU-Es (+6-fold) and CFU-GEMMs (+10-fold) were seen at 6 h after drug administration (73, 74).

Further studies assessed whether plerixafor could enhance responses to G-CSF for the mobilization and collection of $CD34^+$ progenitor cells. In the first part of the study, 18 healthy subjects were given recombinant human G-CSF (10 μg/kg/day s.c. for 4 days) followed by G-CSF (10 μg/kg s.c.), plerixafor (160 μg/kg s.c.) or a combination of the two agents on day 5. Plerixafor significantly increased $CD34^+$ cell counts compared to G-CSF, and the combination was even more effective. Within a cohort of 10 additional subjects enrolled for $CD34^+$ cell collection, 6 followed the treatment schedule outlined above and 4 received a single-dose regimen of plerixafor (240 μg/kg s.c.). Collection of $CD34^+$ cells was comparable between individuals mobilized by single doses of plerixafor and those mobilized with a 5-day G-CSF regimen. However, combined therapy was associated with significantly more circulating $CD34^+$ cells than either agent alone (34, 75, 76).

The safety and clinical efficacy of plerixafor were investigated in patients with MM (n=7) and NHL (n=6). Plerixafor facilitated a rapid and significant increase in total WBC and peripheral blood $CD34^+$ counts at 4 and 6 h after a single injection (160 or 240 μg/kg) in patients who had received prior chemotherapy. The drug was well tolerated (77). The results from this and some of the following studies are summarized in Table I.

Subsequent studies in patients with MM and NHL confirmed that plerixafor + G-CSF-mediated peripheral blood HSC mobilization is superior to G-CSF alone (78-83). U.S. phase II studies enrolled patients with MM or NHL, including heavily pretreated patients, to receive G-CSF (10 μg/kg/day s.c.) and plerixafor (240 μg/kg s.c.) starting on day 4. The results demonstrated superior mobilization of autologous HPCs and fewer apheresis procedures required to reach the target level of $CD34^+$ cells for transplantation. No significant toxicity was reported (78-81). A similar European phase II study showed comparable results (82, 83). Gene expression profiling revealed that the addition of plerixafor to G-CSF for mobilizing $CD34^+$ cells is associated with increased expression of genes potentially associated with superior engraftment after myeloablative therapy (84, 85).

Further examination of MM or NHL patients indicated that greater CXCR4 expression in the bone marrow may predict for lower mobilization efficacy with plerixafor. Patients received G-CSF at 10 μg/kg/day for 4 days and then plerixafor 240 μg/kg on day 4. The number of $CD34^+$ cells detected in peripheral blood on day 5 was inversely correlated with CXCR4 expression, which was significantly decreased on day 5. Pretreatment, CXCR4 expression in bone marrow and peripheral blood was not correlated with $CD34^+$ counts, although CXCR4 expression in bone marrow was significantly elevated in 1 patient considered to be a 'poor mobilizer' (86, 87).

Another phase II study examined the effects of plerixafor (240 μg/kg s.c.) and G-CSF (16 μg/kg) on the mobilization of $CD34^+$, dendritic and lymphoma cells in 10 hard-to-mobilize NHL patients. The results showed that the addition of plerixafor enhances $CD34^+$ and dendritic cell mobilization, but not lymphoma cell mobilization (88).

In another study, the first 10 patients with Hodgkin's disease mobilized with plerixafor (240 μg/kg/day) beginning on day 4 after G-CSF (10 μg/kg/day) showed greater numbers of HPCs collected by apheresis and a reduction in the number of days of apheresis. A median 3.0-fold increase in $CD34^+$ cells was seen after the first dose of plerixafor. Eight patients have been transplanted with the plerixafor + G-CSF-mobilized cells and all showed rapid and stable engraftment (89).

Pilot studies to evaluate the safety and efficacy of plerixafor for the mobilization and transplantation of HLA-matched sibling donor HSCs in patients with advanced hematological malignancies have been ongoing since 2004 (90-95). The most recent communication reported the responses of 11 HLA-identical siblings receiving one or two doses of plerixafor (240 μg/kg s.c.), followed 4 h later by leukapheresis. One week later, the same donors were remobilized with 10 μg/kg/day G-CSF, followed 5 days later by leukapheresis. Plerixafor treatment was associated with a median 7-fold increase in peripheral $CD34^+$ cells within 4-6 h compared to a 2-fold increase after G-CSF. Grafts mobilized after plerixafor treatment had fewer $CD34^+$ cells but more T-, B- and natural killer (NK) cells compared to G-CSF-mobilized grafts. Seven of 8 patients transplanted with plerixafor-mobilized cells were alive without progression at the time of reporting (95).

These studies led to a compassionate-use protocol involving 150 cancer patients classified as 'poor mobilizers'. Data from the first 100 patients were recently presented. Each patient was given G-CSF (10 μg/kg/day s.c.) for at least 5 days combined with plerixafor (240 μg/kg/day s.c.) starting on day 4. Combination therapy effectively increased the number of $CD34^+$ cells in blood, with 50%, 73%, 60% and 69% of NHL, MM, acute myeloid leukemia (AML) and Hodgkin's disease patients, respectively, having $> 2 \times 10^6$ cells/kg collected. The vast majority of these patients subsequently had successful engraftment. Treatment was generally well tolerated (96).

Further compassionate use of plerixafor proved successful in 3 pediatric patients diagnosed with Ewing's sarcoma, acute lymphoblastic leukemia (ALL) and medulloblastoma and classified as 'poor mobilizers'. Treatment

Table I: Clinical studies of plerixafor hydrochloride (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Lymphoma, non-Hodgkin's, Multiple myeloma	Open	Plerixafor, 160 µg/kg (n=6) Plerixafor, 240 µg/kg (n=7)	13	Plerixafor was well tolerated and effectively mobilzed white blood cells and $CD34^+$ cells in patients with multiple myeloma and non-Hodgkin's lymphoma	77
Lymphoma, non-Hodgkin's, Multiple myeloma	Open	G-CSF, 10 µg/kg/d s.c. x 9 d + Plerixafor, 240 µg/kg/d s.c.	20	Plerixafor plus G-CSF showed rapid, significant mobilization of hematopoietic progenitor cells in patients with non-Hodgkin's lymphoma or multiple myeloma, which may reduce the number of apheresis procedures needed to collect an adequate number of cells for engraftment. Twelve patients were successfully transplanted with allografts obtained after plerixafor/G-CSF stem cell mobilization	78
Hematological malignancies	Open	Plerixafor, 240 µg/kg s.c. x 1 → G-CSF, 10 µg/kg/d x 5 d Plerixafor, 240 µg/kg s.c. x 2 → G-CSF, 10 µg/kg/d x 5 d	11	Plerixafor increased peripheral $CD34^+$ cell counts 7-fold from baseline and was associated with successful stem cell transplantation and engraftment in all 8 evaluable patients	95
Cancer	Open	G-CSF, 10 µg/kg/d x ≥ 5 d + Plerixafor, 240 µg/kg s.c. starting on d 4	100	Data from patients treated as part of a compassionate-use protocol demonstrated the ability of plerixafor in combination with G-CSF to induce cell mobilization in cancer patients previously unable to mobilize $CD34^+$ cells	96
Ewing's sarcoma, Medulloblastoma, Leukemia, acute lymphoblastic	Open	G-CSF, 10 µg/kg x ≥ 5 d + Plerixafor, 240 µg/kg/d s.c. starting on day 5	3	Stem cell collection was successful in pediatric cancer patients given plerixafor + G-CSF for mobilization	97
Lymphoma, non-Hodgkin's, Multiple myeloma	Open	Plerixafor [after first apheresis collection] + Cyclophosphamide + G-CSF, 10 µg/kg/d (n=11) Plerixafor [after first apheresis collection] + Rituximab + Methylprednisolone + Cytarabine + Cisplatin + Etoposide or Rituximab + Ifosfamide + Carboplatin + Etoposide + G-CSF, 10 µg/kg/d (n=4)	15	Plerixafor added to chemotherapy and G-CSF mobilization increased circulating levels of peripheral blood stem cells in patients with multiple myeloma and non-Hodgkin's lymphoma undergoing stem cell transplantation	98
Lymphoma, non-Hodgkin's	Randomized Double-blind Multicenter	Plerixafor + Filgrastim Placebo + Filgrastim	340	This phase III study will compare the safety and efficacy of filgrastim alone or combined with plerixafor in mobilizing and collecting the optimal number of stem cells for autologous transplantation in patients with non-Hodgkin's lymphoma	99
Lymphoma, non-Hodgkin's, Multiple myeloma	Open	G-CSF, 10 µg/kg/d x 2-5 d + Plerixafor, 240 µg/kg s.c.		This phase I study will determine the tolerability and efficacy of plerixafor combined with a G-CSF mobilizing regimen in increasing the number of peripheral stem cells in patients scheduled to undergo transplantation	101
Leukemia, acute myeloid, Myelodysplasia	Open	Plerixafor, 80 µg/kg + Busulfan + Fludarabine + [if unrelated/unmatched donor] Thymoglobulin Plerixafor, 160 µg/kg + Busulfan + Fludarabine + [if unrelated/unmatched donor] Thymoglobulin Plerixafor, 240 µg/kg + Busulfan + Fludarabine + [if unrelated/unmatched donor] Thymoglobulin	50	Plerixafor will be evaluated in a phase I/II study as a preparative regimen for eliminating malignant cells before stem cell transplantation and prolonging survival in patients with acute myeloid leukemia or myelodysplastic syndrome	103
Healthy volunteers	Open	Plerixafor s.c.	25	This phase II study will evaluate the efficacy of plerixafor in mobilizing stem cells in healthy volunteers previously mobilized with G-CSF	106

involved a 4-day prephase of 10 μg/kg G-CSF and plerixafor 240 μg/kg s.c. on day 5, and then daily until collection of the required number of $CD34^+$ cells. Following combination therapy, an adequate number of peripheral blood HSCs was successfully collected for all patients to enable re-infusion following myeloablative chemotherapy or sequential tandem high-dose therapy (in the medulloblastoma case), and sustained engraftment was achieved (97).

Clinical studies have also assessed mobilizing regimens of plerixafor with chemotherapy and G-CSF. In 15 patients with MM or NHL, the addition of plerixafor to cyclophosphamide for MM and either rituximab, etoposide, cisplatin, cytarabine and methylprednisone (R-ESHAP) or rituximab, ifosfamide, carboplatin and etoposide (R-ICE) for NHL, along with G-CSF, provided a mean 1.9-fold increase in $CD34^+$ cells collected by leukapheresis. Following transplantation, the patients achieved absolute neutrophil counts of > 500 and platelet independence at a median of 10 and 11 days, respectively (98).

Plerixafor is currently in phase III clinical evaluation for mobilizing stem cells in combination with filgrastim (G-CSF) in patients with NHL and MM (99, 100). Other clinical trials are under way, including phase I studies in combination with G-CSF for patients with MM and NHL failing or probably failing all other conventional stem cell collection therapies (101), and by itself for mobilizing and transplanting HLA-matched sibling donor HSCs for patients with advanced hematological malignancies (102). A phase I/II study is evaluating plerixafor with busulfan, fludarabine and thymoglobulin for allogeneic stem cell transplantation in patients with AML and myelodysplastic syndromes (MDS) (103), and phase II studies are in progress in combination with G-CSF in patients with MM and NHL, including poorly mobilizing patients previously failing conventional stem cell collection methods (104, 105). Another phase II study is assessing peripheral blood HPC mobilization with plerixafor in healthy volunteers previously mobilized with G-CSF (106).

Source

Genzyme Corp. (US) (through its recent acquisition of AnorMED).

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