

## The High-Affinity CXCR4 Antagonist BKT140 Is Safe and Induces a Robust Mobilization of Human CD34<sup>+</sup> Cells in Patients with Multiple Myeloma

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### Abstract

**Purpose:** CXCR4 plays an important role in the retention of stem cells within the bone marrow. BKT140 (4F-benzoyl-TN14003) is a 14-residue bio stable synthetic peptide, which binds CXCR4 with a greater affinity compared with plerixafor (4 vs. 84 nmol/L). Studies in mice demonstrated the efficient and superior mobilization and transplantation of stem cells collected with G-CSF-BKT140, compared with those obtained when using stem cells obtained with each one of these mobilizing agent alone. These results have served as a platform for the present clinical phase I study.

**Experimental Design:** Eighteen patients with multiple myeloma who were preparing for their first autologous stem cell transplantation were included. Patients received a standard multiple myeloma mobilization regimen, consisting of 3 to 4 g/m<sup>2</sup> cyclophosphamide (day 0), followed by granulocyte colony—stimulating factor (G-CSF) at 5 µg/kg/d starting on day 5 and administered between 8 and 10 pm until the end of stem cell collection. A single injection of BKT140 (0.006, 0.03, 0.1, 0.3, and 0.9 mg/kg) was administered subcutaneously on day 10 in the early morning, followed by G-CSF 12 hours later.

**Results:** BKT140 was well tolerated at all concentrations, and none of the patients developed grade 3 and 4 toxicity. A single administration of BKT140 at the highest dose, 0.9 mg/kg, resulted in a robust mobilization and collection of CD34<sup>+</sup> cells ( $20.6 \pm 6.9 \times 10^6$ /kg), which were obtained through a single apheresis. All transplanted patients received  $\sim 5.3 \times 10^6$  CD34<sup>+</sup> cells/kg, which rapidly engrafted ( $n = 17$ ). The median time to neutrophil and platelet recovery was 12 and 14 days, respectively, at the highest dose (0.9 mg/kg).

**Conclusions:** When combined with G-CSF, BKT140 is a safe and efficient stem cell mobilizer that enabled the collection of a high number of CD34<sup>+</sup> cells in 1 and 2 aphaeresis procedures, resulting in successful engraftment. *Clin Cancer Res*; 20(2); 469–79. ©2013 AACR.

### Introduction

High-dose chemotherapy in conjunction with autologous stem cell transplantation (ASCT) has emerged as an established treatment modality for a variety of hematologic malignancies, including multiple myeloma, non-

Hodgkin lymphoma (NHL), and Hodgkin lymphoma (1, 2). Although  $2 \times 10^6$  CD34<sup>+</sup> cells/kg is considered to be the lowest stem cell dose required to ensure hematopoietic engraftment (3, 4), a higher CD34<sup>+</sup> dose ( $>5 \times 10^6$  cells/kg) results in a more rapid, sustainable hematopoietic recovery (5).

Granulocyte colony—stimulating factor (G-CSF) administered in conjunction with chemotherapy is most commonly used for stem cell mobilization (3). However, such mobilization fails to provide a sufficient number of CD34<sup>+</sup> stem cells in approximately 10% to 20% of patients with multiple myeloma and 11% to 55% of patients with NHL, depending on the type and number of precollection therapies applied (6).

The employment of plerixafor (Mozobil, AMD3100; Genzyme Corp.), a small molecule that reversibly inhibits CXCL12 binding to CXCR4, for stem cell mobilization seems to improve mobilization rates and rescue a substantial number of "hard mobilizer" subjects (7). However, even

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### Translational Relevance

The use of peripheral blood as a source of hematopoietic stem cells (HSC or CD34<sup>+</sup> cells) for patients with cancer undergoing both autologous and allogeneic transplantation has largely replaced bone marrow as the preferred source for hematopoietic bone marrow restoration. The primary goal of HSC mobilization, irrespective of the mobilization regimen used, is always to collect sufficient CD34<sup>+</sup> cells for transplantation, preferably in the first mobilization attempt and ideally with a minimum of apheresis sessions (collection events for HSCs). However, although CD34<sup>+</sup> yield serves as a significant factor predicting transplant success, other factors such as the quality of the cell collection composition, rate of engraftment, and subsequent immune reconstitution also contribute, significantly, to long-term patient survival outcomes.

BKT140 (4F-benzoyl-TN14003) is a novel, potent selective inhibitor of the CXCR4 chemokine receptor, which is shown here to, not only bind with high affinity to the CXCR4, but, more importantly, to dissociate from this important receptor in a very slow fashion. In contrast, plerixafor binds in rapidly reversible fashion. As a result, BKT140 has the unique ability, when compared with all other CXCR4 inhibitors, including plerixafor, to shut down completely the normal cell signaling process governing cell trafficking in the bone marrow. This exclusive activity of BKT140 leads to a strong synergistic effect when combined with the granulocyte colony-stimulating factor, resulting in rapid and robust HSC mobilization therapy that is differentiated from current standard of care.

with the use of plerixafor, the mobilization of a sufficient number of stem cells remains a difficult objective in a sizeable proportion of patients (41% NHL and 13% patients with multiple myeloma), particularly those treated with lenalidomide or fludarabine as induction therapy (8–10).

BKT140 (4F-benzoyl-TN14003), a novel CXCR4 antagonist, binds and inhibits the CXCR4 chemokine receptor with high affinity, showing an IC<sub>50</sub> of ~1 nmol/L (11–13) compared with the values obtained with AMD3100 (IC<sub>50</sub> = 651 ± 37 nmol/L; ref. 14). Moreover, BKT140 hinders the cell migration stimulated by CXCL12 within IC<sub>50</sub> values of 0.5 to 2.5 nmol/L (12, 15) compared with the IC<sub>50</sub> value of 51 ± 17 nmol/L for Mozobil (14), suggesting a high mobilization capacity.

Indeed, *in vivo* animal studies have shown that BKT140 rapidly mobilizes a large number of stem cells (16). Furthermore, BKT140 strongly synergizes with G-CSF to rapidly mobilize long-term repopulating stem cells, in addition to BFU-E and GEMM progenitors (16).

This study investigated the dose-dependent mobilization capacity of BKT140 in both mice and humans, hypothesizing that a higher BKT140 dosage will provide

a sufficient amount of highly productive stem cells, with fewer apheresis procedures.

### Materials and Methods

#### Mouse experiments

**Reagents and mice.** Plerixafor was purchased from Sigma, and G-CSF (Neupogen, Filgrastim) was purchased from Amgen. BKT140 was produced and provided by Biokine Therapeutics Ltd. Cyclophosphamide (CPM) was purchased from Baxter Oncology GmbH. Female C57BL/6 mice (7–8 weeks old) were purchased from Harlan. Congenic C57BL/6 (CD45.2) and B6.SJL-Ptcrca Pep3b/BoyJ (B6.BoyJ:CD45.1) mice were purchased from Jackson Laboratory. The mice were maintained under specific pathogen-free conditions at the Hebrew University Animal Facility (Jerusalem, Israel). All experiments were approved by the Animal Care and Use Committee of the Hebrew University. BKT140 (12 mg/kg, equivalent to 0.11 μmol/L/200 μL), plerixafor (AMD3100; 4.4 mg/kg, equivalent to 0.11 μmol/L/200 μL), and G-CSF (5 μg/mouse, for 4 consecutive days) were all reconstituted in PBS and injected subcutaneously in a total volume of 200 μL.

#### *In vitro* experimental protocols

A migration assay was performed using transmigration plates of 6.5 mm/diameter and 5 μm/pore (Costar). Jurkat cells were resuspended in RPMI medium containing 1% Fetal Calf Serum (FCS). Cells (2 × 10<sup>5</sup> cells/well) were added to the upper chambers in a total volume of 100 μL, and 600 μL RPMI supplemented with 100 ng/mL CXCL12 (PeproTech) was added to the lower chambers. Various concentrations of BKT140 (0.6, 1.2, 2.5, 3.6, and 5 nmol/L) were added to the lower chambers. The cells migrating to the bottom chamber of the Transwell within 3 hours were counted using a FACSCalibur Flow Cytometer (BD Biosciences). The data were analyzed using software from CellQuest (version 3.3; BD Biosciences). The IC<sub>50</sub> of BKT140 was calculated using GraphPad Prism software. To analyze the interaction of BKT140 and plerixafor with CXCR4, we used PE-conjugated mouse monoclonal anti-human CXCR4 (clone #12G5; R&D Systems), which recognizes the active conformation of the CXCR4 receptor and can block its interaction with the natural ligand CXCL12. A PE-conjugated mouse IgG2A isotype control was used as a negative control (IC003P; R&D Systems).

#### *In vivo* experimental protocols in mice

BKT140 or plerixafor (AMD3100) was administered alone or following the administration of G-CSF. Blood and bone marrow samples were collected at 4 hours after the last injection of BKT140 or plerixafor. Peripheral blood cells were collected by cardiac puncture, and the total blood counts were tested by American Medical Laboratories (AML) using an automatic multiparameter blood cell counter—Sysmex KX-21. To evaluate the number of progenitor cells in the blood, a colony-forming cell (CFC) assay was performed, evaluating the number of mobilized CFCs following treatment with BKT140 or plerixafor. The

colonies were assayed by plating the cells collected from the blood in Iscove's-modified Dulbecco's Medium containing 1% methylcellulose, 15% FBS, 1% bovine serum albumin, 3 U/mL rh EPO, 104 M 2-mercaptoethanol, 2 mmol/L L-glutamine, 50 ng/mL rmSCF, 10 ng/mL rmIL-3, 10 µg/mL rh insulin, 10 ng/mL rh IL-6, and 200 µg/mL human transferrin (MethoCult GF M3434; StemCell Technologies Inc.). The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The colonies that developed 5 days later were visually scored using a light microscope (using morphologic criteria).

Transplantation was performed by transplanting the mobilized cells into lethally irradiated (900 rad) mice. Mobilized cells were collected from the PB (900 µL) of 2 mice treated 4 hours precollection with BKT140, G-CSF, or both. Four months after the first transplantation, the bone marrow cells recovered from the recipient mice were transplanted ( $2.5 \times 10^6$  cells/mouse) into lethally irradiated secondary recipients. FITC-conjugated anti-mouse CD45.2 and anti-mouse CD45.1 (BD Biosciences) were used to assay the mouse donor cell chimerism of the transplanted C57BL mice.

### Clinical study

**Patients and methods.** A phase I, nonrandomized, open-label, dose escalation, multicenter study was performed in which escalated doses of BKT140 were added to a standard multiple myeloma mobilization protocol, consisting of high-dose CPM/G-CSF (17).

The study was approved by the Human Subjects Committee-Institutional Review Boards of the 2 participating medical centers, Chaim Sheba Medical Center (Tel Hashomer, IL) and Rambam Medical Center and Technion (Haifa, IL), and by the Israeli Ministry of Health. Inclusion criteria for each patient are shown in Supplementary Table S5 and prior therapies of each one of the participants, are shown in Supplementary Table S2. Written informed consent was obtained before enrollment in the study.

Eligible multiple myeloma subjects ( $n = 18$ ) received a standard multiple myeloma mobilization regimen, consisting of 3 to 4 g/m<sup>2</sup> CPM (day 0), followed by G-CSF at 5 µg/kg/d starting on day 5 and administered until the end of stem cell collection (G-CSF was self-administered stem cell each evening, between 8 and 10 pm).

A single injection of BKT140 was administered subcutaneously on day 10 in the early morning, followed by G-CSF 12 hours later. Stem cell collection was performed at WBC more than 1,000 cells. Notably, the protocol was based on the work published by To, and Gianni et al. (18, 19).

The BKT140 dose was increased, starting at 0.006 mg/kg with 2 patients, followed by cohorts of 4 patients: 0.03, 0.1, 0.3, and 0.9 mg/kg. Pharmacokinetic and pharmacodynamic assessments were conducted before BKT140 administration and at 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours postadministration.

Safety monitoring was performed at the same time points and continued for up to 7 days after the completion of stem

cell collection (stem cell collection was permitted for up to 4 days following the study drug administration).

The patients were followed throughout their transplant until myeloid and platelet recovery was documented.

### Eligibility criteria

Newly diagnosed multiple multiple myeloma subjects, aged 18 to 65 years old, who achieved at least partial response following induction (defined according to the international myeloma working group criteria) and were scheduled for peripheral blood stem cell collection followed by ASCT, were included in the study.

### Determination of blood counts and FACS analysis

Venous blood samples were obtained at the designated times for the quantitative evaluation of peripheral blood CD34<sup>+</sup> cells and CD138<sup>+</sup> cells; CD34<sup>+</sup> cells were also measured in leukapheresis products. CD34<sup>+</sup> cells were enumerated by FACS, according to the International Society for Hematotherapy and Graft Engineering protocol, in a single-platform, 2-color assay performed using an Epics XL flow cytometer (Beckman-Coulter).

### Leukapheresis

Peripheral blood mononuclear cells were collected by leukapheresis using a cell separator (Spectra). The mononuclear cell leukapheresis program was used according to the manufacturer's instructions to process 18 L of blood at a flow rate of 50 to 100 mL per min. The blood was anticoagulated with acid-citrate dextrose formula-A (1:22 ACD-A:whole blood ratio) and heparin (5,000 units/500 mL ACD-A). A volume of 28 mL of ACD-A was added to the collection bag before the procedure. The leukapheresis procedures were performed until the achievement of at least  $5 \times 10^6$  CD34<sup>+</sup> cells/kg.

### Transplantation

The patients received 200 mg/m<sup>2</sup> melphalan conditioning, followed by the infusion of the BKT140-collected CD34<sup>+</sup> cells. ASCT was permitted up to 5 weeks following the last aphaeresis. The lowest number of CD34<sup>+</sup> cells allowed for transplantation was  $2 \times 10^6$  cells/kg, as calculated for the actual body weight. The transplantation was performed according to local practice guidelines. G-CSF was started on day 5 or 6 posttransplantation and continued daily until neutrophil engraftment. Neutrophil engraftment was defined as neutrophil count  $\geq 0.5 \times 10^9/L$ , for 3 consecutive days (with no G-CSF support). Platelet (PLT) engraftment was defined as platelet count  $\geq 20 \times 10^9/L$ , without administering PLT transfusions for at least 7 days.

### Statistical methods

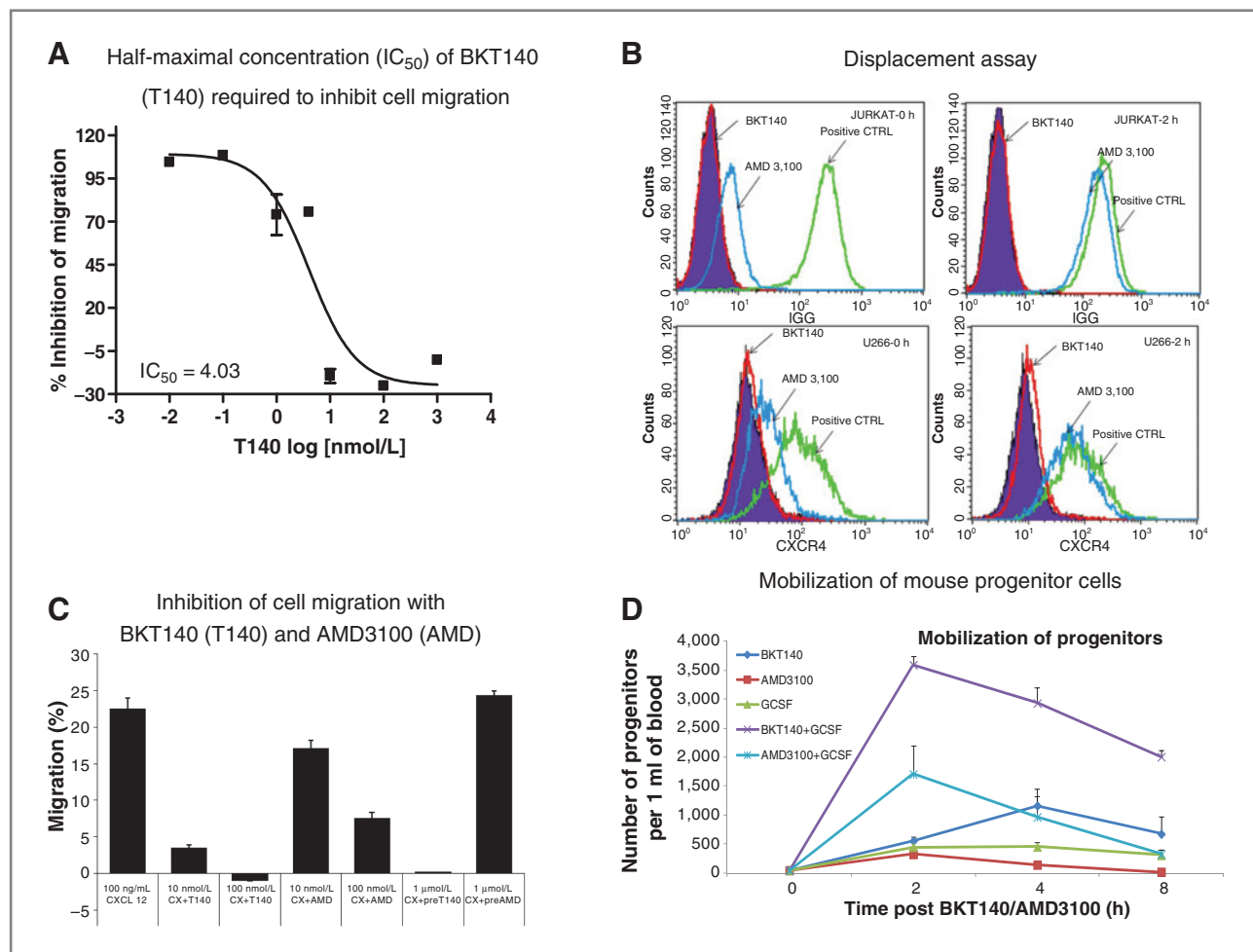
For human data, all measured variables and derived parameters are listed individually and, where appropriate, presented using descriptive statistics. The safety parameters and changes from baseline were examined and summarized for descriptive purposes. Adverse events (AE) were coded according to the MedDRA (version 12.1) system organ class

and preferred term. The individual study drug pharmacokinetic parameters and the mean, SD, values were calculated for each dose group for all subjects. Pharmacodynamic analyses included the values, changes from predose and fold increases of white blood cells (WBCs) (neutrophils, lymphocytes, monocytes, and platelets), CD34<sup>+</sup> and CD138<sup>+</sup> counts, red blood cells (RBCs), and the number of stem cell collections. The individual measurements and changes from baseline by time point are presented in addition to summary tables by dose group. The data were analyzed using SAS version 9.1. A *P* value of less than 0.05 was considered significant, and the significance of the differences between the groups for the stem cell collection was performed using Student *t* test. A paired two-tailed Student *t* test was used to evaluate the significant differences within the groups.

## Results

### *In vitro* characterization of BKT140 binding to CXCR4

The IC<sub>50</sub> value for the inhibition by BKT140 of human Jurkat leukemic T-cell migration toward 100 ng/mL CXCL12 was found to be 4.03 nmol/L (Fig. 1A). To test the ability of BKT140 and plerixafor to compete with 12G5 binding to CXCR4, Jurkat leukemic cells or U266 myeloma cells were incubated with BKT140 (2 μm) or plerixafor (2 μm) for 30 minutes at 4°C and then washed and stained for 15 minutes with an anti-12G5 antibody. When plerixafor or BKT140 were left in the staining medium together with 12G5 (without washing it out), both agents successfully competed with 12G5 for binding to CXCR4 (Fig. 1B). However, when the cells were washed and then stained at 120 minutes postwashing,



**Figure 1.** The half-maximal concentration (IC<sub>50</sub>) of BKT140 (T140) that was required to inhibit the migration of human derived Jurkat cells toward 100 ng/mL CXCL12 is shown in A. The expression of CXCR4 on untreated cells (Green line) was detected with anti-12G5 antibody compared with isotype antibody control staining (bold purple). The expression of CXCR4 by cells that were preincubated with BKT140 (2 μmol/L) is represented by a red line. The expression of CXCR4 by cells that were preincubated with plerixafor (2 μmol/L) is represented by blue line (B). The ability of BKT140 (T140) and plerixafor (10 and 100 nmol/L), added to the lower chamber of the transmigration assay, and that of BKT140 (1 μmol/L) and plerixafor (1 μmol/L), preincubated for 30 minutes with Jurkat cells and then washed, to inhibit the migration of Jurkat cells in response to CXCL12 (100 ng/mL) is shown in C. BKT140 (12 mg/kg) and plerixafor (3.2 mg/kg) were injected once; 5 injections of G-CSF (5 μg/mice daily) alone or together with one injection of BKT140 (12 mg/kg) and plerixafor (3.2 mg/kg) were administered subcutaneously into mice. The number of colony forming cells in the blood was tested after 0,2,4,8 hours (D).

BKT140 (2  $\mu\text{mol/L}$ ) but not plerixafor abrogated the binding of 12G5 to CXCR4 at both time points (Fig. 1B). Similar results were obtained for BL-2 lymphoma cells, primary leukemic cells, prostate PC3 cells, human  $\text{CD4}^+$  cells, and human  $\text{CD34}^+$  cells (data not shown). In agreement with the binding results, the Jurkat cells washed after preincubation with BKT140, but not with plerixafor, lost their ability to migrate in response to CXCL12 (Fig. 1C, T140 = BKT140).

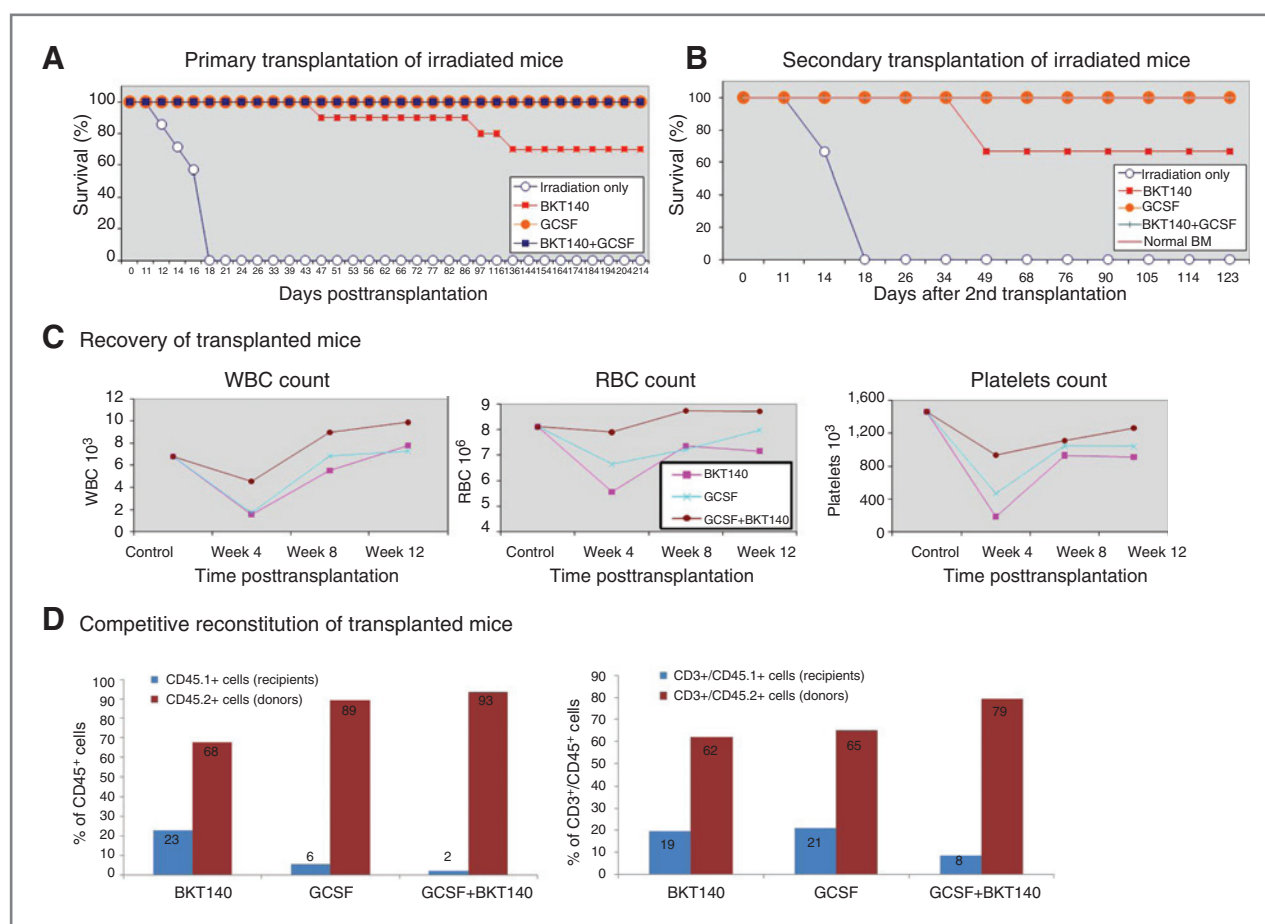
### BKT140 synergizes with G-CSF to mobilize highly efficient hematopoietic stem cells in mice

The mobilization capacity of a high dose of BKT140 (12 mg/kg) administered as a single agent or together with G-CSF was compared with that of a high dose of plerixafor (3.2 mg/kg) using a mouse model. It should be

noted that the above doses are equivalent to 0.9 mg/kg BKT140 and 0.24 mg/kg plerixafor in humans.

A single injection of high-dose BKT140 induced a robust (250-fold) and sustained increase in the number of hematopoietic stem cell (HSC) progenitors in the peripheral blood of treated mice (Fig. 1D). This effect was even more pronounced when high-dose BKT140 was administered together with G-CSF (Fig. 1D) and was higher than that obtained with high-dose plerixafor plus G-CSF (Fig. 1D).

Mouse stem cells were mobilized either with a single injection of 12 mg/kg BKT140 (Fig. 2A and B), 5 injections of G-CSF (5  $\mu\text{g}/\text{mice}$  daily; Fig. 2A and B), or by using a combination of 5 days of G-CSF followed by a single BKT140 injection (Fig. 2A and B). Stem cells were then harvested from 2 mice (900  $\mu\text{L}$  of blood) and transplanted into irradiated recipient mice, resulting in rapid and



**Figure 2.** C57BL/6 mice, serving as donors, were injected with BKT140, G-CSF, or a combination of the 2. G-CSF was subcutaneously injected daily for 3 consecutive days at 5  $\mu\text{g}/\text{mouse}/\text{injection}$ ; 24 hours after the last injection of G-CSF, BKT140 was subcutaneously injected as a single dose (12 mg/kg equivalent). Four hours after the injection of BKT140, the mice were bled, and the peripheral blood cells were collected (900  $\mu\text{L}$ ) and immediately intravenously transferred into C57BL/6 recipient mice that had been pretreated with a lethal dose of irradiation (900 cGy) 24 hours before (A). Four months after the first transplantation, the bone marrow cells recovered from the first recipient mice repopulated by donor cells were injected ( $2.5 \times 10^6$  per mice) intravenously into lethally irradiated secondary mice (B). One month after the first transplantation, the mice were bled, and the number of WBCs, RBCs, and platelets was counted (C). Two months after transplantation of the donor BKT140, G-CSF, BKT140+G-CSF-mobilized cells, FITC-conjugated anti-mouse CD45.2, anti-mouse C45.1 (BD Biosciences), and anti-CD3 antibodies were used to evaluate the mouse donor cell chimerism of the transplanted C57Bl mice (D).

**Table 1.** Summary of the BKT140 pharmacokinetics parameters in humans

0.9 mg/kg, N = 4 Median (SD)	0.3 mg/kg, N = 3 <sup>a</sup> Median (SD)	Pharmacokinetic parameter
866.03 (443.02)	192.32 (213.18)	C <sub>max</sub> (ng/mL)
0.5 (0.00)	0.5 (0.00)	T <sub>max</sub> (h)
726.76 (538.26)	95.22 (196.71)	AUC <sub>last</sub> <sup>b</sup> (ng·h/mL)
735.23 (536.78)	97.57 (197.88)	AUC <sub>inf</sub> (ng·h/mL)
0.72 (0.19)	0.29 (0.20)	T <sub>1/2</sub> (h)
1.23 (1.81)	3.07 (2.46)	Clearance/F (L/h)

<sup>a</sup>One subject (no. 204) was excluded from the analysis because BKT140 was detected in the plasma of this subject only at 30 minutes postdose.

<sup>b</sup>Range is 0 to 24 hours.

sustained engraftment (Fig. 2A). Time to RBCs and platelets recovery were both shorter in mice transplanted with stem cells mobilized with G-CSF (Fig. 2B) versus BKT140-only (Fig. 2B). However, these engraftment outcomes were significantly inferior to those obtained in mice receiving BKT140 together with G-CSF (Fig. 2B) derived stem cells, in which PLT, WBC, and RBC engraftment occurred earlier.

Under normal conditions, stem and progenitor cells are released to the circulation at a low pace and are controlled by the circadian loop through the CXCR4 axis (20). In the bone marrow, there is a small pool of "ready to go cells," which are localized close to the endothelium. Following preconditioning with G-CSF, there is an increase in the "ready to go" cells, which are released to the circulation. It is therefore not surprising that a single administration of BKT140 was inferior to multiple injections of G-CSF. Interestingly, sequential administration of BKT140 injections resulted in an increased number of hematopoietic progenitors and megakaryopoiesis in bone marrow, accompanied with an increased stem cell mobilization and platelet production (21). It is therefore possible, that similar to G-CSF, BKT140 should also be administered as a single agent over few consecutive days.

Four months after the first transplantation, hematopoietic cells were harvested from the bone marrow of mice and injected into lethally irradiated secondary recipient mice. This secondary transplantation resulted in a successful engraftment and the long-term survival of all mice (Fig. 2B). To determine whether the graft obtained following transplantation was of donor origin, a chimerism experiment was performed in which the donor cells were B6.BoyJ (CD45.1) and the recipients were C57Bl/6 (CD45.2).

The combination of BKT140 and G-CSF provided a significantly superior engraftment compared with that obtained using G-CSF or BKT140 alone. Thus, a single administration of a high dose of BKT140 in combination with G-CSF is sufficient to mobilize stem cells with the capacity for rapid and long-term repopulation in mice.

### Phase I clinical study human patient's characteristics

Eighteen patients with multiple myeloma were enrolled, of which 8 (44.4%) were females and 10 (55.6%) were males. The median age was 57.5 years (range 36–62 years), with a median (SD) body mass index of 26.0 (4.8) kg/m<sup>2</sup> (range 21.2–37.5; Supplementary Table S1).

### Pharmacokinetics and product metabolism in humans

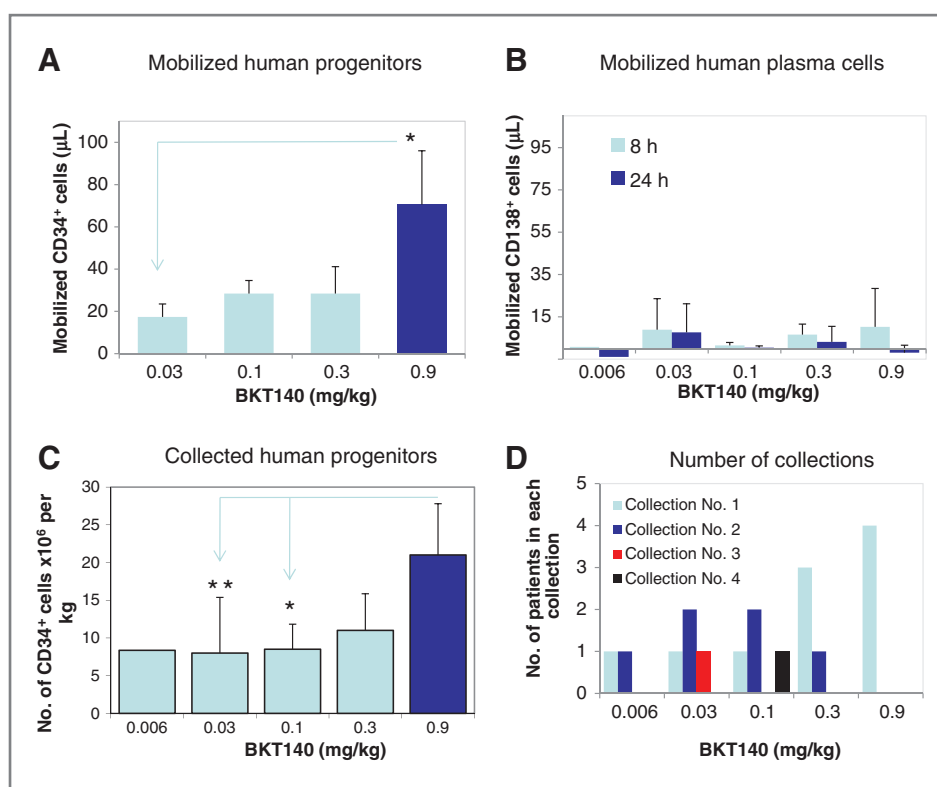
BKT140 was below the quantification limit (<5 ng/mL) for all patients treated with the 0.03 mg/kg dose. In subjects receiving 0.1 mg/kg, BKT140 was detected in the plasma at 30 minutes postadministration only. In patients receiving the 0.3 and 0.9 mg/kg doses, the maximal plasma concentration was observed at 30 minutes after BKT140 administration and then quickly declined. Notably, a 3-fold increase in the BKT140 dose, from 0.3 to 0.9 mg/kg, led to 2.6-, 4.2-, and 2.4-fold increases in C<sub>max</sub>, AUC, and T<sub>1/2</sub>, respectively (Table 1).

### Hematopoietic progenitor cell collection and engraftment

A single administration of BKT140 induced a dose-dependent mobilization of CD34<sup>+</sup> cells into the blood (Fig. 3A, \*P < 0.05; Supplementary Table S4). However, BKT140 did not increase the number of mobilized multiple myeloma cells (as defined by CD138), which remained stably low pre- and post-BKT administration (Fig. 3B). Furthermore, BKT140 induced a dose-dependent increase in the number of CD34<sup>+</sup> cells collected in the first aphaeresis [Fig. 3C, \*P < 0.05 (0.03 mg/kg vs. 0.9 mg/kg), \*\*P < 0.01 (0.1 mg/kg vs. 0.9 mg/kg)].

Moreover, the 2 higher BKT140 doses provided a sufficient number of CD34<sup>+</sup> cells in 1 aphaeresis for 7 of 8 patients (4/4 of those receiving 0.9 mg/kg and 3/4 of those receiving 0.3 mg/kg). In contrast, 3 of 10 patients treated with the same mobilization protocol but with lower BKT140 doses (0.006, 0.03, and 0.1 mg/kg) achieved sufficient CD34<sup>+</sup> cell collection in a single aphaeresis, whereas the remaining subjects in these cohorts required at least 2 aphaeresis procedures (ranging from 2 to 4) to ensure the collection of a sufficient number of cells (Fig. 3D). Fourteen

**Figure 3.** Dose-dependent increase in the absolute number of human CD34<sup>+</sup> cells within 8 hours following the administration of BKT140 ( $P < 0.08$ ) (A). Time- and dose-dependent increase in the absolute number of CD138<sup>+</sup> cells following the administration of BKT140 (B). Dose-dependent increase in the absolute number of CD34<sup>+</sup> cells collected following the administration of BKT140 (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ) (C). Dose-dependent number of CD34<sup>+</sup> cells number of collections following BKT140 administration (D).



out of 18 patients were collected within the first 24 hours following BKT140 administration, 3 were collected within 48, and 1 was collected 72 hours after BKT140 administration (Supplementary Table S3).

The BKT140-mobilized autologous grafts were infused into 17 patients with myeloma following the administration of high-dose melphalan conditioning (200 mg/m<sup>2</sup>). The mean number of CD34<sup>+</sup> cells administered was  $5.3 \times 10^6$  CD34<sup>+</sup> cells/kg. All of the transplanted patients ( $n = 17$ ) engrafted with a median time to neutrophil recovery of 12 days (range 11–14 days). In contrast, platelet recovery was found to be dependent on the BKT140 dosage administered for stem cell collection; the median times for platelet recovery ( $>50,000/\text{mm}^3$ ) for BKT140 doses of 0.006 and 0.030 mg/kg were 20 and 17 days, respectively (range 14–26 days). However, the median for platelet recovery was 14 days (range 11–19 days) at the higher doses of 0.1, 0.3, and 0.9 mg/kg (Table 2).

### WBC, neutrophil, monocyte, and lymphocyte mobilization in human

The single administration of BKT140 induced a dose-dependent mobilization of WBCs, neutrophils, monocytes, and lymphocytes within 8 hours of administration (Fig. 4A).

Repeated lymphocyte measurements performed at 24 hours post-BKT140 administration demonstrated that the lymphocyte counts returned to baseline levels, that is, the pre-BKT140 administration levels, at the lower doses. However, this was not observed in patients receiving the highest

BKT140 dose (Fig. 4B, 0.1 mg/kg vs. 0.3 mg/kg or 0.9 mg/kg, \* $P < 0.05$ ); in these patients, the 24-hour lymphocyte counts were still significantly higher than those measured pre-BKT140 administration (Fig. 4B, 0.3 mg/kg; and Fig. 4D, 0.9 mg/kg).

### Toxicity in human

Application of BKT140 was associated with a favorable safety profile, with no apparent trend toward risk with a specific dose.

A total of 96 AEs were reported in this study, with nearly all subjects (17/18; 94.4%) experiencing at least one AE. The body system class with the highest frequency was "general disorders and administration site conditions" (concentrated in "weakness"), followed by gastrointestinal disorders (concentrated in "nausea"), all anticipated side effects of chemotherapy. Very few AEs were considered to have been "severe" in intensity (8/96; 8.3%). Treatment emergent AEs (i.e., AEs reported to have occurred following administration of the investigational drug) were far fewer (34.4%, 33/96 AEs). About 40% of treatment emergent adverse events (TEAEs) (13/33 reported in 5 subjects) were considered definitely, possibly, or probably drug related. All related TEAEs (13/13) were in the higher dose groups (0.3 and 0.9 mg/kg), 61.5% (8/13) were under MedDRA system class "General Disorder and Administration Site Condition." More than three quarters of the related TEAEs were considered "mild" in intensity (10/13) 23.1% "moderate" (3/13). The investigator did not consider any of the "severe" events as related to the study drug, whereas the sponsor's Medical

**Table 2.** Time- and dose-dependent engraftment following transplantation of human patients

0.9 mg/kg	0.3 mg/kg	0.1 mg/kg	0.03 mg/kg	0.006 mg/kg	Criterion threshold	Dosing group
4	3	4	4	2		Number of patients in group
5.0	5.36	6.15	4.7	5.0		Median No. of infused CD34 <sup>+</sup> cells
12 (11–13)	12 (11–14)	11.5 (11–13)	12.5 (11–13)	12	>500/mm <sup>3</sup>	Median neutrophil recovery, days (range)
11 (0–13)	12 (12)	13 (12–13)	12.5 (12–14)	14.5 (10, 19)	>20,000/mm <sup>3</sup>	Median platelet recovery, days (range)
14 (12–16)	14 (12–19)	14 (11–15)	17 (14–20)	20 (14, 26)	>50,000/mm <sup>3</sup>	

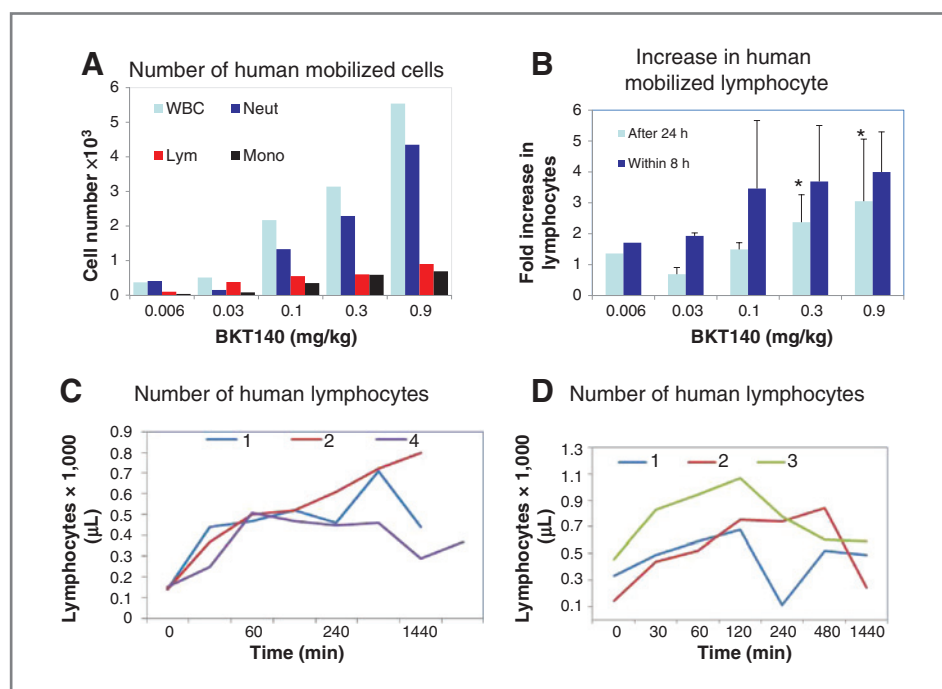
Safety Officer considered one "severe" AE (1/12; 8.3%) as related to the study drug. The median duration of AEs throughout this study was 3 days (range 1–31 days).

There were a total of 6 serious adverse events (SAEs) reported throughout the study, affecting 4 of 18 subjects (22.2%), 1 subject in the 0.006 mg/kg dose group, 2 subjects in the 0.1 mg/kg dose group, and 1 subject in the 0.3 mg/kg group.

Two concurrent SAEs in a 0.3 mg/kg subject (chest pain and dyspnea) were considered by the investigator to be related to investigational study treatment because of proximity of timing to drug administration; however, the sponsor's Medical Safety Officer acknowledged that the events were similar to those anticipated with administration of G-CSF, and therefore these SAEs were considered "unrelated" to study drug. Another SAE, hypokalemia in subject no. 008 (0.1 mg/kg group) was determined to be unrelated by the investigator and as possibly related to the study product by the sponsor's Medical Safety Officer (based on review of the CRF and laboratory data).

The majority of laboratory abnormalities were noted at screening and 24 hours postdosing, improving, or resolving by day 7 postdosing. Notable exceptions in hematology were neutrophil and lymphocyte %WBC values, particularly progressive increases in neutrophils postadministration of study drug, a function of the intended application of the drug. Exceptions in blood chemistry included potassium, where there was a noticeable decrease postadministration of the study treatment and glucose and LDH, for which there were notable elevations postadministration of study treatment in subjects, none associated with sequelae; other clinically significant chemistry parameters considered AEs were determined to not have been related to study treatment, with values returning to normal by study end. There were no notable differences between study dose groups.

Most vital sign parameters (systolic and diastolic blood pressure, heart rate, respiratory rate, oral body temperature, and oxygen saturation) were normal in most subjects at most time points evaluated in this study. Most clinically



**Figure 4.** Increase in the absolute number of human WBCs, neutrophils, lymphocytes, and monocytes within 8 hours following the administration of BKT140 (0.006–0.9 mg/kg) (A). Fold increase in the number of lymphocytes at 4 and 24 hours following the administration of BKT140 (B). Time and BKT140 dose (C = 0.03 mg/kg and D = 0.9 mg/kg)-dependent lymphocyte mobilization following the administration of BKT140.

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significant abnormalities were observed before administration of study drug and resolved by study end; all AEs related to vital sign abnormalities were determined to have not been related to study treatment, and resolved by study end. Electrocardiography results were normal or consistent with predosing profile in most subjects at most time intervals, with no clinically significant abnormalities postadministration of investigational drug or notable dose-dependent difference, all new findings resolved by study end.

Physical examination was normal in most subjects, with all abnormalities observed already predosing or considered to have been unrelated to investigational drug. Weights remained largely unchanged from day 0 to predose day 10. The significance of changes in mean values in the 0.006, 0.03, and 0.1 mg/kg were uninterpretable because of no or low sample size at day 0. There was no weight loss AEs reported in the study.

Karnofsky status remained unchanged or improved among subjects undergoing evaluations, the majority of findings being normal. There were no notable differences between study dose groups.

## Discussion

ASCT following an initial response remains the treatment of choice in transplant-eligible myeloma subjects because it provides long-term responses in a substantial number of patients (1). A sufficient number of CD34<sup>+</sup> cells can be obtained from most patients using a G-CSF/HD CTX mobilization protocol. However, approximately 10% of subjects fail to achieve this goal (22) and require the employment of remobilization strategies. This percentage is even higher in multiple myeloma subjects previously treated with lenalidomide in whom mobilization failure approaches 30% (8). Moreover, the collection success rate in patients mobilized with G-CSF only in an attempt to reduce chemo-mobilization toxicity is even lower, emphasizing the need for new mobilization protocols (17).

The employment of G-CSF in conjunction with plerixafor instead of chemo-mobilization is gradually expanding, providing an efficient and safe method to mobilize stem cells. Nevertheless, plerixafor still fails to provide optimal amounts of stem cells in a substantial percentage of both NHL and patients with multiple myeloma. Indeed, plerixafor failed to provide sufficient stem cells for transplantation in approximately 15% of relapsing NHL subjects, and substandard amounts, less than  $5 \times 10^6$  CD34<sup>+</sup> cells/kg, were harvested in 40% of these patients (23). The plerixafor success rate in patients with myeloma was also substandard, considering that only  $\geq 6 \times 10^6$  CD34<sup>+</sup> cells/kg was ultimately collected in only three quarters of patients (6).

Although still debatable, a "prosperous" stem cell collection has been suggested to be superior to a poor one, providing facilitated platelet and neutrophil engraftment, reducing antibiotic requirements, and decreasing blood product transfusions. More importantly, those patients with lymphoma defined as supermobilizers (achieving an stem cell collection of  $\geq 8 \times 10^6$  CD34<sup>+</sup> HSC/kg) were found to

have a better survival rate compared with their "poorer" counterparts (5), suggesting a clinical advantage in attaining a high number of stem cells.

Because most patients with myeloma experience prolonged survival currently, associated with multiple relapses, a second transplant at an advanced disease stage has become a common approach. Accordingly, a robust stem cell harvest, enabling at least 2 transplants, is desirable (24).

BKT140 demonstrates a high affinity for CXCR4 and had a greater effect on the retention-mobilization balance of bone marrow stem cells when compared with plerixafor in an *in vivo* mice study (25). We postulate that the difference is likely because of the greater affinity of BKT140 for CXCR4, which is highest when administered in conjunction with G-CSF. Notably, this robust mobilization of stem cells resulted in a significant improvement in the time for neutrophil and platelet engraftment in mice. Moreover, a chimeric analysis of allograft mice showed a marked improvement in graft quality in the mice transplanted with the combined BKT140 and G-CSF-induced grafts. This improved engraftment in mice may be ascribed to the higher number of stem cells, to a better cell quality, or possibly to the increased number of lymphocytes and NK cells provided by the BKT140-derived harvests.

Based on these encouraging results, a phase I study in newly diagnosed patients with multiple myeloma scheduled for an autograft was performed, confirming BKT140 to be a highly effective stem cell mobilizer in humans.

The safety data were also remarkably encouraging, whereby none of the patients presented with grade 3 and 4 toxicity. Furthermore, the treatment with BKT140 resulted in the collection of a high number of stem cells, the efficiency of which seemed to be dose dependent. All patients treated with the high BKT140 dosages achieved a remarkably high number of CD34<sup>+</sup> cells that were obtained through a single aphaeresis procedure.

The employment of BKT140 with chemo-mobilization was designed to ensure stem cell collection in all subjects, as this was the first phase I study in humans. The dose dependency effect of BKT140, comparing the mobilization outcomes of subjects receiving low doses (0.006, 0.03, and 0.01 mg/kg) with those obtained in subjects receiving high BKT140 doses (0.3 and 0.9 mg/kg), suggests that the contribution of BKT140 was critical in reducing the number of apheresis procedures, increasing the number of collected CD34<sup>+</sup> cells and reducing the time for neutrophil and platelet recovery.

Furthermore, BKT140 administration resulted in a marked mobilization of lymphocytes, a phenomenon that may prove valuable in patients with multiple myeloma in whom a facilitated immune recovery may result in an improved long-term outcome.

In conclusion, BKT140, a high-affinity CXCR4 antagonist, presented an excellent safety profile with a good efficacy in patients with multiple myeloma, suggesting it to be a highly efficient mobilizer of CD34<sup>+</sup> and lymphocyte cells. CXCR4 antagonists with a more potent

stem and immune cell mobilization capacity may further improve stem cell mobilization, graft quality, and engraftment, thus optimizing stem cell mobilization and transplant outcome.

#### Disclosure of Potential Conflicts of Interest

M. Abraham is employed (other than primary affiliation; e.g., consulting) as a scientist in Biokine Ltd. Y. Ramati is employed (other than primary affiliation; e.g., consulting) as a head of RA in Biokine Therapeutics. A. Nagler has commercial research grant in Biokine and also is a consultant/advisory board member of Biokine. H. Wald, L. Tiomkin, Y. Riback, O. Eisenberg, and S. Aviel are employees of Biokine Therapeutics. A. Peled serves as a consultant for Biokine Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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