The high-affinity CXCR4 antagonist BKT140 is safe and induces a robust mobilization of human CD34+ cells in patients with multiple myeloma

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TRANSLATIONAL RELEVANCE

The use of peripheral blood as a source of hematopoietic stem cells (HSCs or CD34+ cells) for cancer patients 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+ cells for transplantation, preferably in the first mobilization attempt and ideally with a minimum of apheresis sessions (collection events for HSCs). However, while CD34+ 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 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 to 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 G-CSF, resulting in rapid and robust HSC mobilization therapy that is differentiated from current standard of care.
Abstract

Purpose: CXCR4 plays an important role in the retention of stem cells (SCs) within the bone marrow (BM). BKT140 is a 14-residue bio stable synthetic peptide which binds CXCR4 with a greater affinity compared with plerixafor (4nM vs 84nM). Studies in mice demonstrated the efficient and superior mobilization and transplantation of SCs collected with GCSF-BKT140, compared with those obtained when using SCs 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 (MM) who were preparing for their first autologous stem cell transplantation (ASCT) were included. Patients received a standard MM mobilization regimen, consisting of 3-4 gr/m2 cyclophosphamide (Day 0), followed by 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 (SC) on day 10 in the early morning, followed by G-CSF 12 hr later.

Results: BKT140 was well tolerated at all concentrations, and none of the patients developed grade III-IV toxicity. A single administration of BKT140 at the highest dose, 0.9 mg/kg, resulted in a robust mobilization and collection of CD34+ cells (20.6 ± 6.9 x 10^6/kg), which were obtained through a single apheresis. All transplanted patients received ~5.3 x 10^6 CD34+ 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 SC mobilizer that enabled the collection of a high number of CD34+ cells in 1-2 aphaeresis procedures, resulting in successful engraftment.
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 (MM), non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (HL) (1, 2). Although 2 x 10^6 CD34+ cells/kg is considered to be the lowest stem cell (SC) dose required to ensure hematopoietic engraftment (3, 4), a higher CD34+ dose (> 5 x 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 SC mobilization (3). However, such mobilization fails to provide a sufficient number of CD34+ stem cells in approximately 10-20% of MM patients and 11-55% of NHL patients, depending on the type and number of pre-collection therapies applied (6).

The employment of plerixafor (Mozobil, AMD3100; Genzyme Corp., Cambridge, MA), a small molecule that reversibly inhibits CXCL12 binding to CXCR4, for stem cell mobilization appears to improve mobilization rates and rescue a substantial number of "hard mobilizer" subjects (7). However, even with the use of plerixafor, the mobilization of a sufficient number of SCs remains a difficult objective in a sizeable proportion of patients (41% NHL and 13% MM patients), 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 IC₅₀ of ~1 nM (11-13) compared to the values obtained with AMD3100 (IC₅₀ -651 ± 37 nM) (14). Moreover,
BKT140 hinders the cell migration stimulated by CXCL12 within IC$_{50}$ values of 0.5-2.5 nM (12, 15) compared with the IC$_{50}$ value of 51 ± 17 nM for Mozobil (14), suggesting a high mobilization capacity.

Indeed, in vivo animal studies have shown that BK140 rapidly mobilizes a large number of SCs (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).

The present 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 SCs, with fewer aphaeresis procedures.

**Materials and Methods**

**Mouse Experiments**

**Reagents and mice**

Plerixafor was purchased from Sigma, and G-CSF (Neupogen, Filgrastim) was purchased from Amgen (Thousand Oaks, CA). BKT140 was produced and provided by Biokine Therapeutics, Ltd, Rehovot, Israel. Cyclophosphamide (CPM) was purchased from Baxter Oncology GmbH, Frankfurt, Germany. Female C57BL/6 mice (7-8 weeks old) were purchased from Harlan, Rehovot, Israel. Congenic C57BL/6 (CD45.2) and B6.SJL-Ptcrca Pep3b/BoyJ (B6.BoyJ:CD45.1) mice were purchased from Jackson Laboratory, Bar Harbor, Maine. 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 μM/200 μl), plerixafor (AMD3100) (4.4 mg/kg, equivalent to 0.11 μM/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, Cambridge, MA). Jurkat cells were re suspended in RPMI medium containing 1% FCS. Cells (2 x 10^5 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, London, UK) was added to the lower chambers. Various concentrations of BKT140 (0.6, 1.2, 2.5, 3.6, and 5 nM) 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, San Jose, CA, USA). The data were analyzed using software from CellQuest (version 3.3; BD Biosciences). The IC_{50} 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 BM samples were collected at 4 hours (hr) 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 multi-parameter blood cell counter - Sysmex.
KX-21 (Sysmex, USA). 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 (IMDM) containing 1% methylcellulose, 15% FBS, 1% bovine serum albumin (BSA), 3U/ml rh EPO, 104 M 2-mercaptoethanol, 2 mM 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% CO₂. The colonies that developed 5 days later were visually scored using a light microscope (employing 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 pre-collection with BKT140, G-CSF, or both. Four months after the first transplantation, the BM cells recovered from the recipient mice were transplanted (2.5 x 10⁶ 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, non-randomized, open-label, dose escalation, multi-center study was performed in which escalated doses of BKT140 were added to a standard MM mobilization protocol, consisting of high-dose cyclophosphamide/G-CSF (17).

The study was approved by the Human Subjects Committee-Institutional Review Boards of the two 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 5 and prior therapies of each one of the participants, are shown in Supplementary Table 2. Written informed consent was obtained before enrollment in the study.

Eligible MM subjects (n=18) received a standard MM mobilization regimen, consisting of 3-4 gr/m² cyclophosphamide (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 SC each evening, between 8 and 10 PM).

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

The BKT140 dose was increased, starting at 0.006 mg/kg with two patients, followed by cohorts of four patients: 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, and 0.9 mg/kg. Pharmacokinetic (PK) and pharmacodynamic (PD) assessments were conducted prior to BKT140 administration and at 10 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, and 24 hr post-administration.
Safety monitoring was performed at the same time points and continued for up to 7 days after the completion of SC 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 MM 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 (PB) stem cell collection followed by autologous stem cell transplantation (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+ cells and CD138+ cells; CD34+ cells were also measured in leukapheresis products. CD34+ cells were enumerated by FACS, according to the International Society for Hematotherapy and Graft Engineering (ISHAGE) protocol, in a single-platform, two-color assay performed using an Epics XL flow cytometer (Beckman-Coulter, Hialeah, FL).

**Leukapheresis**
Peripheral blood mononuclear cells were collected by leukapheresis using a cell separator (Spectra, Gambro, Lakewood, CO). The mononuclear cell leukapheresis program was employed 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 (5000 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 x 10^6 CD34+ cells/kg.

**Transplantation**

The patients received 200 mg/m^2 melphalan conditioning, followed by the infusion of the BKT140-collected CD34+ cells. ASCT was permitted up to 5 weeks following the last aphaeresis. The lowest number of CD34+ cells allowed for transplantation was 2 x 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 post-transplantation and continued daily until neutrophil engraftment. Neutrophil engraftment was defined as neutrophil count ≥0.5 x 10^9/L , for 3 consecutive days (with no GCSF support). Platelet (PLT) engraftment was defined as platelet count ≥20 x 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. AEs were coded according to the MedDRA (version 12.1) system organ class and preferred term. The individual study drug PK parameters and the mean, SD, values
were calculated for each dose group for all subjects. PD analyses included the values, changes from pre-dose and fold increases of WBCs (neutrophils, lymphocytes, monocytes, and platelets), CD34+ and CD138+ counts, 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’s t test. A paired two-tailed Student’s t test was used to evaluate the significant differences within the groups.
Results

In vitro characterization of BKT140 binding to CXCR4

The IC$_{50}$ value for the inhibition by BKT140 of human Jurkat leukemic T cell migration toward 100 ng/ml CXCL12 was found to be 4.0 nM (Error! Reference source not found.A). 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 min at 4°C and then washed and stained for 15 min with an anti-12G5 antibody (Error! Reference source not found.). 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 (Figure 1B). However, when the cells were washed and then stained at 120 min post-washing, BKT140 (2 μM) but not plerixafor abrogated the binding of 12G5 to CXCR4 at both time points (Figure 1B). Similar results were obtained for BL-2 lymphoma cells, primary leukemic cells, prostate PC3 cells, human CD4$^+$ cells, and human CD34$^+$ cells (data not shown). In agreement with the binding results, the Jurkat cells washed after pre-incubation with BKT140, but not with plerixafor, lost their ability to migrate in response to CXCL12 (Figure 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 to 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 HSC progenitors in the peripheral blood of treated mice (Figure 1d). This effect was even more pronounced when high-dose BKT140 was administered together with G-CSF (Figure 1d) and was higher than that obtained with high-dose plerixafor plus G-CSF (Figure 1d).

Mouse stem cells (SCs) were mobilized either with a single injection of 12mg/kg BKT140 (Figure 2A, B), 5 injections of G-CSF (5 μg/mice daily; Figure 2A, B), or by employing a combination of 5 days of G-CSF followed by a single BKT140 injection (Figure 2A, B). Stem cells were then harvested from 2 mice (900 μl of blood) and transplanted into irradiated recipient mice, resulting in rapid and sustained engraftment (Figure 2A). Time to RBCs and platelets recovery were both shorter in mice transplanted with SCs mobilized with G-CSF (Figure 2B) vs BKT140-only (Figure 2B). However, these engraftment outcomes were significantly inferior to those obtained in mice receiving BKT140 together with G-CSF (Figure 2B) derived SCs, in whom 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 BM there is a small pool of 'ready to go cells' which are localized close to the endothelium. Following pre-conditioning 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 (HPCs) and megakaryopoiesis in BM, accompanied with an increased SC 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 BM 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 (Figure 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 MM patients 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) BMI of 26.0 (4.8) kg/m² (range 21.2-37.5) (Supplementary Table 1).

**Pharmacokinetics and product metabolism in humans**

BKT140 was below the quantification limit (BLQ, < 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 post-administration only. In patients receiving the 0.3 mg/kg 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 mg/kg to 0.9 mg/kg, led to 2.6-, 4.2-, and 2.4-fold increases in Cmax, AUC, and T1/2, respectively (Table 1).

Hematopoietic progenitor cell collection and engraftment

A single administration of BKT140 induced a dose-dependent mobilization of CD34+ cells into the blood (Figure 3A, *p < 0.05, Supplementary Table 4). However, BKT140 did not increase the number of mobilized MM cells (as defined by CD138), which remained stably low pre- and post-BKT administration (Figure 3B). Furthermore, BKT140 induced a dose-dependent increase in the number of CD34+ cells collected in the first aphaeresis (Figure 3C, *p < 0.05 (0.03 mg/kg vs 0.9 mg/kg), (0.1 mg/kg vs 0.9 mg/kg) **p < 0.01). Moreover, the 2 higher BKT140 doses provided a sufficient number of CD34+ cells in 1 aphaeresis for 7 out 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+ cell collection in a single aphaeresis, whereas the remaining subjects in these cohorts required at least 2 aphaeresis procedures (ranging from 2-4) to ensure the collection of a sufficient number of cells (Figure 3D). Fourteen out of 18 patients were collected within the first 24hr following BKT140 administration, 3 were collected within 48 and 1 was collected 72hr after BKT140 administration (Supplementary Table 3).

The BKT140-mobilized autologous grafts were infused into 17 myeloma patients following the administration of high-dose melphalan conditioning (200 mg/m²). The mean number of CD34+ cells administered was 5.3 x 10⁶ CD34+ 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 SC collection; the median times for platelet recovery (> 50,000/mm³) 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 hr of administration (Figure 4A).

Repeated lymphocyte measurements performed at 24 hours post-BKT140 administration demonstrated that the lymphocyte counts returned to baseline levels, i.e., the pre-BKT140 administration levels, at the lower doses. However, this was not observed in patients receiving the highest BKT140 dose (Figure 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 (Figure 4B, 0.3 mg/kg, and Figure 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 the present 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 TEAEs (13/331 reported in 5 subjects) were considered definitely, possibly or probably drug related. All related TEAEs (13/132) were in the higher dose groups (0.3 mg/kg and 0.9 mg/kg), 61.5% (8/133) were under MedDRA system class ‘General Disorder and Administration Site Condition’. More than three-quarters of the related TEAEs were considered ‘mild’ in intensity (10/134) 23.1% ‘moderate’ (3/135). The investigator did not consider any of the 'severe' events as related to the study drug while the sponsor’s Medical Safety Officer considered one ‘severe’ AE (1/12; 8.3%) as related to the study drug. The median duration of AEs throughout the present study was 3 days (range 1-31 days).

There were a total of six SAEs reported throughout the study, affecting 4/18 subjects (22.2%), one subject in the 0.006mg/kg dose group, two subjects in the 0.1 mg/kg dose group, and one 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 due to 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 post-dosing, improving or resolving by Day 7 post-dosing. Notable exceptions in hematology were neutrophil and lymphocyte %WBC values, particularly progressive increases in neutrophils post-administration of study drug, a function of the intended application of the drug. Exceptions in blood chemistry included potassium, where there was a noticeable decrease post-administration of the study treatment and glucose and LDH, for which there were notable elevations post-administration 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 the present study. Most clinically significant abnormalities were observed prior to 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. ECG results were normal or consistent with pre-dosing profile in most subjects at most time intervals, with no clinically significant abnormalities post-administration 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 pre-dosing or considered to have been unrelated to investigational drug. Weights remained largely unchanged from Day 0 to pre-dose Day 10. The significance of changes in mean values in the 0.006, 0.03, and 0.1 mg/kg were uninterpretable due to 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

Autologous stem cell transplantation 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+ 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 re-mobilization strategies. This percentage is even higher in MM 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 SCs. Nevertheless, plerixafor still fails to provide optimal amounts of SCs in a substantial percentage of both NHL and MM patients. Indeed, plerixafor failed to provide sufficient SCs for transplantation in approximately 15% of relapsing NHL subjects, and substandard amounts, under $5 \times 10^6$ CD34+ cells/kg, were harvested in 40% of these patients (23). The plerixafor success rate in myeloma patients was also substandard, considering that only $\geq 6 \times 10^6$ CD34+ cells/kg was ultimately collected in only three quarters of patients (6).

Although still debatable, a “prosperous” SC 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 lymphoma patients defined as super-mobilizers (achieving an SC
collection of \( \geq 8 \times 10^6 \) CD34\(^+\) 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 SCs.

Because most myeloma patients 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 BM SCs when compared to plerixafor in an in vivo mice study (25). We postulate that the difference is likely due to the greater affinity of BKT140 for CXCR4, which is highest when administered in conjunction with G-CSF. Notably, this robust mobilization of SCs 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 MM patients scheduled for an autograft was performed, confirming BKT140 to be a highly effective SC mobilizer in humans.

The safety data were also remarkably encouraging, whereby none of the patients presented with grade III-IV toxicity. Furthermore, the treatment with BKT140 resulted in the collection of a high number of SCs, the efficiency of which appeared to be dose
dependent. All patients treated with the high BKT140 dosages achieved a remarkably high number of CD34+ 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+ 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 MM patients 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 MM patients, suggesting it to be a highly efficient mobilizer of CD34+ 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.

Acknowledgments

We thank Mery Clausen (Gene Therapy Institute, Hadassah Hospital) for technical assistance.
Table 1: Summary of the BKT140 pharmacokinetics parameters in humans.

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>0.9 mg/kg, N=4 Median (SD)</th>
<th>0.3 mg/kg, N=3* Median (SD)</th>
<th>PK parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>866.03 (443.02)</td>
<td>192.32 (213.18)</td>
<td>*</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>0.5 (0.00)</td>
<td>0.5 (0.00)</td>
<td>**</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;last&lt;/sub&gt;† (ng.h/mL)</td>
<td>726.76 (538.26)</td>
<td>95.22 (196.71)</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;inf&lt;/sub&gt; (ng.h/mL)</td>
<td>735.23 (536.78)</td>
<td>97.57 (197.88)</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>0.72 (0.19)</td>
<td>0.29 (0.20)</td>
<td></td>
</tr>
<tr>
<td>Clearance/F (L/h)</td>
<td>1.23 (1.81)</td>
<td>3.07 (2.46)</td>
<td></td>
</tr>
</tbody>
</table>

*One subject (no. 204) was excluded from the analysis because BKT140 was detected in the plasma of this subject only at 30 minutes post-dose.

†Range is 0-24 hr
Table 2. Time and dose-dependent engraftment following transplantation of human patients.

<table>
<thead>
<tr>
<th>Dosing Group</th>
<th>Criterion threshold</th>
<th>Number of patients in group</th>
<th>Median No. of infused CD34+ cells</th>
<th>Median Neutrophil recovery, days (range)</th>
<th>Median Platelet recovery, days (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 mg/kg</td>
<td></td>
<td>4</td>
<td>5.0 (0-13)</td>
<td>11 (11-13)</td>
<td>14 (12-16)</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td></td>
<td>3</td>
<td>5.36 (0-13)</td>
<td>12 (11-14)</td>
<td>17 (12-19)</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td></td>
<td>4</td>
<td>6.15 (0-13)</td>
<td>12.5 (11-13)</td>
<td>14 (12-15)</td>
</tr>
<tr>
<td>0.03 mg/kg</td>
<td></td>
<td>4</td>
<td>4.7 (0-13)</td>
<td>12.5 (11-13)</td>
<td>17 (14-20)</td>
</tr>
<tr>
<td>0.006 mg/kg</td>
<td></td>
<td>2</td>
<td>5.0 (0-13)</td>
<td>12 (11-13)</td>
<td>20 (14, 26)</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1

The half-maximal concentration (IC$_{50}$) 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 to isotype antibody control staining (Bold purple). The expression of CXCR4 by cells that were pre-incubated with BKT140 (2 μM) is represented by a red line. The expression of CXCR4 by cells that were pre-incubated with plerixafor (2 μM) is represented by blue line (B). The ability of BKT140 (T140) and plerixafor (10 and 100 nM), added to the lower chamber of the transmigration assay, and that of BKT140 (1 μM) and plerixafor (1 μM), pre-incubated for 30 min 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 4 hr (D).

Figure 2

C57BL/6 mice, serving as donors, were injected with BKT140, G-CSF, or a combination of the two. G-CSF was subcutaneously (sc) injected daily for 3 constitutive days at 5 μg/ mouse/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 μl) and immediately intravenously (i.v.) 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 BM cells recovered...
from the first recipient mice repopulated by donor cells were injected (2.5 x 10^6 per mice) i.v. 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).

**Figure 3**

Dose-dependent increase in the absolute number of human CD34+ cells within 8 hr following the administration of BKT140 (p < 0.08) (A). Time- and dose-dependent increase in the absolute number of CD138+ cells following the administration of BKT140 (B). Dose-dependent increase in the absolute number of CD34+ cells collected following the administration of BKT140 (*p < 0.05, **p < 0.01) (C). Dose-dependent number of CD34+ cells number of collections following BKT140 administration (D).

**Figure 4**

Increase in the absolute number of human WBCs, neutrophils, lymphocytes, and monocytes within 8 hr following the administration of BKT140 (0.006-0.9 mg/kg) (A). Fold increase in the number of lymphocytes at 4 hr and 24 h 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.
References


Figure 1

A-Half-maximal concentration (IC$_{50}$) of BKT140 (T140) required to inhibit cell migration

B- Displacement assay

C-Inhibition of cell migration with BKT140 (T140) and AMD3100 (AMD)

D-Mobilization of mouse progenitor cells
Figure 2
A-Primary Transplantation of Irradiated mice  B- Secondary Transplantation of Irradiated mice

C-Recovery of transplanted mice

D- Competitive reconstitution of transplanted mice
Figure 3

A-Mobilized human progenitors

B-Mobilized human plasma cells

C-Collected human progenitors

D-Number of collections
Figure 4

A – Number of human mobilized cells

- WBC
- Neut
- Lym
- Mono

B – Increase in human mobilized lymphocyte

- After 24hr
- Within 8hr

C – Number of human lymphocytes

D – Number of human lymphocytes


Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
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