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Novel phosphatidylinositol 3-kinase inhibitor NVP-BKM120 induces apoptosis in myeloma cells and shows synergistic anti-myeloma activity with dexamethasone

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Abstract

NVP-BKM120 is a novel phosphatidylinositol 3-kinase (PI3K) inhibitor and is currently being investigated in phase I clinical trials in solid tumors. This study aimed to evaluate the therapeutic efficacy of BKM120 in multiple myeloma (MM). BKM120 induces cell growth inhibition and apoptosis in both MM cell lines and freshly isolated primary MM cells. However, BKM120 only shows limited cytotoxicity toward normal lymphocytes. The presence of MM bone marrow stromal cells, insulin-like growth factor, or interleukin-6 does not affect BKM120-induced tumor cell apoptosis. More importantly, BKM120 treatment significantly inhibits tumor growth in vivo and prolongs the survival of myeloma-bearing mice. In addition, BKM120 shows synergistic cytotoxicity with dexamethasone in dexamethasone-sensitive MM cells. Low doses of BKM120

and dexamethasone, each of which alone has limited cytotoxicity, induce significant cell apoptosis in MM.1S and ARP-1. Mechanistic study shows that BKM120 exposure causes cell cycle arrest by upregulating p27 (Kip1) and downregulating cyclin D1 and induces caspase-dependent apoptosis by downregulating antiapoptotic XIAP and upregulating expression of cytotoxic small isoform of Bim, BimS. In summary, our findings demonstrate the in vitro and in vivo anti-MM activity of BKM120 and suggest that BKM120 alone or together with other MM chemotherapeutics, particularly dexamethasone, may be a promising treatment for MM.

Keywords

Multiple myeloma; PI3K; BKM120; Apoptosis; Chemotherapy

Introduction

Multiple myeloma (MM) is a malignant B cell tumor characterized by proliferation of plasma cells in the bone marrow [1]. Chemotherapy is the most conventional treatment for MM patients [2]. However, despite the improvement of chemotherapy and introduction of new drugs, MM is still an incurable disease. In the USA, MM accounts for nearly 10% of deaths caused by hematological malignancies [3]. Therefore, developing novel and more effective chemotherapy agents is a continuing effort in MM research.

Phosphatidylinositol 3-kinase (PI3K) plays a central role in cell metabolism [4]. PI3K is activated by growth factors, cytokines, and other stimulatory factors in association with their receptors. Activated PI3K in turn initiates signaling transduction to Akt-mTOR and leads to regulation of cell growth, proliferation, and apoptosis [5]. Dysregulation of the pathway is widely observed in different types of human cancers [6]. Particularly in MM, a number of myeloma growth factors, such as insulin-like growth factor-1 (IGF-1) and interleukin-6 (IL-6), activate PI3K-Akt pathway upon interaction with their receptors on MM cells and promote MM proliferation, survival, and drug resistance [7–9]. Therefore, PI3K-Akt inhibition is expected to exert broad anti-MM activity. Currently, several PI3K-Akt inhibitory compounds are under investigation in pre-clinical studies or phase I and II clinical trials [10–12].

NVP-BKM120 is a novel pan-PI3K inhibitor. The compound has been shown to induce significant cell growth inhibition and induction of apoptosis in a variety of tumor cell lines and is currently being investigated in phase I clinical trials in patients with solid tumors. In this study, we investigated the in vitro and in vivo anti-MM activity of BKM120. Our findings demonstrate a potential use for BKM120 in MM therapy, alone or in combination with other anti-MM chemotherapeutics, specifically dexamethasone, to improve MM treatment.

Materials and methods

Cell lines, primary myeloma cells, BMSCs, PBMCs, antibodies, and reagents

MM cell lines ARP-1, ARK, MM.1S, MM.1R, and U266 were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml

streptomycin at 37°C and 5% CO₂. Primary MM cells and MM bone marrow stromal cells (BMSCs) were isolated or generated from bone marrow aspirates of myeloma patients. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers. The study was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. Anti-caspase-3, caspase-9, caspase-7 PARP, Bim, XIAP, cyclin D1, pp70S6K(Thr389), and p27(Kip1) antibodies were purchased from Cell Signaling. Anti-Bcl-2, Bcl-XL, Akt, pAkt(Thr 308), pAkt(Ser 473), p70S6K, and β -actin antibodies were purchased from Santa Cruz. NVPBKM120 powder was provided by Novartis Oncology Inc. BKM120 was dissolved in DMSO at 10 mM as stock solution. In all experiments, equal amount of solvent, dimethylsulfoxide (DMSO), was added in medium as controls at the final concentration of 0.1%. Dexamethasone and propidium iodide (PI) were purchased from Sigma-Aldrich. Caspase-3 inhibitor Z-DEVD, recombinant human IGF and recombinant human IL-6 were purchased from R&D Systems. FITC-conjugated annexin V was purchased from Invitrogen.

Cell growth assay

The growth inhibitory effects of BKM120 on MM cells or normal PBMCs were assessed by MTS assay following the manufacturer's protocol (Promega).

Apoptosis assays

BKM120-induced cell apoptosis was detected by annexin V binding assay as previously described [13].

Cell cycle analysis

MM cell lines ARP-1, MM.1S, and MM.1R were cultured with or without 1 μ M BKM120 for 24 h. Cells were harvested and permeabilized in 70% ethanol at 4°C overnight, followed by incubation with 50 μ g/ml PI and 20 μ g/ml RNase-A for 15 min. DNA content was analyzed by flow cytometry and FlowJo software.

In vivo effects of BKM120 on established MM

Six- to eight-week-old female severe combined immunodeficiency (SCID) mice were housed and monitored in the MD Anderson Cancer Center animal research facility. All experimental procedures and protocols had been approved by the Institutional Animal Care and Use Committee at The University of Texas MD Anderson Cancer Center. SCID mice were subcutaneously inoculated in the right flank with 1 million ARP-1 or MM.1S cells suspended in 50 μ l phosphate-buffered saline (PBS). After palpable tumor developed (tumor diameter $\geq$ 5 mm), mice were treated with intraperitoneal injection of DMSO/PBS or BKM120 (5 μ M per kg per day) for 15 days. Tumor sizes were measured every 5 days, and blood samples were collected at the same period. Tumor burdens were evaluated by measuring tumor size and detecting circulating human kappa chain or lambda chain.

ELISA

Levels of human kappa chain in mouse serum were measured by a quantitative ELISA (Bethyl Laboratories Inc) following the vendor's protocol.

Statistical analysis

All data were shown as mean±standard deviation. The Student's *t* test was used to compare various experimental groups. A *P*<0.05 was considered significant. Isobologram analysis and interaction index (also known as combination index) were calculated as previously described [14, 15].

Results

BKM120 inhibits the growth of MM cell lines and induces cell apoptosis

To evaluate the effect of BKM120 on myeloma cells, we treated MM cell lines with different doses of BKM120 for 24 or 72 h. BKM120-induced MM cell apoptosis was measured by annexin V binding assay. As shown in Fig. 1a, BKM120 induced MM cell apoptosis in both dose- and time-dependent manners. BKM120 at concentrations 10 μM induced significant apoptosis in all tested MM cell lines at 24 h (*P*<0.05, compared with control). Therefore, we chose 10 μM BKM120 and 24-h treatment in the following experiments if not stated otherwise.

The effect of BKM120 on MM cell growth was tested by MTS assay. As shown in Fig. 1b, BKM120 treatment resulted in a dose-dependent growth inhibition in all tested MM cell lines. BKM120 IC₅₀ (concentration at 50% inhibition) varied among tested MM cells. At 24 h treatment, IC₅₀ for ARP-1, ARK, and MM.1R was between 1 and 10 μM, while IC₅₀ for MM.1S was <1 μM, and IC₅₀ for U266 was between 10 and 100 μM. In summary, our findings indicate that BKM120 treatment resulted in MM cell growth inhibition and apoptosis in dose- and time-dependent manners.

BKM120 induces primary MM cell apoptosis ex vivo

To evaluate BKM120 activity in primary MM cells, we extended our study to CD138⁺ primary MM cells freshly isolated from myeloma patients. According to our previous finding, primary MM cells undergo apoptosis ex vivo unless the cells are cocultured with BMSCs [13]. Therefore, CD138⁺ primary MM cells were cocultured at 1:1 ratio with CD138⁻ BMSCs generated from MM bone marrow aspirates [16]. The cells were treated with different doses of BKM120 from 0 to 1 mM for 24 h. Primary MM cells and BMSCs were identified by APC-CD138 staining. As shown by the representative data obtained from myeloma cells and BMSCs from one out of three patients examined (Fig. 1c), BKM120 induced CD138⁺ primary MM cell apoptosis in a dose-dependent manner. The primary MM apoptosis rate is slightly elevated even in our control group. This is probably because primary MM cells go into spontaneous apoptosis ex vivo after isolation from the tumor-promoting bone marrow microenvironment [13]. Of interest, BKM120 had significant lower cytotoxicity toward CD138⁻ stromal cells. Figure 1d shows BKM120-induced apoptosis of primary MM cells from three different MM patients. Taken together, these data suggest that BKM120 induces primary MM cell apoptosis and has low toxicity toward non-tumoric BMSCs.

BKM120 has low toxicity toward normal blood cells of healthy volunteers

To further examine whether BKM120 induces normal cell apoptosis, PBMCs from different healthy volunteers were incubated with 0–1 mM BKM120 for 24 h. Cells apoptosis rate was measured as described above. As shown in Fig. 2a, BKM120 had comparably low toxicity toward normal PBMCs as to BMSCs. BKM120 at 10 or 100 μ M, which were highly apoptotic to MM cells, only resulted in <40% of PBMC apoptosis. Thus, our findings suggest that BKM120 has low cytotoxicity toward normal PBMCs.

IL-6, IGF, or BMSCs do not protect MM cells from BKM120-induced apoptosis

IL-6 is an important survival cytokine for MM [17]. Previous work has shown that IL-6 promotes MM cell survival under chemotherapy agent dexamethasone treatment [18]. Therefore, we examined whether IL-6 could attenuate BKM120-induced MM cell apoptosis. For this purpose, different MM cell lines were cultured with or without recombinant human IL-6 at a final concentration of 5 ng/ml in the presence or absence of 10 μ M BKM120 for 24 h. As a positive control, MM.1S cells were treated with 40 μ g/ml of dexamethasone with or without IL-6 for the same period of time. As shown in Fig. 2b, IL-6 did not affect BKM120-induced MM cell apoptosis, but promoted MM.1S cell survival under dexamethasone treatment.

Previous researches have also shown that IGF is another MM survival cytokine that activates PI3K-Akt pathway [19]. Therefore, we also tested whether presence of IGF affects BKM120-induced MM cell apoptosis. Different MM cell lines were cultured with or without recombinant human IGF at a final concentration of 10 ng/ml. As shown in Fig. 2c, IGF had no protection on BKM120-induced apoptosis in ARP-1 or U266 cells. Increasing evidence has shown that BMSCs in the myeloma tumor bed provide a tumor promotion microenvironment and protect MM cells from chemotherapy drug-induced apoptosis [20]. Therefore, we also tested whether BMSCs from MM patient bone marrow were able to protect MM cells from BKM120-induced apoptosis. For this purpose, BMSCs generated from MM patients were cocultured with MM cell lines. The cells were treated with or without 10 μ M of BKM120 for 24 h. As a positive control, MM.1S cells were cocultured with or without BMSCs and treated with or without 40 μ g/ml of dexamethasone for 24 h. After treatment, MM cells were identified as CD138⁺ cells by APC-CD138 staining. As shown in Fig. 2d, BMSCs were not able to protect MM cells from BKM120-induced apoptosis, but protected MM.1S cells from dexamethasone-induced apoptosis.

BKM120 causes cell cycle arrest in G1 phase

To study the mechanism of BKM120-induced MM cell growth inhibition and apoptosis, we examined whether BKM120 treatment affects MM cell cycle. As shown in Fig. 3a, ARP-1 cells were cultured with or without 1 μ M BKM120 for 24 h. BKM120 treatment resulted in increased G1-phase cells and decreased S-phase cells. Similar findings were observed in other MM cell lines MM.1S and MM.1R (Fig. 3b).

BKM120 triggers MM cell apoptosis by activating caspases

To elucidate BKM120-induced MM cell apoptosis, MM cell lines, treated with or without BKM120 for 24 h, were assessed for caspase activation by Western blotting analysis. The

results showed the cleavage of caspase 3, caspase 7, and caspase 9 (Fig. 4a). PARP cleavage was also detected after BKM120 treatment in all tested cell lines, indicating activation of the caspase cascade. To examine whether BKM120-triggered cell death depends on caspase activation, ARP-1 cells were treated with BKM120 and caspase-3 inhibitor (Fig. 4b). Our result showed that caspase-3 inhibitor repressed BKM120-induced cell apoptosis. Overall, these findings suggest that BKM120 treatment induces MM cell apoptosis through caspase activation.

BKM120 exposure causes upregulation of BimS and downregulation of XIAP

To analyze the signaling pathways that are modulated by BKM120 exposure in MM cells, we extended our immunoblotting analysis to cell signaling molecules. First, we examined the inhibitory effect of BKM120 on PI3K/Akt-mTOR pathway in MM cells. As shown in Fig. 4c and d, both Akt phosphorylated at Thr473 and Akt phosphorylated at Ser308 were downregulated after BKM120 treatment. Downregulation of total Akt was observed in ARP-1 and MM.1R cells, but not in MM.1S cells (Fig. 4d). This is probably caused by the increase in apoptotic myeloma cells after BKM120 treatment, and/or some cell type-specific regulation of Akt expression/degradation after the treatment. The pP70S6K levels were also decreased after BKM120 treatment in tested MM cells, while total P70S6K expression remained unchanged. Such findings suggest that BKM120 inhibits PI3K-Akt-mTOR pathways in MM cells.

Second, since BKM120 treatment caused cell cycle arrest in G1 phase, we examined the expression of cell cycle regulators. As shown in Fig. 4d, cell cycle repressor p27(Kip1) protein expression was upregulated after BKM120 treatment, while cyclin D1 expression was downregulated.

Next, we examined the expression of apoptosis regulatory factors. Our data showed that the expression of cytotoxic small isoform of Bim, BimS, was upregulated after BKM120 treatment. Bim is a pro-apoptotic factor belonging to the Bcl-2 family [21]. Bim has three major isoforms, BimEL, BimL, and BimS, generated by alternative splicing. The shortest form BimS is the most cytotoxic isoform [22]. Previous work has shown that the transcription of Bim is regulated by the forkhead transcription factor FKHR-L1, a downstream effector of PI3K [23]. In addition to Bim, the expression of anti-apoptotic XIAP and Bcl-XL [24, 25], was downregulated after BKM120 treatment (Fig. 4d). Thus, BKM120-induced MM cell apoptosis may be caused by upregulation of cytotoxic BimS and down-regulation of anti-apoptotic XIAP and Bcl-XL.

Synergistic cytotoxicity of BKM120 and dexamethasone on MM cells

To test whether BKM120 has a synergistic or additive effect with other anti-MM chemotherapy agents, ARP-1 cells were treated with BKM120 (1 μ M) in combination with low doses of melphalan, dexamethasone, lenalidomide, or bortezomib. As shown in Fig. 5a, combinational treatment of BKM120 with dexamethasone or bortezomib, but not with other drugs, had synergistic or additive cytotoxicity in ARP-1 cells. In particular, BKM120 and dexamethasone showed synergistic anti-ARP-1 activity. Next, we extended the experiment to other MM cell lines. As shown in Fig. 5b, although BKM120 or dexamethasone alone at

the low doses had only a limited cytotoxicity, combination of both induced significant cell apoptosis in dexamethasone-sensitive cell lines ARP-1 and MM.1S, but not in dexamethasone-resistant cells MM.1R. Cell growth tests also showed that BKM120 and dexamethasone synergistically inhibited MM.1S cell growth (Fig. 5c). In addition, the same combination of drugs only had limited cytotoxicity toward PBMCs (Supplemental Figure 1).

To examine the minimum doses of each drug for a synergistic effect, we treated MM.1S cells with different doses of BKM120 and dexamethasone for 24 h. The drug synergistic effect was confirmed by Isobologram analysis (Table 1 and Supplemental Figure 2). These results indicated that BKM120 and dexamethasone had synergistic effects when BKM120 was at doses of 0.5 and 1 μM because of the low values of the interaction index.

To elucidate the role of BKM120 and dexamethasone in the synergistic effect on myeloma cells, we treated MM.1S cells with the drugs in a sequential order. MM.1S cells were treated with dexamethasone for the first day, washed, and switched to BKM120 for the second day, or vice versa. Treatments with medium or the single drugs in sequence served as controls. As shown in Fig. 5e, treatment with dexamethasone first followed by BKM120 resulted in higher apoptosis rate than treatments of BKM120 followed by dexamethasone or single drugs alone.

Finally, immunoblotting to analyze caspase-dependent apoptosis was used to elucidate the molecular mechanisms underlying the synergistic effect of the two drugs. As shown in Fig. 5f, BKM120 and dexamethasone combinational treatment resulted in increased PARP and Bcl-2 cleavage and caspase-3 activation. The total Bcl-2 level remained unchanged. This was probably because that cleaved Bcl-2 was only a small part of the total Bcl-2. These findings indicate an enhanced caspase-dependent apoptosis after dual drug treatment. BimS expression was further upregulated in the combinational treatment, which may be the cause of the synergistic effect. In summary, our findings suggest that BKM120 and dexamethasone have synergistic cytotoxicity in dexamethasone-sensitive MM cells.

In vivo effects of BKM120 on established MM

To examine BKM120 in vivo antimyeloma effects, the human MM-SCID mouse model using cell line ARP-1 was established as described previously [26]. When palpable tumors developed (≥ 5 mm in diameter), mice (ten per group) received daily intraperitoneal injections of BKM120 ($5 \mu\text{M kg}^{-1} \text{ day}^{-1}$) or vehicle control (DMSO/PBS). As shown in Fig. 6a and b, mice receiving BKM120 treatment had significantly smaller tumor burdens as compared with control mice, which were measured as tumor volume (Fig. 6a, $P < 0.05$) and level of circulating human kappa chain (Fig. 6b, $P < 0.05$). In addition, BKM120 treatment significantly prolonged the survival of tumor-bearing mice (Fig. 6c, $P < 0.05$).

Next, we examined whether BKM120 and dexamethasone display in vivo synergistic antimyeloma effects. In particular, we wanted to know whether these two drugs could display effective antimyeloma effects in vivo at low doses. SCID mice bearing MM.1S tumor were developed and after palpable tumor developed (tumor diameter ≥ 5 mm), mice (five per group) were treated with intraperitoneal injections of DMSO/PBS, BKM120 ($1 \mu\text{M kg}^{-1}$), dexamethasone ($50 \mu\text{g kg}^{-1}$), or combination of BKM120 and dexamethasone at the

same doses seven times, every other day for 15 days. Although BKM120 or dexamethasone alone at the low doses had no therapeutic effects against established myeloma, combinational therapy using both the drugs at the low doses significantly retarded the growth of myeloma, measured as tumor volume (Fig. 6d, $P<0.05$) and level of circulating human lambda chain (Fig. 6e, $P<0.05$), in treated mice as compared with control mice or mice treated with BKM120 or dexamethasone alone. In addition, combined treatment significantly prolonged the survival of tumor-bearing mice (Fig. 6f, $P<0.05$).

Altogether, our data indicate that BKM120 has a strong tumoricidal activity against MM both in vitro and in vivo in a myeloma mouse model and suggest that BKM120 may be synergistic with dexamethasone for treating MM.

Discussion

In this study, we demonstrated the anti-MM activity of BKM120, a novel pan-PI3K inhibitor developed by Novartis Oncology Inc, by a series of in vitro and in vivo experiments. BKM120 treatment resulted in cell growth inhibition and apoptosis induction in all tested MM cell lines and primary MM cells in a dose-dependent manner. BKM120 has only limited cytotoxicity toward normal PBMCs and BMSCs. In addition, BKM120 had anti-MM activity in vivo in MMSCID mouse model. BKM120-treated myeloma-bearing mice had repressed tumor growth and prolonged survival. BKM120-induced myeloma cytotoxicity overcame drug resistance provided by the presence of BMSCs or IL-6. Previous research has shown that BMSCs in MM bone marrow play a crucial role in MM drug resistance [27]. As a result, most, if not all, MM patients become refractory to conventional chemotherapy agents during the treatment and reestablish myeloma that is more resistant to the drugs [28]. Our findings suggest that BKM120 has potent anti-MM activity and may be used to treat MM patients who have become resistant to conventional chemotherapy drugs.

Our findings also showed a synergistic anti-MM activity of BKM120 and dexamethasone, although this synergistic effect was only observed in dexamethasone-sensitive cells. This suggests that these two drugs can be used to treat dexamethasone-sensitive patients. More importantly, a synergistic antimyeloma effect of BKM120 and dexamethasone could be observed at low doses of the drugs. Furthermore, sequential treatment with BKM120 followed by dexamethasone shows enhanced anti-MM activity compared to treatments with dexamethasone or BKM120 alone. As dexamethasone is widely used in MM treatment, alone or together with other chemotherapeutic drugs [29], it will be beneficial to treat patients with low doses and for short periods with these drugs to minimize side effects without compromising its antimyeloma clinical effects. Thus, our findings suggest that BKM120 and dexamethasone combined treatment may be an effective and less toxic way to treat MM.

In this study we elucidated the signaling and apoptotic pathways and the downstream effectors of PI3K-Akt-mTOR pathway in myeloma cells. It is known that PI3K-Akt inhibition results in cell cycle arrest, cell growth repression, and apoptosis [4]. In our study, the inhibitory effect of BKM120 on PI3K-Akt-mTOR pathway in MM cell lines has been demonstrated. We showed that BKM120 causes MM cell cycle arrest at G1 phase by

upregulation of p27(Kip1) and downregulation of cyclin D1. In addition, BKM120 exposure results in upregulation of apoptotic BimS expression and downregulation of anti-apoptotic XIAP expression, both of which may lead to MM cell apoptosis.

MM is still an incurable disease with a median survival of only about 7 years [30]. Therefore, new therapeutic agents are needed for MM treatment. Constitutive activation of PI3K-Akt pathway in MM has been reported [31]. IGF-1 and IL-6, the two major growth factors in MM, promote myeloma cell proliferation and drug resistance by activating PI3K-Akt pathway [8, 32]. Further mechanistic studies also suggest a feedback loop within PI3K-Akt-mTOR pathway that mTOR/S6K inhibition induces feedback activation of Akt [33]. Thus far, a panel of PI3K-Akt-mTOR pathway inhibitors has been shown to exhibit anti-MM activities both in vitro and in vivo [34, 35]. Therefore, PI3K-Akt-mTOR pathway targeting therapy may be a promising way to treat MM. This study adds BKM120 to the list of PI3K-Akt-mTOR inhibitors that can be used for myeloma therapy.

In conclusion, our study demonstrates the anti-MM activity of BKM120 in vitro and in vivo. BKM120 alone or together with other antimyeloma chemotherapeutics, particularly dexamethasone, may be promising therapeutic agents for MM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Effects of BKM120 on myeloma cell growth and apoptosis. **a** Percentage of apoptotic cells and **b** cell proliferation (percentage of controls) in five established myeloma cell lines examined on days 1 and 3 after treatment with different doses of BKM120. ARP-1, ARK, MM.1S, MM.1R, and U266 were cultured in the presence of 0 (*Med* medium) to 1 mM BKM120, and cell apoptosis or growth were determined on days 1 and 3 by annexin V binding or MTS assays, respectively. Apoptotic cells were determined as annexin V-positive cells. **c** CD138⁺ primary myeloma cells were cocultured with CD138⁻ BMSCs, and treated with BKM120. Representative histograms showing apoptotic (annexin V-positive) CD138⁺ primary myeloma cells and CD138⁻ BMSCs in their cocultures with and **d** percentages of apoptotic primary myeloma cells from three patients (pt) with MM in cultures with different doses of BKM120 for 24 h

Fig. 2.

Effects of BKM120 on normal cell apoptosis and IL-6 and BMSCs on BKM120-induced myeloma cell apoptosis. **a** Percentages of apoptotic PBMCs from three healthy volunteers in cultures with different doses of BKM120 for 24 h. Also shown are percentages of apoptotic myeloma cells induced by BKM120 or dexamethasone in cultures without (*Med* medium) or with **b** human IL-6, **c** human IGF, or **d** BMSCs. **b–d** Myeloma cell lines ARP-1, MM.1S, and MM.1R were cultured with 10 μ M BKM120 or 40 μ g/ml dexamethasone (MM.1S only) without or with human IL-6 (5 ng/ml) for 24 h or cocultured without or with BMSCs with the addition of 10 μ M BKM120 or 40 μ g/ml dexamethasone (MM.1S only) for 24 h. Cells apoptosis was measured by annexin V staining. * $P < 0.05$

Fig. 3.

BKM120 induces myeloma cell cycle arrest at G1 phase. **a** Representative histograms showing the percentages of cells in different cell cycles (G1, S, and G2) of ARP-1 cells treated without (medium) or with 1 μ M BKM120 for 24 h. **b** Percentages of myeloma cells (ARP-1, MM.1S, and MM.1R) in different cell cycles (G1, S, and G2) in cultures without (medium) or with 1 μ M BKM120 for 24 h

Fig. 4.

Effects of BKM120 on myeloma cell signaling and caspase activation. **a** Western blot analysis showing PARP and caspases activation and cleavage; **b** BKM120 (BKM or B) induced cell apoptosis with or without caspase 3 inhibitor (Inh); *Med* medium. Caspase inhibitor inhibited BKM120-induced apoptosis in MM.1S cells. Western blot analysis showing **c** phosphorylation of Akt in ARP-1 myeloma cells treated without (*Med* medium) or with 10 μ M BKM120 for different time points as stated or **d** modulation of signaling pathways including PI3K-Akt-mTOR, cell cycle, and apoptotic regulators in MM cells ARP-1, MM.1S, and MM.1R treated without (*Med* medium) or with 10 μ M BKM120 for 24 h. Cell lysates were used for the analyses

Fig. 5.

Synergistic or additive antimyeloma effects of BKM120 and dexamethasone. **a** Percentages of apoptotic myeloma ARP-1 cells in cultures with BKM120 (BKM; 10 μM), melphalan (Mel; 5 nM), dexamethasone (Dex; 40 $\mu\text{g/ml}$), lenalidomide (Len; 100 μM), or bortezomib (BTZ; 5 nM), alone or in combination for 24 h. **b** Percentages of apoptotic myeloma ARP-1, MM.1S, or MM.1R cells in cultures with 1 μM BKM120 or 40 $\mu\text{g/ml}$ dexamethasone alone, or their combination for 24 h. **c** Cell growth, measured by MTS assay, of myeloma ARP-1, MM.1S, or MM.1R cells in cultures with 1 μM BKM120 or 40 $\mu\text{g/ml}$ dexamethasone alone, or their combination for 24 h. **d** Percentages of apoptotic myeloma MM.1S cells in cultures with different doses of BKM120 (0.1, 0.5, 1, and 5 μM) or dexamethasone (40 or 400 ng/ml, and 4 or 40 $\mu\text{g/ml}$) alone, or their combinations for 24 h. **e** Percentages of apoptotic myeloma MM.1S cells in cultures with BKM120 or dexamethasone for 24 h followed by sequential treatment without (*Med* medium) or with BKM120 or dexamethasone for additional 24 h. In this experiment, MM.1S cells were first treated with 1 μM BKM120 or 4 $\mu\text{g/ml}$ dexamethasone for 24 h, followed by washing once with PBS and culturing in fresh medium alone or medium containing either 1 μM BKM120 or 4 $\mu\text{g/ml}$ dexamethasone for another 24 h. Cell apoptosis was determined by annexin V staining. **f** Western blot analysis showing the protein levels of cleaved PARP (cPARP), caspase-3 (cCaspase-3), and Bcl-2 (cBcl-2), and Bim, Bcl-XL, and Bcl-2 of myeloma MM.1S cells treated without (*Med*

medium) or with dexamethasone (Dex; 4 µg/ml), BKM120 (BKM; 1 µM), or both (B+D) for 24 h. Short exposure time (*short exp.*), about 5 s, was used to detect total Bcl-2 (full length), while long exposure time (*long exp.*), about 1 min, was used to detect cleaved Bcl-2

Fig. 6.

In vivo therapeutic effects of BKM120 on established myeloma in SCID mice. Shown are **a** tumor volumes, **b** levels of circulating human kappa chain in mouse sera detected by ELISA, and **c** survival of tumor-bearing mice treated with vehicle control (DMSO/PBS) or BKM120. SCID mice were inoculated subcutaneously in the right flank with 1×10^6 ARP-1 cells. Three to 4 weeks later, when palpable tumors (≤ 5 mm in diameter) developed, mice (ten per group) were treated intraperitoneally with daily injections of DMSO/PBS or BKM120 ($5 \mu\text{M kg}^{-1} \text{ day}^{-1}$) for 15 days. Tumor burdens were measured as tumor volumes and levels of circulating human kappa chain in mouse sera by ELISA. Also shown are **d** tumor volumes, **e** levels of circulating human kappa chain in mouse sera detected by ELISA, and **f** survival of tumor-bearing mice treated with vesicle control (DMSO/ PBS), BKM120 (BKM), dexamethasone (Dex), or BKM plus dexamethasone (B+D). SCID mice bearing MM.1S tumor were established. After palpable tumors (≤ 5 mm in diameter) developed, mice (five per group) were treated with intraperitoneal injections of DMSO/ PBS, BKM120 ($1 \mu\text{M kg}^{-1}$), dexamethasone ($50 \mu\text{g kg}^{-1}$), or combination of BKM120 and dexamethasone at the same low doses seven times, every other day for 15 days. * $P < 0.05$

Table 1

Synergistic antimyeloema effects of BKM120 and Dexamethasone

BKM120 (μ M)	Dexamethasone (μ g/ml)	Dose ratio (BKM/Dex)	Standardized mean --cell surviving (%)	Interaction index	95% CI (confidence interval)		Conclusion
					Lower limit	Upper limit	
0	0		100				
0.1	0		90.6972				
0.5	0		88.7743				
1	0		87.0985				
5	0		62.6383				
0	0.04		90.1601				
0.1	0.04	2.5	87.2919	0.3695	0.0124	11.0506	Additive
0.5	0.04	12.5	58.2984	0.033	0.0008	1.3752	Synergistic
1	0.04	25	24.7502	0.0025	0	1.5809	Synergistic
5	0.04	125	0.8272	0	0	4.0324	Additive
0	0.4		86.5506				
0.1	0.4	0.25	87.6356	1.8579	0.0096	358.2386	Additive
0.5	0.4	1.25	58.481	0.0336	0.0008	1.3816	Synergistic
1	0.4	2.5	21.0012	0.0016	0	1.4979	Synergistic
5	0.4	12.5	0.8379	0	0	4.0401	Additive
0	4		83.8436				
0.1	4	0.025	89.9345	76.301	0.2226	26155.58	Additive
0.5	4	0.125	55.2798	0.0252	0.0005	1.2826	Synergistic
1	4	0.25	14.3839	0.0006	0	1.3484	Synergistic
5	4	1.25	1.4824	0	0	4.4045	Additive
0	40		75.8513				
0.1	40	0.0025	68.0095	0.066	0.0004	10.2314	Additive
0.5	40	0.0125	51.4878	0.0182	0.0003	1.1744	Synergistic
1	40	0.025	14.588	0.0006	0	1.3532	Synergistic
5	40	0.125	0.6016	0	0	3.8488	Additive

MM.1S cells were treated with different doses of BKM120 (0.1, 0.5, 1, and 5 μ M) or dexamethasone (40 and 400 ng/ml and 4 and 40 μ g/ml) alone, or their combinations for 24 h. Cell survival was calculated from apoptosis rate. Interaction index values of each treatment conditions were calculated as described in "Materials and methods." Drugs with synergistic effects were highlighted in gray