MLN120B, a Novel IκB Kinase β Inhibitor, Blocks Multiple Myeloma Cell Growth In vitro and In vivo

Teru Hideshima,1 Paola Neri,1 Pierfrancesco Tassone,1 Hiroshi Yasui,1 Kenji Ishitsuka,1 Noopur Raje,1 Dharminder Chauhan,1 Klaus Podar,1 Constantine Mitsiades,1 Lenny Dang,2 Nikhil Munshi,1 Paul Richardson,1 David Schenkein,2 and Kenneth C. Anderson1

Abstract

Purpose: The purpose of this study is to delineate the biological significance of IκB kinase (IKK) β inhibition in multiple myeloma cells in the context of bone marrow stromal cells (BMSC) using a novel IKKβ inhibitor MLN120B.

Experimental Design: Growth-inhibitory effect of MLN120B in multiple myeloma cells in the presence of cytokines [interleukin-6 (IL-6) and insulin-like growth factor-1 (IGF-1)], conventional agents (dexamethasone, melphalan, and doxorubicin), or BMSC was assessed in vitro. In vivo anti-multiple myeloma activity of MLN120B was evaluated in severe combined immunodeficient (SCID) – hu model.

Results: MLN120B inhibits both baseline and tumor necrosis factor-α – induced nuclear factor-κB activation, associated with down-regulation of IkBα and p65 nuclear factor-κB phosphorylation. MLN120B triggers 25% to 90% growth inhibition in a dose-dependent fashion in multiple myeloma cell lines and significantly augments tumor necrosis factor-α – induced cytotoxicity in MM.1S cells. MLN120B augments growth inhibition triggered by doxorubicin and melphalan in both RPMI B226 and IL-6-dependent INA6 cell lines. Neither IL-6 nor IGF-1 overcomes the growth-inhibitory effect of MLN120B. MLN120B inhibits constitutive IL-6 secretion by BMSCs by 70% to 80% without affecting viability. Importantly, MLN120B almost completely blocks stimulation of MM.1S, U266, and INA6 cell growth, as well as IL-6 secretion from BMSCs, induced by multiple myeloma cell adherence to BMSCs. MLN120B overcomes the protective effect of BMSCs against conventional (dexamethasone) therapy.

Conclusions: Our data show that the novel IκB inhibitor MLN120B induces growth inhibition of multiple myeloma cells in SCID-hu mouse model. These studies provide the framework for clinical evaluation of MLN120B, alone and in combined therapies, trials of these novel agents to improve patient outcome in multiple myeloma.
Although the precise role of NF-κB activation in pathogenesis of multiple myeloma has not been fully characterized, we have shown previously that multiple myeloma cell adhesion to bone marrow stromal cells (BMSC) induces NF-κB-dependent up-regulation of transcription of IL-6 (14, 15). In addition, TNF-α secreted by multiple myeloma cells activates NF-κB in BMSCs, thereby directly up-regulating IL-6 transcription and secretion in BMSCs, and also activates NF-κB in multiple myeloma cells, thereby up-regulating intracellular adhesion molecule-1 (CD54) and vascular cell adhesion molecule-1 (CD106) expression on both multiple myeloma cells and BMSCs, thereby increasing multiple myeloma cell to BMSC binding (12). Because IL-6 is a major growth and survival factor in multiple myeloma cells (16, 17) and adherence of multiple myeloma cells to fibronectin confers resistance to drug-induced apoptosis, specific blockade of NF-κB signaling represents a novel therapeutic strategy in multiple myeloma.

We and others have shown already that a variety of novel agents with both preclinical and early clinical anti-multiple myeloma activity, including proteasome inhibitor bortezomib (PS-341; ref. 13), thalidomide and immunomodulatory derivatives (18), histone deacetylase inhibitors (19), transforming growth factor-β inhibitor (20), lysophosphatidic acid acyltransferase-β inhibitor (21), and 1-acetoxychavicol acetate (22) inhibit both NF-κB activation and multiple myeloma cell growth. Importantly, we have also shown that IKKβ inhibitor PS-1145 blocks multiple myeloma cell growth in the context of BMSCs, associated with down-regulation of IL-6 secretion from BMSCs (14).

MLN120B is a novel specific and stable small-molecule inhibitor of IKKβ (23). In this study, we show that MLN120B, a more selective and stable novel IKKβ inhibitor, blocks TNF-α-induced NF-κB activation in multiple myeloma cells through inhibition of IκBα phosphorylation and degradation of IκBα. Importantly, MLN120B inhibits both IL-6 secretion from BMSCs triggered by multiple myeloma cell adhesion and proliferation of multiple myeloma cells adherent to BMSCs. Most importantly, MLN120B inhibits multiple myeloma cell growth in a clinically relevant severe combined immunodeficient (SCID)–hu mouse model. These studies therefore confirm a central role for IKKβ in regulating growth and survival of multiple myeloma cells in the bone marrow milieu and further suggest the potential utility of novel therapeutics targeting IKKβ or NF-κB in multiple myeloma.

**BMSC cultures.** Bone marrow specimens were obtained from patients with multiple myeloma. Mononuclear cells separated by ficoll-hypaque density sedimentation were used to establish long-term BMSC cultures as described previously (14). When an adherent cell monolayer had developed, cells were harvested in Hank’s buffered saline solution containing 0.25% trypsin and 0.02% EDTA, washed, and collected by centrifugation.

**Reagents.** An IKKβ inhibitor MLN120B (C18H15ClN4O2, MW = 367; Millennium Pharmaceuticals, Cambridge, MA) was dissolved in DMSO and stored at −20°C; it was diluted in culture medium immediately before use; MLN120B control medium contained <0.1% DMSO. For the animal studies, MLN120B was dissolved in vehicle (0.5% methylcellulose) at a final concentration of 15 mg/mL TNF-α, IL-6, and IGF-1 were purchased from R&D Systems (Minneapolis, MN). Dexamethasone, melphanal, and doxorubicin were purchased from Sigma Chemical.

**DNA synthesis.** Multiple myeloma cells (3 × 10⁵ per well) were incubated in 96-well culture plates (Costar, Cambridge, MA) in the presence of medium, MLN120B, and/or other agents for 72 hours at 37°C. DNA synthesis was measured by [3H]thymidine ([3H]Tdr; Perkin-Elmer, Boston, MA) uptake. Cells were pulsed with [3H]Tdr (0.5 μCi/well) during the last 8 hours of 48-hour cultures. All experiments were done thrice in quadruplicate.

**Cytotoxic assay.** The inhibitory effect of MLN120B on multiple myeloma cell growth was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye absorbance as described previously (13). All experiments were done thrice in quadruplicate. Absorbance was measured at 570/630 nm using a spectrophotometer (Molecular Devices Corp., Sunnyvale, CA).

**Immunoblotting.** Multiple myeloma cells were cultured with MLN120B, harvested, and lysed using lysis buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% NP40, 5 mmol/L EDTA, 5 mmol/L NaF, 2 mmol/L Na3VO4, 1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 5 μg/mL aprotinin]. Whole-cell lysates were subjected to Western blotting using phosphorylated IκBα, IκBα, phosphorylated p65 NF-κB, and p65 NF-κB antibodies (Cell Signaling, Beverly, MA).

**Electrophoretic mobility shift analysis.** Electrophoretic mobility shift analyses (EMSA) were carried out as we’ve done previously (12, 14). Briefly, multiple myeloma cells were preincubated with MLN120B (1.25-20 μmol/L for 60 minutes) before stimulation with TNF-α (5 ng/mL) for 20 minutes. Cells were then pelleted, resuspended in 400 μL hypotonic lysis buffer [20 mmol/L HEPES (pH 7.9), 10 mmol/L KC1, 1 mmol/L EDTA, 0.2% Triton X-100, 1 mmol/L Na3VO4, 5 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 5 μg/mL aprotinin], and kept on ice for 20 minutes. After centrifugation at 4°C, the supernatant was collected as nuclear extract. Double-stranded NF-κB consensus oligonucleotide probe (5′-GGGGACCATTTCCC-3′; Santa Cruz Biotechnology, Santa Cruz, CA) was end labeled with [γ-32P]ATP (50 μCi at 22 TBq/mmol/L; Perkin-Elmer). Binding reactions containing 1 ng oligonucleotide and 5 μg nuclear protein were conducted at room temperature for 20 minutes in a total volume of 10 μL binding buffer [10 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 1 mmol/L MgCl2, 0.5 mmol/L EDTA, 0.5 mmol/L DTT, 4% glycerol (v/v), and 0.5 μg poly(dioxyninosinic-deoxyctydylid acid) (Pharmacia, Peapack, NJ)]. The samples were loaded onto a 4% polyacrylamide gel, transferred to Whatman paper (Whatman International, Maidstone, United Kingdom), and visualized by autoradiography.

**Effect of MLN120B on paracrine multiple myeloma cell growth in the bone marrow.** To evaluate growth stimulation and signaling in multiple myeloma cells adherent to BMSCs, 3 × 10⁴ MM.1S cells were cultured in BMSC-coated 96-well plates for 48 hours in the presence or

---

**Materials and Methods**

**Multiple myeloma–derived cell lines.** Dexamethasone-sensitive (MM.1S) and dexamethasone-resistant (MM.1R) human multiple myeloma cell lines were provided by Dr. Steven Rosen (Northwestern University, Chicago, IL). RPMI 8226 and U266 human multiple myeloma cells were obtained from American Type Culture Collection (Rockville, Maryland). IL-6-dependent INA6 cell line was kindly provided by Dr. Renate Burger (University of Kiel, Kiel, Germany). Melphanal-resistant RPMI-LRS and doxorubicin-resistant RPMI-DoxR cell lines were provided by Dr. William Dalton (H. Lee Moffitt Cancer Center, Tampa, FL). All multiple myeloma cell lines were cultured in RPMI 1640 containing 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO), 2 μmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies, Grand Island, NY).
absence of MLN120B. DNA synthesis was measured as described above. The DuoSet ELISA (R&D Systems) was used to measure IL-6 in supernatants of 48-hour cultures of BMSCs, with or without MM.1S cells, in the presence or absence of MLN120B.

SCID-hu mouse model. Human fetal long bone grafts were implanted into SCID mice (SCID-hu mice) as described previously (24, 25). Approximately 4 weeks following bone implantation, 2.5 × 10⁶ INA6 multiple myeloma cells in 50 µL PBS was injected directly into human bone within SCID-hu hosts. Soluble human IL-6 receptor (shuIL-6R) released from INA6 cells was assessed in mouse sera by ELISA (R&D Systems) as in our prior studies (26). Mice were treated orally with vehicle alone or MLN120B 50 mg/kg (twice daily) for 3 weeks after detection of measurable shuIL-6R in mouse sera.

Statistical analysis. Statistical significance of differences observed in drug-treated versus control cultures was determined using the Wilcoxon signed-ranks test. The minimal level of significance was P < 0.05.

Results

MLN120B inhibits IκBα phosphorylation and NF-κB activation in multiple myeloma cells. We first examined baseline NF-κB activation in multiple myeloma cell lines by EMSA. Although there is variation, constitutive NF-κB activation was observed in all multiple myeloma cell lines (Fig. 1A). Because IKKβ is believed to be upstream kinase, which phosphorylates IκBα, we next examined whether MLN120B could sufficiently block phosphorylation of IκBα. MLB120B inhibits baseline phosphorylation of IκBα in RPMI 8226 and INA6 cells; however, no significant inhibition was observed in MM.1S cells (Fig. 1B). Consistent with inhibitory effect on IκBα phosphorylation, NF-κB inhibition was triggered by MLN120B in RPMI 8226 and INA6 cells in a dose-dependent fashion, whereas no inhibition was recognized in MM.1S cells (Fig. 1C).

Because NF-κB activation requires phosphorylation, ubiquitination, and degradation of IκBα, we next examined whether MLN120B could inhibit phosphorylation and degradation of IκBα using Western blotting. MM.1S cells pretreated with either DMSO control medium or MLB120B (1.25-20 μmol/L for 90 minutes) were stimulated with TNF-α (5 ng/mL for 20 minutes). TNF-α-induced phosphorylation and degradation
of IκBα were completely abrogated by MLN120B in a dose-dependent fashion. Phosphorylation of p65 NF-κB induced by TNF-α was also blocked by MLN120B (Fig. 1D). We next examined whether MLN120B also could block TNF-α-induced NF-κB activation in MM.1S cells. As seen in Fig. 1E, MLN120B completely abrogated TNF-α-induced NF-κB activation in a dose-dependent fashion in MM.1S cells. No blockade of phosphorylation of extracellular signal-regulated kinase, signal transducers and activators of transcription 3, Akt, p38 mitogen-activated protein kinase, or c-Jun NH2-terminal kinase by MLN120B was observed (data not shown). These results indicate that MLN120B potently blocks phosphorylation and degradation of IκBα, thereby blocking NF-κB activation in multiple myeloma cells.

**MLN120B inhibits proliferation of multiple myeloma cell lines.** To study the growth-inhibitory effect of MLN120B on multiple myeloma cells, we next did both MTT assay and [3H]thymidine uptake in MM.1S, MM.1R, U266, RPMI 8226, RPMI-LR5, RPMI-Dox40, and INA6 cell lines cultured for 72 hours in the presence of MLN120B (2.5-40 μmol/L). Five percent to fifty percent and 18% to 70% inhibition in proliferation was observed at doses >20 μmol/L MLN120B, assessed by MTT assay (Fig. 2A) and [3H]thymidine uptake (Fig. 2B), respectively. We further examined cell cycle profile by propidium iodide staining using flow cytometry; no accumulation of sub-G0-G1 phase cells was recognized (data not shown), suggesting that MLN120B does not trigger apoptosis.

Because we and others have shown that IL-6 (7, 16, 17, 27, 28) and IGF-I (29–31) are major growth and antiapoptotic factors in multiple myeloma, we next examined whether MLN120B could overcome the growth-stimulatory effects of these cytokines. U266 cells were cultured with 10 μmol/L MLN120B in the presence or absence of IL-6 or IGF-I (5 and 10 ng/mL) for 48 hours. Importantly, MLN120B almost completely abrogated the growth-stimulatory effect of these cytokines (Fig. 2C). TNF-α is known to stimulate both NF-κB and cell death signaling cascades. We therefore hypothesized that MLN120B could induce cytotoxicity triggered by TNF-α, by blocking NF-κB activity in M cells. When MM.1S cells were cultured with TNF-α (0.25 and 0.5 ng/mL) in the presence of MLN120B (10 μmol/L) for 48 hours, MLN120B significantly augmented TNF-α-induced cytotoxicity (Fig. 2D). Similar results were observed in INA6 cells (data not shown). These results show that MLN120B triggers growth inhibition, which is not overcome by growth and antiapoptotic factors (IL-6 or IGF-I).

**MLN120B enhances cytotoxicity of conventional (doxorubicin, melphalan, and dexamethasone) agents.** We next examine whether MLN120B could enhance cytotoxicity of conventional therapies. RPMI 8226 (Fig. 3A) and INA6 (Fig. 3B) cells were cultured with doxorubicin (125 and 250 nmol/L), melphalan (5 and 10 μmol/L), or dexamethasone (125 and 250 nmol/L) for 48 hours in the presence (10 μmol/L) or absence of MLN120B. MTT assay at 48 hours revealed that doxorubicin, melphalan, and dexamethasone alone each significantly inhibited RPMI 8226 and INA6 cell growth in a dose-dependent fashion; moreover, MLN120B additively enhanced their growth-inhibitory effects. Importantly, no additive or synergistic effect of MLN120B with these agents was observed in MM.1S cells. This result was consistent with that MLN120B does not inhibit baseline NF-κB activity in this cell line. Freshly isolated tumor cells from bone marrow aspirates of multiple myeloma patients were also cultured with doxorubicin (250 nmol/L) or dexamethasone (500 nmol/L) in the presence or absence of MLN120B (5-20 μmol/L) for 48 hours. As is true
in multiple myeloma cell lines, MLN120B augmented cytotoxicity of doxorubicin and dexamethasone in a dose-dependent fashion in primary tumor cells (Fig. 3C). These results indicate that inhibition of IKK activity enhances conventional agent-triggered cytotoxicity in both multiple myeloma cell lines and primary tumor cells.

**MLN120B inhibits paracrine multiple myeloma cell growth associated with down-regulation of IL-6 in the bone marrow microenvironment.** We have shown that the bone marrow microenvironment confers growth and drug resistance in multiple myeloma cells (9, 14). We therefore next studied the effect of MLN120B on paracrine multiple myeloma cell growth in the bone marrow milieu. We first examined the direct inhibitory effect of MLN120B on baseline IL-6 secretion from patient BMSCs (n = 3) using ELISA as in our previous studies (14). MLN120B significantly (64-71%; P < 0.01) down-regulated IL-6 secretion from BMSCS in a dose-dependent fashion (Fig. 4A). Furthermore, MLN120B also significantly (P < 0.01) inhibited TNF-α-induced up-regulation of IL-6 secretion from BMSCs (Fig. 4B). Because we have shown that IKK inhibitor PS-1145 inhibits multiple myeloma cell growth in the presence of BMSCs (14), we similarly examined growth-inhibitory effect of MLN120B on multiple myeloma cells in the presence of BMSCS. Multiple myeloma cell lines (MM.1S and INA6) were cultured with MLN120B (2.5-10 μmol/L) for 48 hours in the presence or absence of BMSCs. Adhesion of MM.1S (Fig. 4C) and INA6 (Fig. 4D) cells enhanced (1.7- to 2-fold; P < 0.01) IL-6 secretion from BMSCs; however, MLN120B (10 μmol/L) significantly (P < 0.01) inhibited this effect. Importantly, proliferation of MM.1S (3-fold; Fig. 4E) and INA6 (3.8-fold; Fig. 4F) triggered by adherence to BMSCs was significantly (P < 0.01) inhibited by MLN120B in a dose-dependent fashion. Because binding of multiple myeloma cells to BMSCs triggers increased secretion of IL-6 in culture supernatants (13, 14), these results are consistent with the observed inability of exogenous IL-6 to overcome the growth-inhibitory effects of MLN120B (Fig. 2).

**MLN120B abrogates the protective effect of BMSCs against dexamethasone-induced apoptosis.** To define the functional sequelae of MLN120B-related NF-κB blockade in MM.1S cells, we examined its inhibitory effect on growth of multiple myeloma cells, in the presence of dexamethasone as well as with or without BMSCS. Dexamethasone significantly inhibited...
MM.1S cell growth in a dose-dependent fashion, and BMSCs completely abrogated dexamethasone-induced cytotoxicity. Importantly, MLN120B triggers dose-dependent growth inhibition even in the presence of BMSCs (Fig. 5). These data suggest that MLN120B retains its antitumor activity against multiple myeloma cells in the bone marrow milieu.

**MLN120B inhibits human multiple myeloma cell growth in vivo.** Having shown the signaling mechanisms mediating the anti-multiple myeloma effects of MLN120B in vitro, we next determined whether MLN120B mediates anti-human multiple myeloma cell activity in vivo using a novel SCID-hu model, in which multiple myeloma cells grow in vivo in the context of the human bone marrow microenvironment (26, 32). Eight SCID mice were implanted with human fetal bone chips (SCID-hu), into which human IL-6-dependent INA6 cells were directly injected. These mice were treated orally with either MLN120B (50 mg/kg) or vehicle control twice daily for 3 weeks. As seen in Fig. 6, treatment with MLN120B induced a reduction of shuIL-6R, marker of tumor growth, whereas shuIL-6R continued to increase in mice treated with vehicle control. We also observed a trend toward prolonged survival in animals treated versus control. Mice of the control group have been sacrificed 6 days before the treated group for the occurrence of deaths and paralysis (data not shown). These results confirm activity of MLN120B as an antitumor agent in vivo in this SCID-hu model of human multiple myeloma. Evaluation of terminal bleeds did not reveal any differences in hematologic variables in drug-treated mice versus control mice (data not shown).

**Discussion**

In this study, we characterize the biological sequelae of NF-κB inhibition in multiple myeloma cells in the context of the bone marrow microenvironment. Specific NF-κB blockade using the IKKβ inhibitor MLN120B confirms the role of NF-κB in growth, survival, and drug resistance in multiple myeloma cells within the bone marrow milieu and suggests the therapeutic benefit of targeting NF-κB in multiple myeloma. We first determine baseline NF-κB blocking activity by EMSA in multiple myeloma cell lines, including MM.1S, MM.1R, RPMI 8226, RPMI-LR5, RPMI-Dox40, U266, and INA6 cells. Although some variation is recognized, all multiple myeloma cell lines show baseline NF-κB activity. For example, the highest and lowest NF-κB activity is observed in MM.1R and RPMI-LR5 cells, respectively. We next show the inhibitory effect of MLN120B on phosphorylation and degradation of IκBα in MM.1S, RPMI 8226, and INA6 cells. Although MLN120B inhibits baseline phosphorylation of IκBα and NF-κB activity in RPMI 8226 and INA6, inhibition of neither IκBα phosphorylation nor NF-κB activity by MLN120B is recognized in MM.1S cells. Importantly, TNF-α-induced IκBα phosphorylation and NF-κB activation are completely blocked by MLN120B in MM.1S cells. Our results suggest that baseline NF-κB activity in MM.1S cells may not be mediated via IKKβ, whereas, inducible (i.e., TNF-α stimulation) NF-κB activity is triggered by IKKβ. Based on NF-κB inhibitory effect, we next examined whether MLN120B inhibits multiple myeloma cell proliferation. MLN120B (20 μmol/L) induced up to 35% and 75% inhibition, assessed by MTT assay and [3H]thymidine uptake, respectively. There is no relationship between baseline level NF-κB activity and susceptibility to MLN120B in these multiple myeloma cell lines.

Both IL-6 and IGF-I are abundant in the bone marrow microenvironment and promote multiple myeloma cell growth and survival (9, 33); importantly, MLN120B almost completely abrogates the growth-stimulatory effect triggered by these cytokines. Because TNF-α induces both NF-κB activation and cytotoxic signaling cascades, we hypothesized that NF-κB blockade by MLN120B would augment TNF-α-induced multiple myeloma cell cytotoxicity. As expected, MLN120B significantly augments TNF-α-induced cytotoxicity, suggesting that NF-κB mediates protection against TNF-α-induced apoptosis in multiple myeloma cells. Because NF-κB also regulates transcription of cell cycle- and apoptosis-regulating proteins
Inhibition of IKK,β in Myeloma

(34, 35), we further examined whether MLN120B augments cytotoxicity of conventional anti-multiple myeloma agents. Importantly, MLN120B additively augments doxorubicin-, melphalan-, and dexamethasone-induced cytotoxicity in multiple myeloma cell lines, as well as in freshly isolated tumor cells from multiple myeloma patients, suggesting potential clinical utility of IKKβ or NF-κB inhibitors with other chemotherapy in multiple myeloma.

The bone marrow microenvironment plays a crucial role in multiple myeloma cell proliferation, survival, migration, and drug resistance, due to both multiple myeloma cell adherence to BMSCs and the cytokine circuit between multiple myeloma cells and BMSCs (9, 33). For example, IL-6 secretion from BMSCs is significantly enhanced both by multiple myeloma cell adhesion (15, 36) and by cytokines secreted from multiple myeloma cells [i.e., vascular endothelial growth factor (37), TNF-α (12, 14), and transforming growth factor-β (20, 38)]. Specifically, IL-6 transcription and secretion from BMSCs is regulated by NF-κB. Indeed, baseline, TNF-α-induced, and multiple myeloma cell adhesion induced IL-6 secretion from BMSCs is significantly blocked by MLN120B. Most importantly, multiple myeloma cell growth stimulation by BMSCs is markedly inhibited by MLN120B, suggesting that multiple myeloma cell growth stimulation by BMSCs is, at least in part, mediated by IKKβ activity.

We have shown that dexamethasone-induced apoptosis in multiple myeloma cells is mediated via related adhesion focal tyrosine kinase activation; second mitochondria-derived activator of caspases, but not cyt.c release from mitochondria; and caspase-9 activation (39–41). Conversely, IL-6 protects against dexamethasone-induced apoptosis via phosphatidylinositol 3-kinase/Akt signaling (6–8) and activation of SH2 domain containing protein tyrosine phosphatase SHP2, thereby blocking related adhesion focal tyrosine kinase activation (39). We therefore examined whether MLN120B could overcome protective effect of BMSCs against dexamethasone-induced apoptosis. Dexamethasone significantly inhibits [3H]thymidine uptake of MM.1S cells in a dose-dependent fashion, which is abrogated in the presence of BMSCs. Importantly MLN120B almost completely abrogates this protective effect of BMSCs against dexamethasone-induced apoptosis, suggesting its utility to either sensitize or overcome resistance to dexamethasone therapy.

Finally, we evaluated the in vivo effects of MLN120B on multiple myeloma cell growth within the human bone marrow milieu using SCID mice implanted with a human fetal bone chip, into which human IL-6-dependent IN6A cells were injected. In our models, tumor growth has been monitored by measurement of serum shuIL-6R levels, released from IN6A, progressive increased during the progression of the disease, and predictably detected after 4 weeks from cell injection. Whereas the detection of human κ chain, which is produced in very small amounts when cells are cultured in vitro (42), is associated only with the presence of a high burden of disease and does not represent a sensitive marker of early tumor growth. In this model, we observed a reduction of shuIL-6R levels released from IN6A in mice treated with MLN120B. These results therefore suggest that MLN120B can target both multiple myeloma cells and the bone marrow microenvironment in vivo. In summary, our data show that NF-κB activation promotes growth, survival, and drug resistance of multiple myeloma cells in the bone marrow microenvironment and provide the framework for clinical trials of novel agents, such as MLN120B, specifically targeting IKKβ in multiple myeloma.

References


MLN120B, a Novel IκB Kinase β Inhibitor, Blocks Multiple Myeloma Cell Growth *In vitro* and *In vivo*

Teru Hideshima, Paola Neri, Pierfrancesco Tassone, et al.


**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/19/5887

**Cited articles**
This article cites 42 articles, 26 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/19/5887.full.html#ref-list-1

**Citing articles**
This article has been cited by 23 HighWire-hosted articles. Access the articles at:
/content/12/19/5887.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.