PRIMA-1\textsuperscript{Met}/APR-246 Displays High Antitumor Activity in Multiple Myeloma By Induction of p73 and Noxa

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Abstract

Targeting p53 by the small-molecule PRIMA-1\textsuperscript{Met}/APR-246 has shown promising preclinical activity in various cancer types. However, the mechanism of PRIMA-1\textsuperscript{Met}-induced apoptosis is not completely understood and its effect on multiple myeloma cells is unknown. In this study, we evaluated antitumor effect of PRIMA-1\textsuperscript{Met} alone or its combination with current antimyeloma agents in multiple myeloma cell lines, patient samples, and a mouse xenograft model. Results of our study showed that PRIMA-1\textsuperscript{Met} decreased the viability of multiple myeloma cells irrespective of p53 status, with limited cytotoxicity toward normal hematopoietic cells. Treatment of multiple myeloma cells with PRIMA-1\textsuperscript{Met} resulted in induction of apoptosis, inhibition of colony formation, and migration. PRIMA-1\textsuperscript{Met} restored wild-type conformation of mutant p53 and induced activation of p73 upregulating Noxa and downregulating Mcl-1 without significant modulation of p53 level. siRNA-mediated silencing of p53 showed a little effect on apoptotic response of PRIMA-1\textsuperscript{Met}, whereas knockdown of p73 led to substantial attenuation of apoptotic activity in multiple myeloma cells, indicating that PRIMA-1\textsuperscript{Met}-induced apoptosis is, at least in part, p73-dependent. Importantly, PRIMA-1\textsuperscript{Met} delayed tumor growth and prolonged survival of mice bearing multiple myeloma tumor. Furthermore, combined treatment of PRIMA-1\textsuperscript{Met} with dexamethasone or doxorubicin displayed synergistic effects in both multiple myeloma cell lines and primary multiple myeloma samples. Consistent with our in vitro observations, cotreatment with PRIMA-1\textsuperscript{Met} and dexamethasone resulted in enhanced antitumor activity in vivo. Our study for the first time shows antmyeloma activity of PRIMA-1\textsuperscript{Met} and provides the rationale for its clinical evaluation in patients with multiple myeloma, including the high-risk group with p53 mutation/deletion. Mol Cancer Ther; 12(11); 2331–41. ©2013 AACR.

Introduction

Multiple myeloma is a neoplasm of terminally differentiated B cells, characterized by aberrant expansion of plasma cells within the bone marrow as well as into extramedullary sites (1). Modern chemotherapy schedules have improved the outcome of patients with multiple myeloma except the p53-mutated/deleted high-risk group (2, 3). At diagnosis, the incidence of p53 gene mutations/deletions in multiple myeloma seems to be low (~10%), however, it increases as the disease progresses (4–6). Importantly, p53 mutations/deletions are considered an adverse risk factor in multiple myeloma as patients with p53 mutation/deletion are resistant to current therapies including stem cell transplantations (4–8). Despite increased response rates in the p53-aberrant patients with clinically approved drugs, more than half display no response to therapy and relapse early, stressing an urgent need for alternative treatment options for this patient subgroup (9–12).

We have previously evaluated two small molecules, nutlin and RITA, which can restore function of wild-type p53 in multiple myeloma (13–16). However, these molecules have been shown to be inactive in multiple myeloma cells with p53 mutation/deletion. PRIMA-1/PRIMA-1\textsuperscript{Met} has been identified as a nongenotoxic agent that can target mutant/deleted p53 (17–20). PRIMA-1, a nongenotoxic candidate drug for mutant p53 reactivation, shows a significant preference for growth inhibition in several human tumor cell lines containing mutant p53, rather than those containing wild-type p53 (17–29). Because of its positive effect on animal models and efficient killing of tumor cells in ex vivo experiments, PRIMA-1\textsuperscript{Met} (or APR-246), a more efficient analog of PRIMA-1 (Fig. 1A), is being tested in phase I/II clinical trials (29).
The mutant p53-dependent antitumor activity of PRIMA-1Met has been shown in vitro and in vivo using human solid tumor xenografts (17, 19–23). In hematologic malignancies, cytotoxic activities of PRIMA-1 have been shown in the samples of patient with chronic lymphocytic leukemia (CLL) and acute myelogenous leukemia (AML). However, none of these studies on PRIMA-1 addressed the underlying mechanisms of its antileukemic effect (18, 24, 25). Interestingly, a recent study showed that PRIMA-1Met could also activate wild-type p53 and trigger a wild-type p53-dependent apoptosis in malignant melanoma cells in three dimensional culture and in melanoma xenografts in vivo (26). Moreover, PRIMA-1Met has been shown to induce apoptosis in a human prostate cancer and a mouse leukemia cell line lacking p53 expression (27, 28). Induction of apoptosis by PRIMA-1Met in both mutant and wild-type p53 or even in the absence of p53 indicates that multiple signaling pathways related or unrelated to restoration of p53 activity may be involved in this process.

In this study, we investigated in vitro and in vivo antitumor potential of PRIMA-1Met in multiple myeloma and explored the molecular mechanisms associated with this process.

Materials and Methods

Primary human myeloma samples and cell lines

Freshly isolated multiple myeloma samples were collected from newly diagnosed patients. Multiple myeloma cell lines, MM.1S, NCI-H929 (H929), RPMI-8226 (8226), LP1, and U266 used in this study were obtained from American Type Culture Collection. No authentication was done by the authors. 8226R5 cell line that do not express p53 was kindly provided by Dr. R. Buzzeo (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL) as described previously by us (15, 16). The cell lines were maintained as described previously (13–16). Peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMNC) were obtained from healthy donors.
volunteers and processed as described previously (15). This study was approved by the research ethics committee of University Health Network, Toronto, Canada, in accordance with the declaration of Helsinki.

**Drug treatment**

PRIMA-1Met was purchased from Cayman Chemical and dissolved in dimethyl sulfoxide (DMSO) to create a 10 mmol/L stock solution and stored at −20°C. In each experiment, the final DMSO concentration was kept constant and did not exceed 0.05% (v/v). In some experiments, cells were simultaneously exposed to PRIMA-1Met and dexamethasone or doxorubicin. The pan-caspase inhibitor, ZVAD-FMK was purchased from Biovision Inc. After drug treatment, cells were harvested and subjected to further analysis as described later.

**Cell viability, apoptosis, colony formation, and migration assay**

Cell viability in cell lines and patient samples was assessed by MTT assay as previously described (13–16). To examine apoptotic cell death, multiple myeloma cells were treated with various concentrations of PRIMA-1Met and stained for flow cytometry with Annexin V–FITC (Abcam) and propidium iodide (Sigma-Aldrich). Apoptotic cells were analyzed on a BD FACSCanto II (BD Biosciences) using FACSDiva (BD Biosciences) as described by us previously (16). For colony formation assays, multiple myeloma cells (5 × 10³ cells/mL) were plated into 6-well plates in 1 mL RPMI medium (20% FBS) containing 1% methylcellulose and maintained with DMSO control or the indicated concentration of PRIMA-1Met. Ten days after plating, the total number of colonies was calculated and enumerated by morphologic assessment, as previously described (30, 31). Migration assays were conducted in triplicate with 24-well Transwell insert chambers (8 μm insert; Costar, Corning Inc.) according to the manufacturer's instruction. In brief, multiple myeloma cells (5 × 10⁴ cells/mL) in were added to the upper chamber in the presence or absence of PRIMA-1Met at the indicated concentrations and allowed to migrate for 4 hours at 37°C. The migration of control DMSO-treated cells on the Transwell was normalized to 100% (32).

**Western blot analysis and immunoprecipitation**

Western blot analysis was conducted as described elsewhere (13–16). Primary antibodies used were those against p53 (DO-7; Sigma-Aldrich); Noxa (Abcam); Mcl-1, caspase-3, and PARP (Cell Signaling Technology); c-Jun, EGR1, and p73 (Signalway Antibody); p53 (pAb1620) and p53 (pAb240; Calbiochem); and α-tubulin (BioLegend). Goat anti-mouse and anti-rabbit secondary antibodies conjugated to horseradish peroxidase were purchased from Cell Signaling Technology and Santa Cruz Biotechnology, respectively. Immunoprecipitation using anti-p53 antibody was carried out by using PureProteome Protein A Magnetic Beads (Millipore) as described by the manufacturer.

**Gene expression analysis by microarray and quantitative PCR**

Protocols for microarray analysis and quantitative PCR (qPCR) are described in the supplementary section of the article (Supplementary Data). All of our microarray data are deposited to the gene expression omnibus repositories (accession no. GSE46609) following the minimum information about a microarray gene experiment guidelines.

**Genetic knockdown of selective target genes**

MM.1S, U226, or 8226R5 cells were transfected with target-specific siRNAs against p53, p73, and Noxa (Sigma), c-Jun (Qiagen) or EGR1 (Dharmacon), and control scrambled siRNA (Sigma) using the Cell Line Nucleofection Solution Kit V (Amaxa, GmbH) according to the manufacturer’s instruction with the Amxna Nucleofector II device (Amaxa; ref. 16). siRNAs synthesized for wild-type or mutant p53 were previously shown to differentiate between wild-type and mutant p53 (33). Following transfection, cells were treated with PRIMA-1Met and analyzed for inhibition of expression of p53, p73, Noxa, c-Jun, and EGR1 and apoptotic targets including caspase-3 and PARP. The effect of apoptosis induction by PRIMA-1Met following knockdown of p53, p73, Noxa, EGR1, or c-Jun was analyzed for Annexin V–positive cells by flow cytometry.

**Multiple myeloma mouse xenograft model**

Animal studies were carried out according to the guidelines of the Institutional Review Board. A total of 3 × 10⁷ 8226 cells mixed with Matrigel (BD Biosciences) were subcutaneously injected into the right flank of 6-week-old severe combined immunodeficient mice (SCID) mice. In first experiment, mice were randomized into two groups when tumors are palpable, 5 mice for treatment with 100 mg/kg PRIMA-1Met and 5 mice used as control groups. In second experiment, mice were divided into four groups. Each group contained 5 mice, with 50 mg/kg of PRIMA-1Met or 1.0 mg/kg dexamethasone or with their combination. Control group was treated with PBS. Mice were monitored for body weight and tumor volume by caliper measurements every alternate day. Tumor volumes were calculated with the formula $V = \frac{a \times b^2}{2}$, where $a$ is the long and $b$ is the short diameter of the tumor. Mice were euthanized in the event of tumor size of more than 1.5 cm or major compromise in their quality of life. Tumors were harvested for further assay and tumor weight was recorded. For Western blot analysis and immunohistochemical analysis, tumors (two from each group, after three injections) were snap frozen or fixed in 10% formalin solution and processed routinely. TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling; Promega) and Ki-67 (Novus Biologicals) staining of the tumor sections were conducted as described by the manufacturer. A group of mice was followed up until the mice in the treated or control groups die or event occurs (tumor exceeds the allowable size).
Survival of mice between the two treatment groups was
determined by the Kaplan–Meier test using the log-rank
analyses to compare the mean overall mouse survival
with 95% confidence intervals.

Statistical analysis
The synergistic effect [combination index (CI) < 1.0] of
the combination of PRIMA-1Met and dexamethasone or
doxorubicin was analyzed using CalcuSyn (Biosoft), a
software program based on the Chou–Talalay method,
as described by us previously (13–16). Statistical signifi-
cance levels were determined by two-tailed Student t test
analysis. P values of more than 0.05 were considered
significant.

Results
PRIMA-1Met induces apoptosis in multiple myeloma
cells but has minimal cytotoxicity on normal
hematopoietic cells
We first studied in vitro activity of PRIMA-1Met against
different multiple myeloma cell lines harboring wild-type
(MM.1S, H929), mutant (LP1, U266, and 8266), or null
p53 (8266R5). The effect of PRIMA-1Met on viability of
multiple myeloma cells was determined by MTT assays.
Multiple myeloma cell lines of different p53 status were
cultured in the presence of increasing doses of PRIMA-
1Met (0–40 μmol/L) for 48 hours. As shown in Fig. 1,
PRIMA-1Met treatment resulted in dose-dependent de-
crease in the viability of multiple myeloma cells. The
IC50 of PRIMA-1Met varied among the cell lines tested
(Fig. 1B and C). By interphase FISH analysis we detected
hemizygous p53 deletions in U266 (~90%) and LP1
(~20%) cells (data not shown). To evaluate the cytotoxic
effect of PRIMA-1Met in patient with multiple myeloma
cells, we isolated CD138+ tumor cells from 9 newly
diagnosed patients with multiple myeloma, three (sample
#2, #5, and #9) of the nine samples carried hemizygous
p53 deletions (data not shown). PRIMA-1Met treatment
resulted in a dose-dependent cytotoxicity of patient-
derived multiple myeloma cells (Fig. 1D). However,
PRIMA-1Met showed only a limited cytotoxicity in normal
PBMCs and BMMNCs obtained from healthy donors (Fig.
1E). These results indicate that PRIMA-1Met preferentially
kills malignant myeloma cells. We next examined wheth-
er the declining of the viability of multiple myeloma cells
upon PRIMA-1Met stimulation was due to apoptosis
induction in these cells. Results showed that PRIMA-1Met
induced apoptosis in multiple myeloma cells as evi-
denced by enhanced Annexin V binding in cells treated
with PRIMA-1Met. The percentage of apoptotic MM.1S,
H929, U226, and 8226R5 cells increased in a dose-depen-
dent manner (Fig. 1F). The sensitivity of wild-type, mutant,
or p53-null cells to PRIMA-1Met suggest that it
can induce apoptosis irrespective of p53 functional status
in multiple myeloma cells.

PRIMA-1Met inhibits the clonogenic and migratory
properties of multiple myeloma cells
To further explore the antmyeloma effects induced
by PRIMA-1Met, we carried out a clonogenic assay to
study the colony formation activity of multiple myeloma
cells treated with PRIMA-1Met. We found a 30% and 33%
reduced MM.1S and 8226 colony formation, respectively
(Fig. 2A). As migration plays an important role in the
progression of multiple myeloma, the effect of PRIMA-
1Met on cell migration was then examined using Transwell
inserts system. As shown in Fig. 2B, treatment of either

![Image](https://example.com/image.png)

**Figure 2.** PRIMA-1Met inhibits colony formation and cell migration of multiple myeloma cells. A, colony formation assay using multiple myeloma cells. RPMI-
8226 and MM.1S cells (5 × 10⁴ cells/mL) were seeded into 12-well plate in methylcellulose-based medium. After 10 days, colony formation capacity was
evaluated by counting colonies including more than 50 cells. B, MM.1S and 8226 cells treated with DMSO control or PRIMA-1Met were grown in 24-well
Transwell migration plates and the migratory potential of the cells were determined as described in Materials and Methods. Data are expressed as mean ± SD
of triplicate cultures. *, P < 0.05; **, P < 0.01.
MM.1S or 8226 cells with PRIMA-1 Met significantly decreased the number of migrating cells. These findings indicate that PRIMA-1 Met inhibits clonogenic and migratory properties of multiple myeloma cells irrespective of p53 status.

**PRIMA-1 Met** treatment results in caspase-dependent apoptosis of multiple myeloma cells

To understand the mechanism of PRIMA-1 Met–induced apoptosis, we investigated the effect of PRIMA-1 Met on multiple myeloma cells using immunoblotting analysis. Western blot analysis revealed that PRIMA-1 Met triggered time- (Fig. 3A) and dose-dependent (Fig. 3B) activation of p73 and HSP70 followed by caspase-3 and PARP cleavage in MM.1S, U266, and 8226R5 cells. Moreover, activation of caspase was associated with upregulation of a proapoptotic marker, Noxa and downregulation of an antiapoptotic marker, Mcl-1 in a time- and dose-dependent manner (Fig. 3A and B). Furthermore, to determine PRIMA-1 Met–induced cell death is dependent on caspase activity, we treated U266 and MM.1S cells with the pan-caspase inhibitor ZVAD-FMK (20 μmol/L) before treatment with 50 μmol/L for 2 hours before treatment with ZVAD-FMK pretreatment, evidenced by inhibition of cleavage of caspase-3 and PARP induced by PRIMA-1 Met in U266 cells (Fig. 3C) and confirmed by increase in survival by MTT assay in MM.1S and U266 cells (Fig. 3D; P < 0.05). These results suggest that apoptosis induced by PRIMA-1 Met in multiple myeloma cells is caspase-dependent.

**Apoptosis induction of multiple myeloma cells by PRIMA-1 Met is not totally dependent on p53 activation**

Having shown that PRIMA-1 Met induces apoptosis in multiple myeloma cells harboring either wild-type or mutant p53 or even in p53 null cells, we further examined whether selective knockdown of p53 expression by siRNA has any effect on the induction of apoptosis in multiple myeloma cells. MM.1S and U266 cell lines were transfected...
with siRNAs specific for wild-type or mutant p53, respectively, treated with PRIMA-1Met (10 and 20 \( \mu \)mol/L, respectively), and examined for its apoptotic activity by Western blot and flow cytometry analysis. The results showed that knockdown of p53 in either MM.1S or U266 cell lines had little effect on the cleavage of PARP induced by PRIMA-1Met (Fig. 4A) as well as on the binding of Annexin V (Fig. 4B), indicating that PRIMA-1 Met–induced apoptosis in multiple myeloma cells is not totally dependent on p53.

**Effective knockdown of p73 and Noxa attenuates the apoptotic activity of PRIMA-1Met in multiple myeloma cells**

Because treatment of multiple myeloma cells with PRIMA-1Met resulted in increased p73 protein levels in multiple myeloma cells carrying wild-type, mutant, or null p53 we next sought to determine whether PRIMA-1Met–induced apoptosis is specifically dependent on activation of p73. To this aim, p73 expression was silenced by siRNA. p73 knockdown using siRNA specific to p73 resulted in more than 90% inhibition of p73 expression, as measured by densitometry. Having shown upregulation of the proapoptotic protein Noxa in multiple myeloma cells irrespective of p53 status, we also silenced the expression of Noxa. Knockdown of p73 diminished expression of Noxa, however, silencing Noxa did not significantly modulate the expression of p73, indicating that Noxa is the downstream target of p73 (Fig. 4C).

Functionally, knockdown of either p73 or Noxa resulted in attenuation of PRIMA-1 Met–induced apoptosis as shown by decrease in percentage of Annexin V-positive cells (Fig. 4D). Taken together, our data suggest that induction of p73 is required for PRIMA-1Met–mediated apoptosis in multiple myeloma cells.

**PRIMA-1Met treatment retards growth of multiple myeloma tumors harboring mutant p53 in vivo**

Next, we sought to establish whether our in vitro observations would translate to antimyeloma activity in vivo by studying the antitumorigenic potential of PRIMA-1Met in multiple myeloma xenograft SCID mouse models. Of note, 8266 cells, which are capable of forming tumors, were inoculated into SCID mice and...
the animals received intraperitoneal injections of either 100 μL PBS (control) or 100 mg/kg PRIMA-1Met once daily for 15 days after tumor formation was evident. Administration of PRIMA-1Met resulted in significant inhibition of multiple myeloma tumor growth (Fig. 5A; \( P < 0.05 \)) and increased the survival (Fig. 5B; \( P = 0.007 \)). There were no obvious toxic effects of the treatments as evaluated by mouse body weight data (Fig. 5C).

To assess \textit{in vivo} activation of p73 signaling, we examined whether PRIMA-1Met could induce apoptosis through activation of p73 in human tumor xenografts. Treatment of 8226 tumor xenografts with PRIMA-1Met resulted in induction of p73 and cleavage of caspase-3 (Fig. 5D) in accordance with our \textit{in vitro} results (Fig. 3). Given the therapeutic efficacy of PRIMA-1Met in multiple myeloma mouse xenograft model, we next examined the associated histologic events. As seen in Fig. 5E, treatment of PRIMA-1Met resulted in decrease of the Ki-67–positive and increase of TUNEL–positive cells in mice tumor samples. These findings therefore support our \textit{in vitro} data showing induction of apoptosis in multiple myeloma cells by PRIMA-1Met.

\textbf{PRIMA-1Met has synergistic or additive antimyeloma activity when combined with current therapeutics}

Tumors with alterations of p53 or its family usually do not respond adequately to genotoxic chemotherapeutic agents including glucocorticoids (e.g., dexamethasone) or topoisomerase inhibitors (e.g., doxorubicin) that act through the p53 pathway. We, therefore, examined whether combination of PRIMA-1Met with dexamethasone or doxorubicin can enhance their cytotoxic response in multiple myeloma cells. MM.1S cells were treated with 5 μmol/L PRIMA-1Met, dexamethasone (0.25 μmol/L), or doxorubicin (0.25 μmol/L) alone or in combination of these doses of PRIMA-1Met plus dexamethasone or PRIMA-1Met plus doxorubicin. The cytotoxicity of the cells was assayed by MTT at 48 hours. A significant decrease in cell viability was observed in response to treatment with combined low doses of PRIMA-1Met and dexamethasone or PRIMA-1Met and doxorubicin than with either agent alone (\( P < 0.05 \)) in MM.1S cell line (Fig. 6A). Synergism was noted at these combinations of the drugs tested, evidenced by CI of less than 1. Combination of PRIMA-1Met and dexamethasone in two primary

\begin{figure}[h]
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\caption{PRIMA-1Met inhibits the growth of multiple myeloma tumors with mutant p53 \textit{in vivo}. SCID mice (\( n = 5/\text{group} \)) were injected subcutaneously with 8226 cells (\( 3 \times 10^7 \)) in RPMI medium along with Matrigel matrix. Following detection of tumor, mice were divided into two groups: the control group that received daily intraperitoneal injection of vehicle (PBS) and the treatment group that received daily intraperitoneal injection of PRIMA-1Met (100 mg/kg). A, tumors were measured in two perpendicular dimensions every alternate day and tumor volumes were calculated (\( \cdot P < 0.05 \)). B, survival was evaluated from the first day of treatment until death or an event occurred by using the Kaplan–Meier curve (\( P = 0.007 \)). C, body weight was measured every alternate day till day 15. D, tumors were isolated from PRIMA-1Met–treated and control mice and harvested for analysis by Western blot analysis for the expression of p73 and caspase-3. E, immunohistochemical analysis of tumor sections showed that treatment of mice bearing multiple myeloma tumors with PRIMA-1Met resulted in decrease in the proliferation index (Ki-67) and increase in the apoptotic index (TUNEL), further verifying the ability of PRIMA-1Met to induce apoptosis in multiple myeloma cells \textit{in vivo}.}
\end{figure}
multiple myeloma specimens with hemizygous p53 deletions displayed at least additive cytotoxic response (Fig. 6B).

Finally, to test whether enhanced myeloma cell apoptotic response in combination with PRIMA-1\textsuperscript{Met} and dexamethasone could be extended \textit{in vivo}, we used similar subcutaneous 8226 xenograft model of human multiple myeloma in mice as described above. As shown in Fig. 6C, PRIMA-1\textsuperscript{Met} or dexamethasone alone showed moderate inhibition of tumor growth. In contrast combination of PRIMA-1\textsuperscript{Met} and dexamethasone displayed significant growth inhibition versus control (\(**, P < 0.01\)) and versus low-dose PRIMA-1\textsuperscript{Met}/dexamethasone alone (on day 15; \(*, P < 0.05\)). Importantly, overall survival of PRIMA-1\textsuperscript{Met} and dexamethasone-treated mice was significantly prolonged as compared with mice treated with dexamethasone (\(P = 0.043\)) or PRIMA-1\textsuperscript{Met} (\(P = 0.010\)) alone or control (\(P = 0.006\); Fig. 6D). In addition, treatment with either PRIMA-1\textsuperscript{Met} alone or combination with dexamethasone did not affect body weight (Fig. 6E), indicating the doses used for the treatment were tolerable to the mice.

Discussion

In this report, we show that PRIMA-1\textsuperscript{Met} exerts a powerful antitumor activity in multiple myeloma cell lines and malignant plasma cells derived from patients as well as in a clinically relevant xenograft model of human multiple myeloma. \textit{In vivo} results were complemented by \textit{in vitro} experiments where PRIMA-1\textsuperscript{Met} showed significant anti-proliferative activity, apoptotic effects, and modulation of gene expression. To our knowledge, this is the first experimental evidence of antitumor activity of PRIMA-1\textsuperscript{Met} in preclinical models of multiple myeloma.

Treatment of multiple myeloma cells with PRIMA-1\textsuperscript{Met} resulted in significant inhibition of viability, colony

![Graph showing viability and survival](Image)

**Figure 6.** PRIMA-1\textsuperscript{Met} in combination with dexamethasone or doxorubicin display synergistic cytotoxic response in a multiple myeloma cell line and two primary multiple myeloma samples. A, MM.1S cells were treated with PRIMA-1\textsuperscript{Met} (5 \(\mu\)mol/L) in combination with dexamethasone (0.25 \(\mu\)mol/L) or doxorubicin (0.25 \(\mu\)mol/L). Forty-eight hours after treatment, cells were assessed for survival by MTT viability assay (*, \(P < 0.05\)). Close bar (■), PRIMA-1\textsuperscript{Met}; open bar (□), dexamethasone or doxorubicin; and open hatched bar ( ), combination of PRIMA-1\textsuperscript{Met} plus dexamethasone or doxorubicin. B, primary multiple myeloma samples from 2 newly diagnosed patients carrying hemizygous p53 deletion were treated with PRIMA-1\textsuperscript{Met} (5 \(\mu\)mol/L) and dexamethasone (1 \(\mu\)mol/L) for 48 hours and viability was determined as described above (*, \(P < 0.05\); **, \(P < 0.01\)). PRIMA-1\textsuperscript{Met}-enhanced antitumor activity of dexamethasone \textit{in vivo}. SCID mice were inoculated subcutaneously with \(3 \times 10^7\) 8226 cells in 100 \(\mu\)L RPMI medium together with Matrigel matrix. Twenty tumor-bearing mice were randomly assigned to four groups and treated for 15 days with control (PBS), PRIMA-1\textsuperscript{Met} (50 mg/kg), dexamethasone (1 mg/kg), or the combination once daily. At day 15 the treatment was discontinued and mice were monitored for tumor dynamics and body weight. C, PRIMA-1\textsuperscript{Met} and dexamethasone combination therapy triggered more potent inhibition of tumor growth in mice treated with PRIMA-1\textsuperscript{Met} or dexamethasone alone (**, \(P < 0.05\)) or control (***, \(P < 0.001\)). D, combination therapy markedly prolonged survival compared with mice treated with control (\(P = 0.006\)), PRIMA-1\textsuperscript{Met} (\(P = 0.010\)), or dexamethasone (\(P < 0.043\)) alone. E, mice body weight was used to assess toxicity of the treatment. C, control; P, PRIMA-1\textsuperscript{Met}; D, dexamethasone; P + D, PRIMA-1\textsuperscript{Met} + dexamethasone.
Antimyeloma Activity of PRIMA-1\textsuperscript{Met}
synthesis and Myc overexpression has been proposed to be associated with progression of multiple myeloma (1, 2). Recent reports have shown that c-Myc–dependent apoptosis of multiple myeloma cells by small-molecule inhibitors such as pazopanib (41) and 10058-F4 (42) or a growth factor, bone morphogenetic proteins (43). Therefore, in addition to activation of p73 and/or Noxa, downregulation of c-Myc may play a role in PRIMA-1Met–induced apoptosis of multiple myeloma cells. Further studies will be required to understand the significance of PRIMA-1Met–induced downregulation of c-Myc in the apoptosis of multiple myeloma cells.

To study the clinical relevance of PRIMA-1Met in vivo, mice bearing multiple myeloma tumors were treated with PRIMA-1Met or PRIMA-1Met in combination with dexamethasone. In both in vivo studies, PRIMA-1Met induced a significant tumor growth delay and prolonged survival compared with control mice. In keeping with our in vitro observation, the apoptotic effect of PRIMA-1Met was related to p73 activation in mouse xenograft model. From a therapeutic perspective, it is interesting to note that the doses of PRIMA-1Met that induces apoptosis in multiple myeloma cells in vitro are achievable in vivo, as previously shown by their potent antitumor effect in mouse models of human cancer (17, 19, 23). PRIMA-1Met has recently been administered safely to patients with AML and prostate cancer (29). Furthermore, our in vitro and in vivo studies have shown that apoptosis induced by PRIMA-1Met can synergistically be enhanced by combining it with dexamethasone. This suggest that it could be possible to lower the doses of PRIMA-1Met as well as of the chemotherapeutic drugs used in the treatment of multiple myeloma and thus reduce cytotoxicity to normal cells. Importantly, our findings warrant the clinical evaluation of PRIMA-1Met in a broader patient group, as antimyeloma activity of PRIMA-1Met is not restricted to only mutant p53.

On the basis of the data presented here we conclude that treatment of multiple myeloma cells with PRIMA-1Met lead to induction of p73-mediated apoptosis by upregulating Noxa and downregulating Mcl-1 irrespective of p53 status. Our study provides the preclinical framework to initiate clinical trial of PRIMA-1Met either alone or in combination with a conventional antimultiple myeloma agent, dexamethasone to increase response, overcome drug resistance, reduce side effects, and improve patient outcome in multiple myeloma including the high-risk group with p53 mutation/deletion.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M.N. Saha, H. Chang
Development of methodology: M.N. Saha, H. Jiang, Y. Yang, H. Chang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Yang, D. Reece, H. Chang
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Writing, review, and/or revision of the manuscript: M.N. Saha, D. Reece, H. Chang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Jiang
Study supervision: H. Chang

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References

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